“Now this is not the end.
It is not even the beginning of the end.
But it is, perhaps, the end of the beginning.”

Winston Churchill (1874-1965)
The skin as a barrier in physiotherapy
FACULTY OF PHYSICAL EDUCATION AND PHYSIOTHERAPY
DEPARTMENT OF HUMAN BIOMETRY AND BIOMECHANICS

THE SKIN AS A BARRIER IN PHYSIOTHERAPY

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THESIS SUBMITTED IN FULLFILMENT OF THE REQUIREMENTS FOR THE
DEGREE OF DOCTOR IN PHYSIOTHERAPY

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<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preface and Acknowledgements</td>
<td>I</td>
</tr>
<tr>
<td>Scientific curriculum: Publications in international peer reviewed journals</td>
<td>III</td>
</tr>
<tr>
<td>List of abbreviations</td>
<td>VI</td>
</tr>
<tr>
<td>Summary</td>
<td>VIII</td>
</tr>
<tr>
<td>1. Background and introduction of the thesis</td>
<td>1</td>
</tr>
<tr>
<td>1.1. The skin</td>
<td>5</td>
</tr>
<tr>
<td>1.1.2 The dermis</td>
<td>7</td>
</tr>
<tr>
<td>1.1.3 The subcutaneous tissue</td>
<td>8</td>
</tr>
<tr>
<td>1.2 The skin as an insulating barrier</td>
<td>9</td>
</tr>
<tr>
<td>1.3 The skin as a barrier for topical drug application</td>
<td>11</td>
</tr>
<tr>
<td>1.3.1 Skin penetration pathway</td>
<td>16</td>
</tr>
<tr>
<td>1.3.2 Reservoir function of the SC</td>
<td>17</td>
</tr>
<tr>
<td>1.4 Penetration enhancement methods</td>
<td>18</td>
</tr>
<tr>
<td>1.4.1 Occlusion</td>
<td>19</td>
</tr>
<tr>
<td>1.4.2 Iontophoresis</td>
<td>19</td>
</tr>
<tr>
<td>1.4.3 Sonophoresis</td>
<td>21</td>
</tr>
<tr>
<td>1.5 Non-invasive measurements of skin properties</td>
<td>23</td>
</tr>
<tr>
<td>1.5.1 Skin temperature</td>
<td>23</td>
</tr>
<tr>
<td>1.5.2 Skin colour</td>
<td>24</td>
</tr>
<tr>
<td>1.5.3 Transepidermal water loss</td>
<td>27</td>
</tr>
<tr>
<td>1.5.5 Perfusion of skin microcirculation measurement</td>
<td>28</td>
</tr>
<tr>
<td>1.5.6 Models to study percutaneous absorption non- invasively</td>
<td>30</td>
</tr>
<tr>
<td>1.6 Summary evaluation</td>
<td>32</td>
</tr>
<tr>
<td>1.6.1 Research questions</td>
<td>35</td>
</tr>
<tr>
<td>2. MATERIALS AND METHODS</td>
<td>36</td>
</tr>
<tr>
<td>2.1 Measurements techniques</td>
<td>36</td>
</tr>
<tr>
<td>2.1.1 Skin temperature</td>
<td>36</td>
</tr>
<tr>
<td>2.1.2 Skin colour measurements</td>
<td>36</td>
</tr>
<tr>
<td>2.1.3 Perfusion of the Microcirculation</td>
<td>37</td>
</tr>
<tr>
<td>2.1.4 Transepidermal water loss</td>
<td>38</td>
</tr>
<tr>
<td>2.1.5 Blood pressure and heart rate measurements</td>
<td>38</td>
</tr>
</tbody>
</table>
### 3. Experimental stipulations

3.1 Methyl nicotinate induced skin erythema

3.2 Corticosteroid induced blanching

3.3 Structure and chemical properties of the used indicator molecules

### 4. Results

4.1 Changes of skin characteristics during and after local paraphango therapy as used in physiotherapy

4.2 The effects of iontophoresis in the treatment of musculoskeletal disorders – A systematic review and meta-analysis

4.3 Determination of the in vivo bioavailability of iontophoretically delivered diclofenac using a methyl nicotinate skin inflammation assay

4.4 In vivo determination of the diclofenac skin reservoir: comparison between passive, occlusive and iontophoretic application

4.5 Influence of the timing of ultrasound application on the penetration of corticosteroids

### 5. General Discussion

### 6. General Conclusion and suggestions for further research

### 7. References

### 8. Dutch summary
Preface and Acknowledgements

After finishing my master degree in Physical Therapy Science in 2005, I became the opportunity to continue my work as scientific collaborator at the Department of Human Biometry and Biomechanics from the faculty of Physical Education and Physiotherapy at the “Vrije Universiteit Brussel”. Over the past eight years I experienced and learned a lot about being a faculty member and doing research in the field of transdermal drug delivery. Completion of this doctoral thesis was only possible with the support of several people. I would like to express my sincere gratitude to all who have contributed to the completion of this work.

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Scientific curriculum: Publications in international peer reviewed journals


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### List of abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>AU</td>
<td>Arbitrary unit</td>
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<tr>
<td>BPM</td>
<td>Beats per minute</td>
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<td>CCT</td>
<td>Controlled clinical trail</td>
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<td>CER</td>
<td>Ceramides</td>
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<td>CHOL</td>
<td>Cholesterol</td>
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<tr>
<td>CI</td>
<td>Confidence interval</td>
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<tr>
<td>COX</td>
<td>Enzyme cyclooxygenase</td>
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<td>CTS</td>
<td>Carpal tunnel syndrome</td>
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<tr>
<td>DBP</td>
<td>Diastolic blood pressure</td>
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<tr>
<td>DF</td>
<td>Diclofenac</td>
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<tr>
<td>DSP</td>
<td>Dexamethasone sodium phosphate</td>
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<tr>
<td>EN</td>
<td>Ethyl nicotinate</td>
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<tr>
<td>ESWT</td>
<td>Extracorporal shock wave therapy</td>
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<tr>
<td>ET</td>
<td>Electrotherapy</td>
</tr>
<tr>
<td>FFA</td>
<td>Free fatty acids</td>
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<td>FSS</td>
<td>Functional status scale</td>
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<td>IRT</td>
<td>Infra red thermography</td>
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<tr>
<td>JSS</td>
<td>Steady state flux</td>
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<tr>
<td>LDV</td>
<td>Laser Doppler velocimetry</td>
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<tr>
<td>MCS</td>
<td>Microcirculation of the skin</td>
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<td>MN</td>
<td>Methyl nicotinate</td>
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<tr>
<td>MW</td>
<td>Molecular weight</td>
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<tr>
<td>NSAID</td>
<td>Non-steroid anti-inflammatory drug</td>
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<td>PCS-12</td>
<td>Physical component Summery-12</td>
</tr>
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<td>PEDro</td>
<td>Physiotherapy Evidence Database</td>
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<tr>
<td>Abbreviation</td>
<td>Description</td>
</tr>
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<td>--------------------------------------------------</td>
</tr>
<tr>
<td>PGD2</td>
<td>Prostaglandin D2</td>
</tr>
<tr>
<td>PU</td>
<td>Perfusion Units</td>
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<tr>
<td>QoL</td>
<td>Quality of life</td>
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<tr>
<td>RCT</td>
<td>Randomized controlled trails</td>
</tr>
<tr>
<td>ROM</td>
<td>Range of motion</td>
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<tr>
<td>RTD</td>
<td>Resistance temperature detector</td>
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<tr>
<td>SBF</td>
<td>Skin blood flow</td>
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<tr>
<td>SBP</td>
<td>Systolic blood pressure</td>
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<tr>
<td>SC</td>
<td>Stratum corneum</td>
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<tr>
<td>SD</td>
<td>Standard deviation</td>
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<tr>
<td>SI</td>
<td>Steroid iontophoresis</td>
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<td>SP</td>
<td>Stratum papillare</td>
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<tr>
<td>SR</td>
<td>Stratum reticulare</td>
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<tr>
<td>SSS</td>
<td>Symptom severity scale</td>
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<tr>
<td>ST</td>
<td>Skin temperature</td>
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<tr>
<td>TACA</td>
<td>Triamcinolone acetonide</td>
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<tr>
<td>TENS</td>
<td>Transcutaneous electrical nerve stimulation</td>
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<td>TEWL</td>
<td>Transepidermal water loss</td>
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<td>TMD</td>
<td>Temporomandibular disorders</td>
</tr>
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<td>TS</td>
<td>Tape stripping</td>
</tr>
<tr>
<td>US</td>
<td>Ultrasound</td>
</tr>
<tr>
<td>VAS</td>
<td>Visual analog scale</td>
</tr>
</tbody>
</table>
Summary
This last decade, the professional societies and educational programs in physiotherapy have included the paradigm of Evidence Based Practice (EBP) within the defining of the professional competencies of the physiotherapist. Furthermore, this last decade physiotherapy has made reasonable progress to support its treatments with evidences. Despite, several gaps remain towards evidences for electrophysical applications. In this doctoral thesis we investigated aspects of physiological processes related to physical applications in physiotherapy from the point of view of the skin as a limiting barrier in particular concerning the therapeutic implications of phango, iontophoresis and ultrasound.

Paraphango
Local paraphango applications are used to treat musculoskeletal problems. The vasodilation induced by local heat increases the blood flow in the muscle tissue, reducing ischemia and pain sensation. Cardiovascular problems are often considered as a contraindication for local heat application due to redistribution of blood towards the heated area. Despite these claims, the physiological mechanisms controlling vasomotion and thermoregulation are insufficiently understood and information on heat induced changes in skin parameters is limited.
Our study revealed that there were strong local effects of paraphango with increased skin temperature, microcircular perfusion and skin erythema. The systemic (cardiovascular) effects were weak and remained in physiological range.

Physiotherapeutic support of topical transdermal applications
Topical transdermal applications are commonly used in the treatment of various (rheumatic) inflammatory dysfunctions and acute soft tissue injuries. In physiotherapy various transdermal enhancements techniques, such as sonophoresis, iontophoresis and occlusion are used in order to decrease the skin barrier and to achieve a clinical
effective drug concentration in the target tissues.

The literature study and meta-analysis on the treatment effects of iontophoresis in musculoskeletal disorders provided quantitative evidence that iontophoresis might be effective to cope with pain. Although clinical studies claim an enhanced healing process, the evidences concerning iontophoretic drug delivery lacked solid research designs and often did not take account of the basic principles of percutaneous penetration. The iontophoretic delivery of an active substance only has been compared with placebo iontophoresis without an active substance, which provides no proof for the penetration enhancement effect of a current.

In our study investigating the bioavailability of iontophoretically delivered diclofenac with the methylnicotinate test, we did not find an increased bioavailability after electrically assisted delivery of diclofenac as compared with the passive percutaneous penetration under the contact sponge. Our results suggest that the electromotive forces may have been overestimated during iontophoretic delivery in those experimental designs whereby the passive uptake and occlusion effects were not evaluated.

The building up of a stratum corneum reservoir is an important component of the pharmacodynamics of topically applied substances. There is scarce information concerning the pharmacodynamic behaviour of topical substances used in the physiotherapy setting, for which reason we aimed to estimate the formation and emptying of the diclofenac skin reservoir after passive, semi occlusive and electrically assisted applications of diclofenac. The results of this study indicated that the formation and emptying of a DF skin reservoir was found to be dependent on the DF application mode. Penetration enhanced delivery resulted in a faster emptying of the reservoir. Our results assume that the application with the fastest reservoir building up conditions may result in a
greater delivery of active substances to the viable tissues, increasing the effectiveness of the physiotherapeutic treatment.

**Ultrasound** is used to support topical treatment of NSAIDs or corticosteroids and can be applied in a “pre”- application treatment or a “simultaneous” application modus. Although low frequency sonophoresis (frequencies 20-100 KHz) is up to three orders of magnitude more effective in enhancing skin permeability compared to the 1MHz ultrasound, the most common sonophoretic application in physiotherapy involves the use of high frequency or therapeutic sonophoresis (frequencies ≥ 0.7 MHz). The aim of this study was to estimate the influences of sonophoretic treatment on delivery of halcinonide, using a blanching procedure and to compare the efficacy of “pre” and “simultaneous” ultrasound treatment.

The “pre”- application modus of ultrasound resulted in a significant blanching response of the skin while under the condition of “simultaneous” ultrasound no blanching was found two hours after the initial application of halcinonide.

The clinical implication of this study is that better bioavailability results may be expected when ultrasound is applied before application of the drug.

Evidenced based practice in physiotherapy is relatively new, the positive impacts of which are just becoming to be validated. Within the context of evidence based practice, continued research in physiotherapy is essential to improve the effectiveness of treatment strategies. Within the paradigm of evidence based practice, the results of this research support the competence of the physiotherapist as a clinical practitioner to optimize the efficacy of his/her treatment strategies.
1. Background and introduction of the thesis

Physiotherapy interventions encompass training of motor skills, muscle strengthening, joint mobilisation and manipulation, massage, hydrotherapy, relaxation and the use of physical modalities such as heat, electrical stimulation and ultrasound [71]. In treatments such as massage, electrotherapy and thermocryotherapy, the skin acts as a mechanical or insulation barrier. Despite, the skin as an organ received up to now scarce attention in the physiotherapy literature. Furthermore, musculoskeletal disorders are frequently treated with topical application on the skin of non-steroidal anti-inflammatory drugs (NSAID) or corticosteroids, with the intention to locally treat the tissues underlying the skin, such as the muscles, tendons and/or synovial fluid. Again, the skin and in particular the stratum corneum (SC) is the major barrier for these topical applied substances.

The number of electrophysical agents for physiotherapeutic applications has grown remarkably over the past three decades. Selecting the most appropriate therapy modality for a patient depends on the practitioner decision-making process within an evidence based practice philosophy. This change in paradigm of the physiotherapeutic decision making process from an empirical approach based on experience to the evidence based practice philosophy has led to a shift how physiotherapists view and use therapy modalities.

Despite the widespread use of physical applications in physiotherapy, the physical and physiological principles substantiating the treatment effects are often not well understood and sometimes inappropriately applied. Additionally, the lack of scientific evidence contains the risk of suboptimal treatment protocols, thereby reducing the treatment’s efficacy [164]. This lack of evidence does not implicate that there is evidence for absence of effect. Watson (2002) stated that the basic tenets
of modern electrotherapy are essentially correct, but also made a plea for clinical
decision making in context of evidence based practice by which evidences should
guide the practitioner to replace common electrotherapy protocols by those with a
substantive evidence of efficacy [164].

Consequently, evaluation of the physiological effects of specific applications on the
skin may be an important step in the quest for therapeutic evidences since integrity
and functional properties of the skin barrier are factors that influence the penetration
process. The integrity of the skin barrier can be instrumentally evaluated by means
of skin hydration and transepidermal water loss (TEWL) [63][27][70]. Non-invasive
quantification of the skin response after penetration of vaso-active substances can
be carried out instrumentally, measuring skin temperature (ST), skin colour, and
perfusion of the microcirculation. This approach has been proven valid. The non-
invasive quantification of skin erythema provoked by a methylnicotinate (MN) skin
inflammation assay can be used as a method to evaluate its bioavailability in the
skin [22][66][87][150][151]. Similarly, the vasoconstriction provoked after
penetration of corticosteroids (skin blanching assay) can be used to study the
penetration processes of this substance [31][35]. In the present study, non-invasive
skin measurements were used to evaluate the effectiveness of some common
treatment modalities as used in physiotherapy: local heat application, ultrasound
and iontophoresis. Although these treatments modalities are frequently used in
physiotherapy setting the evidence of effectiveness is poor or lacking. Therefore
investigation of the underlying physiological mechanisms, even though it concerns
but a limited part of the total picture, can support physiotherapists with their
evidence based decision-making process.
Paraphango therapy such as ‘Paraphango di Battaglia’ is a commonly used modality in physical therapy with described but not objectively quantified effects on ST, and superficial microcirculation of the skin [115] [53] [122] [50] [153]. Due to vasomotion of the capacitance vessels of the deeper layers of the skin, the blood circulation increases in order to maintain the body core temperature. The latter may explain the better diffusion of catabolic waste with phango therapy. Heat induced interruption of the so called „pain-spasm-pain cycle“ can prevent further tissue injury and reduce the sensation of pain [109]. The redistribution in blood circulation may influence systemic cardiovascular parameters. Although in physiotherapy practice cardiovascular diseases are often considered as a contra-indication for local heat applications, the evidence for this assumption is not well substantiated. In order to get a better understanding of the physiological effects of local paraphango application, its systemic effects on heart rate, blood pressure and core temperature were evaluated together with a quantification of its effect on the skin.

In the physiotherapeutic treatment of inflammatory dysfunctions and soft tissue injuries, topical therapy is commonly used [41] [94] [112]. It is well known that permeability of the skin for topically applied substances is low. Penetration enhancement techniques such as occlusion, iontophoresis, sonophoresis have been developed with proven efficacy [106]. These experimental designs are not always applicable under clinical physiotherapeutic condition. Nevertheless there is a plethora of literature reporting the use of these techniques in a clinical setting [2] [3] [11] [5] [41] [61]. Although several studies using skin penetration enhancement techniques claim clinical effects in the treatment of musculoskeletal disorders, controversial reports related to the effectiveness of these treatment modalities are found in the literature [14] [76] [13]. In most in vivo studies the principles of
percutaneous penetration are not always taken into account. The effectiveness of an iontophoretic delivered active substance is often compared with placebo iontophoresis without an active compound, which is no valid proof for the enhanced penetration effect of a current. Clinical studies evaluating the effectiveness of electrically assisted drug delivery often use multiple treatments strategies, without objective outcome measures or control groups. Consequently, it is impossible to differentiate between the independent treatment effects of the factors of influence on percutaneous penetration, specifically to draw conclusions on the effective outcome of iontophoresis / ultrasound as a treatment on its own. To avoid overestimation of the enhancement factor of a current, or ultrasound it is important to control for passive diffusion and occlusion. Therefore two models were developed to study respectively iontophoresis and ultrasound enhancement as commonly used under clinical physiotherapeutic conditions. In the first model we estimated the effect of current on percutaneous penetration and skin reservoir building of DF. This model allowed us to control for passive penetration and occlusion. In the second model we compared two different modes of ultrasound assisted corticosteroids application. Again the model allowed us to control for passive penetration.

The results of present research may contribute to the evidence-based practice of frequently used applications in physiotherapy and may equally contribute to the fundamental knowledge on the percutaneous penetration to topically applied substances in physiotherapy.

The introduction of the thesis provides an overview of the structure of the skin, the skin as an insulating barrier and the skin as a barrier for topically applied substances. It equally describes several penetration enhancement methods and finally the applicability of non-invasive measurements of skin properties for the study of percutaneous absorption is discussed.
1.1. The skin

The skin, the outmost largest single organ of the human body, acts as an interface between the organism and the external environment, protecting and supporting the life it encloses [77] [99]. The skin is relatively complex in structure and composed of two main layers: the epidermis, an epithelial layer of ectoderm origin; and the dermis, a layer of connective tissue of mesoderm origin [77].

The SC, the uppermost layer of the epidermis comprises 25-30 layers in which flattened death corneocytes embedded in a crystalline lipid lamellar matrix fill the extracellular space. The corneocytes contain keratin and minor quantities of involucrine and fillagrine. The intercellular matrix consists of lipids (e.g. ceramides, fatty acids and cholesterol) [77] [89], composed of long chain ceramides (CER), free
fatty acids (FFA) and cholesterol (CHOL) as main lipid classes by which it differs markedly from other biological membranes [17].

The SC has been well recognized as the principal barrier of the skin [17]. This two-way barrier minimizes water and electrolytes losses and prevents the entrance of foreign substances from the external environment into the deeper layers of the skin [174]. Maintenance of the SC structural integrity is critical for the barrier function of the skin. Occlusion by covering the skin with an impermeable wrap enhances skin hydration by obstruction of the transepidermal water loss from the skin surface.

Increased SC hydration can enhance the transcutaneous diffusion of specific topically applied substances through the SC [157] [159] [174] and can accelerate the formation and emptying of a drug reservoir within the SC [132].

The intercellular lipid matrix of the SC Several hypothetical models of the SC intercellular lipid organization have been proposed: “the brick and mortar model” [52], "the domain mosaic model" [57], "the sandwich model“ [16] and "the single gel phase model“ [113]. More recently, Iwai et al. (2012) stated the molecular organization of the skin’s lipid matrix as “stacked bilayers of fully extended CER with CHOL molecules associated with the CER sphingoid moiety“. This proposed CER bilayer organization with asymmetric CHOL distribution rationalizes the skin’s low permeability towards water and towards hydrophilic substances, as well as the skin barrier’s robustness towards hydration and dehydration, environmental temperature and pressure changes, stretching, compression, bending, and shearing [74].
1.1.2 The dermis

The dermis connects the epidermis to the underlying tissues, and is entirely comprised of living cells. The dermis accounts for more than ninety percent of the skin mass. The dermis is composed of connective tissue made up of arc-shaped elastic fibres and undulated nearly inelastic collagen fibres [126]. These flexible and strong composite materials are responsible for the elasticity and physical strength of the dermis and passively protect the skin against mechanical skin deformation and trauma. Based on the differences in the quantity of elastic fibers and collagen tissue, the dermis can be divided into the papillary layer and the reticular layer. The stratum papillare (SP) is the thin upper layer of the dermis, which is clearly demarcated from the epidermis by an irregular undulated border which expands the contact area with the epidermis. The SP contains loose elastin and collagen fibers and connective tissue cells such as fibroblasts, mast cells and macrophages and numerous capillary loops and several nerve endings. Dermal papillae increase with their finger like appearance the contact surface with the epidermis and are responsible for exchange of oxygen, nutrients and waste products between the dermis and the epidermis. Anchoring collagen fibrils insert into the basal lamina of the epidermis securing the dermis to the epidermis [77].

The stratum reticulare (SR) is thicker and composed of irregular dense connective tissue containing large multidirectional bundles of collagen and elastic fibers providing the skin its elasticity and strength. Compared to the papillary layer, the SR contains more fibers and less cells [77]. The reticular dermis is connected to the hypodermis by a network of fibers.
1.1.3 The subcutaneous tissue

The thickest and deepest layer of the skin, the hypodermis is composed of loose connective tissue, the ‘white adipose tissue’ [77], which consists of grouped adipocytes separated by fibrous walls of collagen and elastin. The subcutaneous tissue layer invaginates into the dermis and loosely binds the skin to the subjacent organs. Its thickness varies according to the individual nutritional status and the area of the body in question. The panniculus adiposus provides the body a thermal insulation and delivers the skin plasticity to absorb external impacts [77] [18].

In contrast to the avascular epidermis the dermis is highly vascularized by a three-dimensional plexus of both small and large vessels [77]. The microcirculation by the arterioles and the venules is organised in a superficial and profound horizontal plexuses in the dermis. The superficial plexus is situated 1-1.5mm below the skin surface in the papillary dermis. Arterial branches from the superficial plexus generate the nutritive capillary loops of the dermal papillae. The lower horizontal cutaneous plexus is situated at the dermal-subcutaneous interface and shaped by perforating vessels from the underlying muscles and subcutaneous fat. The profound plexus is directly connected to the superficial horizontal plexus [18]. Each dermal papilla is supplied by a single capillary loop arising from a terminal arteriole in the horizontal papillary plexus. The capillary loop is perpendicular to the surface of the skin and composed of an ascending branch, an intrapapillary loop having a hairpin turn, and a descending branch that connects with a postcapillary venule in the horizontal plexus. The venous descending limb can be 1.5 times as wide as the arterial ascending limb. Vertical arteriovenous anastomoses with glommerae connect the two plexuses.
The horizontal vessels of the profound and superficial plexus are of utmost physiological importance for the skin’s microcirculation and the skin’s thermoregulation (see figure 2) [77] [126].

![Schematic Diagram representing of microvascular organization in human skin.](image)

**Fig.2.** Schematic Diagram representing of microvascular organization in human skin.

### 1.2 The skin as an insulating barrier

Physiotherapy interventions applied on the skin such as massage, cryotherapy, thermotherapy, electrotherapy and manual therapy can induce afferent stimulation of thermo- and or mechanosensors, which by “gate control” reduce the cortical perception of nocisensoric stimuli [102] [46] [43]. With topical application of thermo / cryotherapy, the skin and the subcutaneous adipose tissue act as an insulating barrier. The skin plays an important role to maintain the core temperature of the body by regulation of the local skin blood flow (SBF) and the secretion of sweat. This thermoregulation by means of increase or decrease in local blood flow is a
physiological process used in physiotherapeutic treatment of musculoskeletal disorders [109]. In addition to the local effects of temperature in non-glabrous skin (limbs, head, and trunk), reflex changes in SBF are mediated by dual sympathetic neural control mechanisms encompassing noradrenergic vasoconstrictor nerves and cholinergic active vasodilator nerves, which are unique to humans. They effect the major aspects of thermoregulatory responses over most of the human body's surface [79].

During periods of normothermia the SBF represents approximately 5% of the cardiac output and the cutaneous vessels receive relatively minor neural inputs from active vasoconstrictor and vasodilator nerves. In a state of hypothermia with a decreased core and ST, the reflex activity of the sympathetic active vasoconstrictor nerve increases in order to reduce SBF and conserve the central body temperature. In response to hyperthermia with an increased local skin and core temperature, an increase in sympathetic active vasodilator activity is induced to increase SBF. During periods of severe heat stress SBF can reach up to 60 % of the cardiac output. To maintain a constant core temperature, the increased SBF will reduce body heat by conduction and sweat evaporation [79]. The heat transported from the body core to the skin’s surface has to pass through the subcutaneous adipose tissue, which inappropriately is a poor conductor of heat. Even a thin layer confers insulation against heat loss. The insulating effect of the adipose tissue layer can be bypassed through vasodilation of the cutaneous arteriovenous vessels. Increasing the blood flow in the dermis and reducing the ST by evaporation of sweat on the skin is the primary mechanism by which muscle heat during exercise and heat of a local thermo application is released from the body [114].
1.3 The skin as a barrier for topical drug application

The skin acts as a protective barrier to maintain our internal milieu separated from extraneous materials [123]. The cornified envelope of corneocytes is almost impermeable for diffusing substances. Since the lipid regions in the SC constitute the only continuous structure, penetrating substances applied onto the skin always have to pass these regions [17]. Therefore, the skin’s barrier capacity is a function of the molecular architecture of the lipid structure in the extracellular space between the cells of the SC [16].

The biological mechanism of dermal absorption is the complex process of penetration (substances entering a skin layer), permeation (migration through skin layers) and the resorption (take up of the compound by the circulation or lymph system) into the viable tissue of the body. Topical formulations can be designed to exert their action on the skin; in the skin (epidermis and dermis); systemically (absorption by the vascular system); or in deeper tissues such as muscles, tendons and synovia [152]. The rate and amount of percutaneous absorption of a compound depend highly on both the physiologic characteristics of the skin and the physico-chemical properties of the permeating agent [60].

The transport of a substance through the skin occurs mainly by passive diffusion. The diffusion process of a compound through the skin can be divided into a lag phase and a linear phase. The lag phase is defined as the period between drug application and the linear phase. In the lag phase the substance flux rate increases progressively. The linear phase is characterised by a steady state process whereby a constant gradient of compound is present in the membrane [65]. The theoretical curve as depicted in figure 4 indicates that in the lag phase of drug application no or very little drug penetrates the skin. When the number of molecules entering the
membrane increases, the flux of the drug gradually grows until it reaches a steady state level [56]. The lag time has been defined as the linearly extrapolated intercept with the time axis of the steady state section of the penetration profile [65].

Fig. 4. Penetration profile obtained under infinite dose conditions. Lag time phase (a); the amount penetrating the skin attains a steady state (B) where the amount penetrating per unit time is constant; the gradient of this line can be used to calculate the steady state flux (JSS) often expressed as (g.cm⁻².h⁻¹) (Chilcott, RP. and Price S. Principles and Practice of Skin Toxicology 2008) [25].

The linear steady state phase occurs when the drug concentration gradient across the membrane is constant, i.e. when the drug rate entering the skin equals the rate that it exits.
According to Fick’s first law, the flux of a permeant through the SC is constant if the permeability coefficient and concentration difference is constant.

\[ J = K_p \Delta C = D \cdot K \cdot \Delta C / L \]

- **J** = flux of the permeant through the SC (µg.cm\(^{-2}\).s\(^{-1}\))
- **Kp** = permeability coefficient of the permeant in the SC (cm. s\(^{-1}\))
- **K** = partition coefficient between the SC and the vehicle (concentration in the skin / concentration in the vehicle)
- **D** = diffusion coefficient of the permeant in the SC cm\(^{-2}\).s\(^{-1}\)
- **ΔC** = concentration difference across the membrane (µg.cm\(^{-3}\))
- **L** = length of diffusion pathway of the SC

Factors influencing the efficiency of compound absorption through the skin include: area of contact, duration of exposure, lipophilicity, molecular weight (MW) concentration of the compound, integrity of the SC and thickness of the epidermis [56]. To achieve a high concentration of a permeant in the first layers of the SC, the permeant should have a high tendency to exit its vehicle and to permeate into the dermis. The potential for depletion of the permeant from its vehicle is expressed in the partition coefficient (K) of the permeant. As the barrier in the SC is mainly lipoid, a high lipid solubility of the compound is necessary for a maximal input of the permeant into the SC. As the underlying layers of the epidermis, dermis and circulatory regions are more hydrophilic than the SC, extremely hydrophobic permeants will encounter difficulties to permeate into the dermis.
The magnitude of the K is affected by the composition of the support as well as the chemical structure and the charge of the permeant [166] [146]. In transdermal drug delivery the choice of vehicle is crucial as it influences the tendency of permeant release [39]. The diffusion coefficient, also called diffusivity, (D) is an important indicative parameter of the diffusion mobility of a molecule within a medium, in this case the intercellular lipids in the SC [171]. The molecular size (MW) and the interaction of the substance with cutaneous molecules are depending parameters of diffusivity. Non-specific and specific binding may occur in both the epidermis and dermis, reducing diffusivity and thereby decreasing skin permeability [166]. The diffusion of the compound through the skin is inversely related to its molecular size. The diffusivity for a large molecule is lower than that of a small molecule [10]. Molecules with a MW less than 500 Daltons and an adequate solubility in oil and water, will penetrate efficiently and rapidly though the skin. It is generally accepted that molecules with a molecular mass less than 5000 Daltons are able to migrate the skin barrier. The use of penetration enhancing techniques or delivery molecules, increases the possibilities of molecules with a high MW (in particular proteins) to penetrate the skin [12].

In the therapeutic treatment of musculoskeletal inflammatory disorders NSAID’s and corticosteroids are frequently used to reduce pain and to inhibit the inflammation process. Target tissues are muscle, tendon, synovia, ligaments and skin. As the skin is a heterogeneous multilayer tissue consisting of the lipophilic SC and the much more aqueous viable epidermis and dermis the permeant should have balanced partitioning criteria. Once the topical applied compound is absorbed through the skin, part of the substance is taken up into the microcirculation for systemic distribution. In physiotherapy transdermal drug applications aim to achieve
high drug concentrations in the target tissues. Therefore, the used compound should have a low dermal retention and a low systemic uptake.

The drug permeation after transdermal administration is controversially debated in the literature. Panus et al. (1999) investigated the local tissue permeation depth (in swine) during in vivo ketoprofen iontophoresis and demonstrated that a single iontophoretic administration of ketoprofen resulted in clinical relevant higher ketoprofen concentration in the superficial muscle layer (at 1cm depth) compared to the drug concentration in the skin [117]. The study of Hui et al. (2001), investigating the in vivo local and systemic uptake of iontophoretically administrated DF in rabbits, also found significant higher drug concentrations in the skin, subcutaneous tissue and muscle directly under the site of application compared to plasma concentrations and concentration in comparable tissues at an untreated location. These results indicated that iontophoretic administration of DF leads to higher drug concentrations in the tissues under the site of application independently of systemic distribution [73]. Radermacher et al. (1991) investigating the DF plasma and synovia concentrations after topical administration of DF on the knee could not confirm these findings. Although the DF synovia concentration was higher in the treated knee compared to the untreated knee, the local DF synovia concentration was lower than the systemic plasma concentration. The authors concluded that the transdermal uptake of DF in synovial fluid is not the effect of direct local tissue uptake but mainly due to effect of the systemic uptake [129].
1.3.1 Skin penetration pathway

Drug substances can permeate the SC by means of a transepidermal route or a route via pores [171]. As stated by Williams and Barry (1992), the transepidermal route provides a transcellular and an intercellular pathway through the human SC (Figure 5) [171].

Fig. 5. Simplified diagram of SC and two microroutes of drug penetration (Barry, 2001) [10].

Polar or hydrophilic substances are believed to pass the SC through polar channels, also called the transcellular route, crossing the skin by directly passing through both the lipid structures of the SC and the cytoplasm of the dead keratinocytes. Although the transcellular route may provide a direct pathway through the SC, polar molecules may encounter significant resistance to permeation because they have to cross both lipophilic and hydrophilic structures [56] [171] [152]. The more common route for lipophilic materials to permeate the skin is the lipophic intercellular route [67]. Here the permeant overcomes the SC by passing through the lipid rich intercellular space between the corneocytes.

Besides the transepidermal and intercellular pathways, there is a third potential pathway for the penetration of topically administered substances. The transappendageal penetration route transports the substances into the
subepidermal layers through the sweat glands and via hair follicles with their associated sebaceous glands [152]. The transappendageal pathway may be important for particles that slowly permeate through the SC such like ions, polymers, colloidal particles and large polar molecules [10]. Recent publications suggest that drug penetration through the follicular shunts of the pilosebaceous units could be more significant than previously believed [100] [172] [82].

![Possible drug penetration pathways across the SC schematically. Adapted from Lippold [92.]](image)

**1.3.2 Reservoir function of the SC**

As first demonstrated by Vickers (1963), the human SC has besides its function as a barrier the property to store previously topically applied substances for a prolonged time. In Vickers’ experiment, small quantities of corticosteroid were applied and occluded with a plastic film. When after 16 hours the occlusion was removed, blanched vasoconstricted application areas appeared. Four days after the initial corticosteroid application, the skin application sites were occluded a second time. Without any product application, the re-occlusion provoked a new blanching vasoconstriction of the pre treated skin areas. This effect was not seen when the SC was removed by tape stripping (TS) or bypassing the tissue with an intra-dermal
injection. Vickers suggested that this phenomenon was due to the occlusion-stimulated release of corticosteroids out of a SC reservoir [160]. A SC reservoir is an accumulation of topically applied compound within SC for a longer period of time. More recently, reservoir properties have been equally ascribed to deeper skin layers [132]. The formation and the duration of a reservoir can affect the biopharmaceutical efficacy of passive delivered compounds and can influence the drug concentration permeating through the skin and the concentration reached in the target tissue [32] [134] [135]. The existence of a reservoir within the SC has been documented for several topically applied solutes [160] [32] [118] [149].

1.4 Penetration enhancement methods

In physiotherapy the transdermal delivery of drugs is a routinely used topical treatment to reduce pain and inflammation in musculoskeletal disorders [136] [93] [11] [94] [62] [20]. Transdermal transport has potential advantages compared to traditional drug delivery methods including oral delivery and injections. Transdermal drug delivery bypasses the hepatic first pass effect, avoids gastrointestinal problems and facilitates patient’s compliance [104]. The lamellar bilayer organization of the lipid matrix offers the major barrier to transdermal drug delivery [16]. In order to achieve a clinical effective drug concentration in the target tissues such as muscle, tendon and synovial fluid, various studies have been carried out to find safe and suitable techniques to promote the percutaneous absorption of drugs [104].

Penetration enhancers encompass chemical methods by means of substances or physical methods that facilitate the absorption of a penetrant through the skin by temporarily diminishing the poor permeability of the skin [145].
Several studies revealed that the permeation of a drug through the skin can be enhanced significantly by chemical or physical penetration enhancement methods [88] [64] [137] [9] [45] [163]. Physical methods like occlusion, iontophoresis and sonophoresis are frequently used in physiotherapy in order to increase transport of molecules across the skin [78] [104] [7] [143] [130]. Physical penetration enhancement techniques as used in physiotherapy aim to reduce the barrier properties of the skin in different ways, some of the techniques act on the lipid bilayer while other techniques act on the keratinized structures or cutaneous hydration [24].

1.4.1 Occlusion

Occlusion is defined as the insulation of the skin with a water evaporation-limiting barrier [140]. With this obstruction of the TEWL from the skin surface, the normal water content of the SC (10-20%) can be increased up to 50%. Skin occlusion and enhanced SC hydration both increase the skin's permeability for many molecules in vivo as well as in vitro and thereby affect the transdermal absorption of topically applied drugs [174]. In physiotherapy the administration of DF by a topical patch is a frequently used method indicated for the treatment of acute pain, sprains and contusions [96] [91] [120].

1.4.2 Iontophoresis

To enhance the delivery of (pro) drugs through the skin, physiotherapeutic iontophoresis involves the application of a direct current of low ampere rating up to 0.5 mA/cm² by means of bipolar electrodes [88] [64] [137] [9]. Ionized molecules are stated to migrate along the lines of the applied electric field with the positive ions
(cations) driven away from the anode and attracted by the cathode, with the opposite action for the negative ions (anions). After overcoming the main SC barrier the penetrated molecules become available for the viable tissues [45].

The penetration of unionized molecules is stated to occur by means of a convective flow from the anode to the cathode due to an electrochemical NaCl gradient. This convective flow carries with it these unionized molecules. This electrolytic process results in an increase of pH at the cathode and a decrease of pH at the anode [163].

If the ampere dosage is too high, the involved pH changes can provoke discomfort and irritation of the skin. In physiotherapeutic iontophoretic application the electrode surface ranges from 7 to 44cm² [44] [138] [87] and the reported current varies from 4 to 11mA [88] [64] [137] [44] [87] applied during 10 up to 20 minutes [88] [64] [137] [44] [169] [141]. The electrodes can be placed parallel on to the treated area, [44] [169] or the active (compound) electrode can be placed above the treated area while the return electrode is generally positioned at the opposite site [137] [9] [138].

Fundamental as well as clinical iontophoretic experiments have been published. Fundamental experiments have been carried out to gain insight in the mechanisms and influencing factors of the percutaneous penetration process. They have been carried out on healthy volunteers and on animals and in vitro on animal skin.
Evaluation methods were directly invasive (e.g. dialysis, biopsies) or indirect by means of radioactive substances. The methods used in fundamental research are not applicable during iontophoretic treatment in the physiotherapy setting. The duration of the treatment in fundamental experiments can last for hours whereas the duration of iontophoretic therapy normally lasts for about 20 minutes. Current densities applied in the in vitro studies were often unrealistic high and not comparable or applicable under clinical physiotherapeutic conditions.

### 1.4.3 Sonophoresis

Therapeutic ultrasound is one of the most frequently used electro physical modulations in clinical physiotherapy and sport medicine practice [26] [125]. Although little evidence supports the use of ultrasound therapy in the treatment of musculoskeletal disorders, therapeutic ultrasound is a predominant application in the clinical practice to treat musculoskeletal injuries [158] [59]. Ultrasound therapy can also be used to enhance the transdermal penetration of pharmacologic agents through the skin, in context called sonophoresis or phonophoresis [97] [105]. In physiotherapy the sonophoretic delivered drugs commonly are anti-inflammatory drugs such as dexamethasone, diclofenac (DF) and salicylates, but also anaesthetics such as lidocaine [3] [133] [28] [170]. The mechanism of skin permeabilization induced by high-frequency ultrasound is stated to be provoked by cavitation effects [105]. The cavitation effects are predominantly invoked within the skin, either in skin appendages or at locations near the keratinocytes of the SC [104]. It has been hypothesized that the collapse of cavitation bubbles near the SC increases the skin permeability due to a disruption in the lipid barrier organization of the SC [106]. Although cavitation during sonophoresis is the primary mechanism to enhance skin permeability, non-cavitational mechanisms related to sonophoresis
have been proposed, such as convection, mechanical or radiation pressure and thermal effects [104] [144] [90] [103]. Although high frequency sonophoresis (1–3 MHz) is less effective to enhance skin permeability than low frequency sonophoresis (20–100 KHz) the lowest value for the high frequency band (1MHz) may equally have penetration enhancing effects. Sonophoresis as used in physiotherapy is commonly applied at a frequency of 1MHz and aims the regional and topical delivery of drugs [105]. There are two modes of sonophoretic treatment: i) a pre-treatment method, with the skin treated with ultrasound prior to the drug administration or ii) a modus with the ultrasound applied simultaneously through a coupling media containing the pharmacologic substances [133] [128].

Although the sonophoretic delivery of therapeutic compounds is a common procedure in the physiotherapeutic practice, there are scarce evidences concerning the clinical effectiveness of this transdermal delivery technique. Most physiotherapists apply sonophoresis using the simultaneous technique whereby the treatment time depends on the size of the treatment area [128]. After the sonophoretic treatment, the coupling media including the pharmacologic components is usually immediately removed from the skin. Saliba et al. (2007) proposed an additional exposure under occlusive dressing after sonophoretic treatment [139].
1.5 Non-invasive measurements of skin properties

Cadaver skin has been used within the context of fundamental research. In dermatologic practice and in vivo clinical research, examination of skin properties can be performed by visual inspection and by palpation. However, visual inspection is subjective, and some important skin properties are not visually perceptible. Due to the easy accessibility of the skin, researchers tended to use invasive traditional assessments like harvesting skin biopsies, which are time consuming, and painful [58]. So for in vivo evaluation of the skin, there is a certain need for objective reproducible and standardized non-invasive skin assessment techniques. Over the last decades the study of the biophysical parameters of the skin has been undergoing an enormous transformation. Advances in technology increased the possibilities of non-invasive measurements of the skin. New commercially available instruments have facilitated the study of many of the skin’s physiological and biophysical properties including barrier properties water content, TEWL, temperature, elasticity, and blood microcirculation of the skin. These reproducible, standardized and valid measurement procedures have contributed to a better understanding of the skin characteristics and skin function. The following chapter only discusses non-invasive biophysical skin measurement techniques used in the experimental design of the study.

1.5.1 Skin temperature

Since the human skin defines the interface between the human body and its thermal environment, the ST is an essential to control and to quantify the heat transfer. The ST is influenced by outside factors such as environmental temperature, the air dynamics (chill factor) and the relative humidity but also by endogenous factors
including metabolic activity, psychological factors and hormone release [80] [81]. SBF changes dynamically due to the continuous interplay between the vasoconstricting and vasodilatating mechanisms influencing the ST. Consequently, ST measurements can be used as an indicator of superficial skin perfusion or to study induced skin changes.

Several methods to measure skin surface temperature exist such as contact thermometry (thermocouple, thermistors, thermo chromic liquid crystals) and non-contact techniques like infrared thermometry.

The **thermocouple** technology is based on the Seebeck principle whereby in a closed circuit of two dissimilar metals an electric current is induced due to a difference in temperature between the two ends of the junction. The thermoelectric voltage created depends on the metals that create the thermocouple and the temperature difference at the junction [131].

Clarys et al. (1991), evaluating different topical vasodilatory products with non invasive techniques, demonstrated that thermocouple thermography measurements have a high reliability within a context of low inter-subject variability (mean 32.4 °C ± 0.09 °C; C.V. = 0.26% for n=12) [31].

### 1.5.2 Skin colour

The major biochemical determinants of skin colour are the pigments carotene, hemoglobin and melanine. The primary determinant of the variability in colour of the human skin is the amount, density, and distribution of the pigment melanin. The quantification of experimentally induced colour changes of the human skin is a widely used in vivo dermato-cosmetic research method, since the magnitude of the
colour response can be used as an indicator of skin properties (integrity of the skin barrier and sensitivity), drug properties (concentration, bioavailability), vehicle properties (formulations, enhancers) and skin protection properties (sun screens) [147] [29]. Although the human eye can easily distinguish differences in skin colour, it is inadequate to make quantitative comparisons at different times or at distant sites [147]. The ‘Commission Internationale de l’Eclairage’ (CIE) developed a colour system attuned to the non-linear characteristic of the colour perception of the human eye. The L*, a*, b* system colour space system is defined by a three-dimensional perpendicular axis system. The chromaticity coordinates comprises two radius coordinates on a circular plane: (a) a zero angle radius coordinate representing colours and hues ranging from red (+a*) at one end to green (-a*) (b) a 90° angle radius coordinate representing colours and hues ranging from yellow (+b*) at one end to blue (-b*). Perpendicular to this disk plane lies the L* coordinate standing for the brightness and varying between white (L*=100) and black (L*=0). The three radial axes define a sphere of colours and hues.

![Figure 8](image.png)

**Fig. 8.** The CIELAB L* a*b* color space system. The chromaticity coordinates a* (varying between red and green) and b* (varying between yellow and blue) represented on a circle with the colour value perpendicular to the disk plane.
Instrumental colour assessment provides consistent and objective quantification of induced skin changes. Most instruments developed to measure colour are **reflectance meters**, measuring the emitted and reflected spectrum [165].

Up to now the Chromameter is the most used instrument to objectively quantify surface colour changes. The Minolta CR 200 Chromameter® (Minolta Camera CO., Ltd., Osaka, Japan) is a compact tristimulus colour analysis system with a pulsed xenon arc lamp. The instrument consists of six high sensitive silicon photocells, which are used by the meter’s double beam feedback system to measure both the emitted and the reflected light from colour surfaces.

The Chromameter CR 200 consists of a data processing unit and a measuring head with an 8 mm diameter measuring area.

Several researcher confirm the positive correlation in quantification of MN induced erythema between the a* parameter of the Chromameter reflectance measurement and perfusion of the skin microcirculation by laser Doppler velocity measurements [87] [86] [31].

The skin blanching assay is a non invasive frequently used bioscreening technique to evaluate topical corticosteroid potency in human skin [23]. The intensity of the blanching response varies directly with the L* coordinate and inversely with the a*, as the skin pallor becomes lighter and its redness fades. For measuring the skin blanching response with the Chromameter, the coordinates L* and a* have been proven to be more discriminative compared to the b*coordinate [23] [127]. The overall colour evaluation of the blanching response is often expressed by ΔE, (total colour difference) which is calculated by the sum of colour difference between baseline value and the blanching value for the L*,a* and b* parameter [167].
1.5.3 Transepidermal water loss

The epidermal layer serves as a permeability barrier regulating the transcutaneous movement of water and the transport of electrolytes [89] [63] [111]. Transepidermal water loss (TEWL) is a measure of the quantity of water that passes through the SC to the surrounding atmosphere via diffusion and evaporation processes [63] [111]. Measurement of the TEWL is a non-invasive technique to assess SC integrity and used as a reliable indirect measure of the barrier function of the skin. TEWL evaporimeter measurements are generally expressed in g/m².h. In normal healthy skin the barrier is quite effective and the low water loss rates from the living tissue result in low TEWL values. Compromised barrier function due to increased hydration or physical damage of the SC is directly related to higher TEWL values [85] [108].

Various types of evaporimeters have been developed, the most widely used is the open chamber device with calculation of TEWL based on Fick’s law of diffusion [111]. The open chamber evaporimeter probe is on top open to the environment and consists of a cylindrical capsule, equipped with two hygrosensors to detect the water evaporation gradient. The hygrosensors are coupled with temperature sensors [36]. Limitations of the open chamber evaporimeters are relatively long recording times and the fact that the probe must be held in the vertical position in order for the water vapor to flow unimpeded over both sensors. More recently closed chamber evaporimeters are available on the market, claiming to have shorter reading times and the ability to measure TEWL at any angle to the skin surface [4] [42]. The Tewameter® is an open chamber device (Courage and Khazaka electronic GmbH Cologne, Germany) and is widely used in both research and dermacosmetic industry.

Various studies used TEWL measurements to demonstrate the relationship
between barrier disruption and the application of penetration enhancing techniques. Choi et al. (1999) studied the effect of pre-treatment of chemical skin penetration enhancers in transdermal drug delivery by means of iontophoresis. Pre-treatment with chemical enhancers induced high TEWL values in correlation with barrier impairment [27]. Recently, Herwadkar et al. (2012) used TEWL measurements to demonstrate that low frequency sonophoresis at 20 kHz was effective in enhancing the delivery of ketoprofen into and across the skin in rats. Drug concentration levels in skin increased from $34.69 \pm 7.25 \mu g$ following passive permeation to $212.62 \pm 45.69 \mu g$ following sonophoresis. TEWL values increased from $31.6 \pm 0.02$ (passive) to $69.5 \pm 12.60$ (sonophoresis) indicating disruption of barrier properties. Performed under standardized environmental conditions, TEWL measurement is an accurate and sensitive method to evaluate the effectiveness of penetration enhancing techniques [70].

1.5.5 Perfusion of skin microcirculation measurement

The microcirculation has an important function in thermoregulation (vasodilatatory- or vasoconstrictive reactions). The perfusion can equally be influenced by other physiological mechanisms and vaso-active substances. Skin perfusion can be quantified using Laser Doppler Velocimetry. The laser Doppler technique is based on the measurement of the Doppler shift, induced by the migrating red blood cells within the coherent monochromatic laser beam. The laser Doppler measures the total local microcirculatory blood perfusion including the perfusion in capillaries (nutritive flow), arterioles, venules and shunting vessels. A beam of laser light (633nm or 780 nm) carried by a fiber-optic probe is placed on the skin area under investigation. When the laser beam illuminates a small area of tissue, photons will
be scattered and partly absorbed by the tissue. The velocity of the moving blood cells under the laser beam provokes a change in the wavelength of the beam (Doppler shift) with the light reflected by the static tissue (not laser shifted) remaining unchanged.

**Laser Doppler velocimetry** (LDV) is a frequently used non-invasive diagnostic method to quantify the microperfusion of the skin. The magnitude and frequency distribution of the wavelength changes are directly related to the number and velocity of the moving blood cells in the illuminated sample volume (Flux). The laser light penetrates the skin to a depth of approximately 1 mm, enabling perfusion measurements of the dermal capillaries and arteriovenous shunts at a depth of 0.5-1 mm with a measurement volume of about 1 mm$^3$. The output signal from the laser Doppler Flowmeter is expressed in relative and dimensionless arbitrary Perfusion Units (PU). There are limitations of the technique, the Doppler laser technique does not evaluate the perfusion at a single point quantitatively in real time, but relatively. No current laser Doppler instrument provides absolute perfusion values. The lack of absolute quantification for perfusion, the lack of knowledge of the depth of measurement and the biological zero signal (perfusion measured at no flow condition) are the major limitations of the laser Doppler technique [95] [161].

Recent developments enable to measure in a single session the perfusion of the microcirculation from a larger area by scanning the laser beam over the area of interest, providing a perfusion map output on a heterogeneous tissue. The advantage of the laser Doppler perfusion imaging is that there is no need of tissue surface contact, making it specifically useful in clinical situations were contact with the tissue is undesirable [54].
1.5.6 Models to study percutaneous absorption non-invasively

Molecules eliciting a physiological reaction on the microvascular system can be used in models to study percutaneous penetration. The use of non-invasive instrument allows objective quantification of different aspects of the response kinetics. Substances such MN, hexynicotinate and capsaicin provoke a localised vasodilatory response whilst corticosteroids induce a vasoconstrictive reaction. Moreover the inhibition of the MN response can equally be used to indicate the presence of anti-inflammatory agents such as NSAID’s [49] [48] [150] [75].

Evaluation of the response using objective colour measurement allows the comparison of responses under different application modalities. The topical application of MN results in a localized vasodilatatory response mediated by the release of arachidonic acid and prostagladin D2 (PGD2) from niacin responsive cells that resides in the skin. The produced prostaglandins act on the neuroreticular tissue of the arteriovenous anastomoses of the dermal vascular plexuses by means of an endothelium relaxant factor. The latter provokes a relaxation of the vascular smooth muscles resulting in an augmentation of the cutaneous circulation and a flooding of the superficial veins. This immediate contact reaction is visible as an erythema [22] [49]. The release of PGD2 in response to topically applied MN occurs in a dose-dependent manner over the concentration range of 0.001 to 1.0 M [83] [107]. Tur et al. (1991,1994) evaluated the in vivo percutaneous absorption of MN at different anatomic sites by using laser Doppler flowmetry. The authors concluded at different body regions that the variation in erythema response was related to regional differences in percutaneous absorption of MN, regional variations in vascular sensitivity and regional differences in density of the microvascularisation [154] [155]. Quantified MN responses with chromameter provided, similar findings [87].
Topical corticosteroid application results in a blanching response, as first described by McKenzie and Stoughton (1962). This so-called skin blanching assay has been used commonly to classify the potency of topical applied corticosteroid formulations [98]. Although the exact mechanism behind the skin blanching assay is not fully understood, it is thought that blood flow reduction due to local vasoconstriction in the skin microvasculature initiates this phenomenon [68]. The skin blanching assay has also been used to demonstrate the reservoir building properties of the SC. Vickers (1964) demonstrated that re-occlusion after application of a corticosteroid formulation provoked a new blanching response indicating the presence of active corticosteroid in the SC up to one week after the initial application [159]. When using a physiological response such as a blanching response after corticosteroid penetration, it is generally accepted that the initial part of the response curve can be considered as an indication for the percutaneous penetration process while the total response is an indication for the amount of compound reaching the viable tissue [34].

A more pronounced blanching response to the occluded application is an indication of weaker barrier properties of the hydrated SC [30]. The skin-blanching assay has proven to be a valid non-invasive method to demonstrate reservoir properties and bioavailability of corticosteroids in the SC. Consequently, the skin blanching test can be used to evaluate the effectiveness of penetration enhancement techniques as used in physiotherapy.

Finally, quantification of the inhibition of the MN is an elegant method to study the presence of anti-inflammatory substances in the dermal tissues.

Wilkin et al. (1985) used laser Doppler velocimetry to study the effect of oral administration of anti-inflammatory drug on the MN induced erythema response. The inhibited local erythema responses indicated the involvement of prostaglandin
release in the cutaneous vascular reaction [168]. The studies from Treffel & Gabard (1993b) and Treffel et al. (1993) demonstrated an inhibition of the MN induced inflammation reaction after topical application of DF and ibuprofen. The in vivo results of the Chromameter measurements and the Laser Doppler flowmetry measurements correlated significantly with the in vitro determination of the drug levels in the SC [151] [150]. These findings demonstrated a proportional inhibition of an MN provoked erythema by the bioavailability of anti-inflammatory drugs in the SC. The inhibition of the MN induced inflammation assay is a valid non-invasive method to measure the effectiveness of topical formulations containing anti-inflammatory agents [118] [87] [151] [150] [66] [22] [168].

1.6 Summary evaluation

Although little evidence exists for the long term effectiveness of physical therapy applications, its use in the management of musculoskeletal disorders is widespread. Local heat applications such as paraphango are used in the treatment of ischaemic muscle pain. The vasodilation induced by local heat increases the blood flow in the muscle tissue, reducing the ischaemia and pain sensation. As the redistribution of blood towards the heated area could have an impact on the patients cardiovascular system, cardiovascular problems are often considered as a contraindication for local heat application. Although it is claimed that local heat application influences the perfusion in the underlying soft tissue, the physiological mechanisms controlling vasomotion and thermoregulation are not well understood and information on heat induced changes in skin parameters is limited. Evaluation and quantification of the changes in ST and the local effects on the arterial and venous microcirculation of the skin could provide important insights and define contraindications for
paraphango therapy. Cardiovascular adaptations such as heart rate, DBP and SBP in reaction to paraphango could also provide insights in the impact of blood redistribution on the cardiovascular system and to define possible contraindications.

**Topical transdermal applications** are commonly used in the treatment of various (rheumatic) inflammatory dysfunctions and acute soft tissue injuries [62] [94] [136] [173]. In physiotherapy various transdermal enhancements techniques, such as sonophoresis, iontophoresis and occlusion are used in order to decrease the skin barrier and to achieve a clinical effective drug concentration in the target tissues [15] [47] [51] [87] [156]. Although several clinical studies claim an advanced healing process after the use of transdermal techniques, [69] [44] [41] the evidence related to the effectiveness is controversially discussed in the literature and basic principles of transdermal penetration are not always taken into account.

Screening the literature on **iontophoretic delivery** as used in the physiotherapy revealed that most human in- vivo studies take no account of the basic principles of percutaneous penetration. The effectiveness of iontophoretic delivery of an active substance is usually only compared with placebo iontophoresis without an active substance, which provides no proof for the penetration enhancement effect of a current.

Studies investigating clinical outcome of iontophoretic drug delivery did not compare the effect of iontophoresis with the effect of passive penetration. To obtain valid results on the enhancement factor of a current, data of iontophoretic delivery should be compared with data of passive drug penetration, as iontophoretic delivery can be substantially influenced by passive penetration.

Beside its function as a barrier, the human stratum corneum has the property to store previously topically applied substances during a prolonged time. A stratum corneum reservoir is an accumulation of a topically applied compound within the
skin or within a skin layer for a longer period of time. The building up of a SC reservoir is an important component of the pharmacodynamics of topically applied substances. Rougier et al. (1985) used the reservoir effect of the stratum corneum after 30 minutes of application to predict the total amount absorbed [134]. Up to now there is little information concerning the reservoir properties of DF on different application modalities as used in physiotherapy.

In the treatment of musculoskeletal disorders, ultrasound is one of the most frequently used physical treatment modalities in physiotherapy [26] [125]. When ultrasound is used in combination with topical treatment of NSAIDs or corticosteroids a sonophoretic drug enhancement is aimed. In physiotherapy, sonophoresis is applied to the skin in a pre-treatment modus and a simultaneous treatment. Although low frequency sonophoresis (frequencies 20-100 KHz) is up to three order of magnitude more effective in enhancing skin permeability compared to the 1MHz ultrasound, the most common sonophoretic application in physiotherapy involves the use of high frequency or therapeutic sonophoresis (frequencies ≥ 0.7 MHz) [105] [104] [124]. In recent years, several in vivo studies revealed the clinical effectiveness of high frequency sonophoresis with NSAID’s and corticosteroids in the treatment of musculoskeletal disorders [72] [8] [84] [11] [21]. As hypothesized in the review of Polat et al. (2011), the pre-treatment method causes enhancement of the drug transport mainly due to structural changes in the skin (cavitation) whereas the simultaneous technique enhances the skin permeability by structural changes and through convention-related mechanisms (thermal radiation, pressure effects, etc.) [124].
1.6.1 Research questions

Review of the literature indicated that non-invasive bioengineering techniques can be utilized to unravel different aspects of topical treatment as used in physical therapy. The aim of this doctoral project comprised the following research questions:

1. What are the effects of paraphango therapy on skin temperature, perfusion of the microcirculation and skin colour?
2. What are the systemic effects of local paraphango therapy?
3. Is the clinical applicability of iontophoresis in the physiotherapeutic treatment of musculoskeletal disorders evidence based?
4. Is the application of iontophoresis effective in reducing pain in patients with musculoskeletal disorders?
5. Is there an increased bioavailability of DF in the skin after electrically assisted delivery in comparison to the passive percutaneous penetration and occlusion?
6. Is the formation and emptying of a DF skin reservoir dependent of the DF application mode?
7. Does the timing of sonophoretic application influences the availability and the penetration process of corticosteroids?

To answer the research questions 1 and 2 an original study comprised the investigation of the changes of skin characteristics and cardiovascular parameters during and after local paraphango therapy. For the research questions 3 and 4 a systematic review and meta-analysis was done on the effects of iontophoresis in the treatment of musculoskeletal disorders. To investigate the research questions 5 and 6 an original study assessed the bioavailability of iontophoretically delivered DF.
Question 7 was evaluated by means of an original study comparing the halcinonide bioavailability between the ultrasound pre-treatment and the simultaneous treatment method.

2. MATERIALS AND METHODS

2.1 Measurements techniques

2.1.1 Skin temperature
Skin surface temperature was measured in °C with a thermocouple (NiCr-Ni) thermometer, the Testoterm 9010 ® (Testoterm GmbH & Co, Lenzkirch, Germany). The Testoterm 9010 ® consists of a measuring unit with digital display and 2 simultaneously recording temperature probes. Measurement rate is 1 measurement a second with an accuracy of 0.1 °C in the temperature range of -20 °C to 50° C.

2.1.2 Skin colour measurements
To assess the skin surface colour the Minolta Chromameter CR200® (Minolta Camera Co., Ltd., Osaka, Japan) was used. The skin colour measurements were performed using the CIE 1976 (L*, a*, b*) system mode (see fig.8). Immediately in advance of each measurement session the device was calibrated to a standard white reflective plate (CR-A43). The centre of the reading head was gently and precisely positioned perpendicular on the skin surface in alignment with the application site. Three pulsed xenon beams illuminated the sample. One measurement represented the mean value of three subsequent readings and was automatically calculated by the instrument. The device permits absolute colour measurements in different colour systems (Yxy, L*,a*,b*, L*,C*, H°). Baseline readings (zero time) were taken at all sites (including the untreated control sites).
prior to the application of the formulations. During the experiments a* parameter values were collected on scoring sheets.

2.1.3 Perfusion of the Microcirculation

Perfusion of the skin microcirculation was assessed with the laser Doppler flow meter Periflux PF3® (Perimed KB, Stockholm, Sweden). The instrument consists of 3 units: i. The processor section, containing the laser, optical fibers and signal processing circuits. ii. The display/control section containing the controls necessary to operate the instrument and the various analog and digital displays. iii. The output section contains the RS 232 connection, which delivers the measurement parameters as standardized digital values for external computer handling, and two outputs give out the same parameter as analogue voltages. The periflux PF3® instrument uses a 2 mW helium-neon laser source, with a wavelength of 632.8 nm. For the flowmetric measurements the multipurpose probe (PF3008) with probe holder PF 104 was used. Prior to the measurements the Periflux PF3® was allowed to warm up for 30 minutes. Calibration of the perfusion value was verified daily using the PF100 motility standard provided by the manufacturer. Measurements were performed in the WIDE BAND mode to cover all Doppler frequencies of clinical significance. To prevent occlusion of the perfusion, the probe was placed on the skin without pressure between the probe and the tissue. During the experiments 5 successive perfusion values were collected on scoring sheets. The average of these 5 readings was defined as the perfusion value.
2.1.4 Transepidermal water loss

Measurements of Transepidermal water loss were performed with the Tewameter TM 210® (Courage & Khazaka, GmbH, Cologene, Germany). The Tewameter TM 210® is a commercial available TEWL instrument based on the open chamber method, measuring the water evaporation gradient on skin surfaces. The measuring head is composed of a cylindrical chamber (10 mm diameter, 20 mm height) with humidity and temperature sensors measuring TEWL values (0 – 90 g/m².h), relative humidity (0 – 100%) and probe temperature (0 – 50 °C). Since the probe temperature influences the TEWL values measured, although minimally, the probe was placed on an adjacent skin area in order to obtain thermal equilibrium with the skin before performing measurements. Measuring TEWL, the cylindrical chamber of the probe was placed on the skin with a low and constant pressure, in a horizontal plane with the probe parallel to the surface. The TEWL score was defined as the mean value over the 60-120 seconds time interval after application of the measuring head to the skin.

2.1.5 Blood pressure and heart rate measurements

For blood pressure and heart rate monitoring a full-automatic oscillometric device (Omron®, RX Classic, Kyoto, Japan) was used. The Omron® RX Classic is a clinically validated wrist blood pressure monitor with a measurement range of 0 to 299 mmHg for blood pressure and 40 to 180 beats/min for heart rate. The accuracy for blood pressure is within ±3 mmHg and for pulse rate within ±5% of the reading. Before, during and after the paraphango treatment, blood pressure and heart rate measurements were performed with an interval of 3 minutes. All measurements were conducted at the left wrist with the arm flexed in front of the breast, holding the
measuring unit at heart level. Throughout the measurement session the subjects were asked not to move or to talk and to keep the hand in a neutral position until the measurement was completed. The SBP, DBP and heart rate values were logged in the scoring sheet.

3. Experimental stipulations

The participants voluntary took part in the experiments. The total sample (n=112) consisted of 39 healthy male and 73 female, Caucasians aged between 18 and 32 years. 18 subjects were involved in the phango study (see chapter 4.1); 12 for the iontophoretic study (see chapter 4.3); 60 for the skin reservoir study (see chapter 4.4) and 22 for sonophoresis study (see chapter 4.5). The subjects had healthy skin and were free from local or systemic treatment with anti-inflammatory drugs. Before the experiments, all volunteers were informed about the research protocol, the aim of the measurements and signed an informed consent. The ethical committee of the Vrije Universiteit Brussel approved the project.

All measurements were performed under standardized temperature (20 ± 2°C) and relative humidity (45 ± 5%) conditions. Prior to the measurements, the volunteers were submitted to a 30 minutes acclimatization period of supine position with coverage of a blanket (Phango experiment) or seated in a chair with the skin region under investigation exposed to environmental conditions (iontophoresis and sonophoresis experiment). During the duration of the iontophoresis, sonophoresis and reservoir experiments the volunteers were asked to maintain their daily activities but to abstain intensive showering, swimming and not to use any body lotions or creams at the skin regions under investigation.
During the entire period of the phango experiment, subjects were covered by a cotton and woollen blanket as during standard paraphango treatment. The first part of the experiment consisted of the relaxation period in which the subjects were resting in a supine position. During this period noise was avoided and the light in the laboratory was dimmed in order to ensure a relaxing atmosphere. At a three minute interval before (relaxing period), during and after the paraphango application the following measurements were directed: the heart rate, SBP and DBP using an automatic monitor (Omron®; RX Classic, Koyoto, Japan), ST (thermo-couple thermometry), and perfusion of the skin microcirculation Laser-Doppler. Due to the dimension of the measuring probe, measurement of skin redness (Chromameter; Minolta, Japan) was only possible before and after the phango application.

3.1 Methyl nicotinate induced skin erythema

In our experiments, the method of Duteil et al. (1990) and Treffel and Gabard (1993) was applied to estimate the bioavailability of diethylammonii diclofenac (Voltaren Emulgel®) in the skin. Measurements were taken on the same subject after iontophoretic delivery, passive diffusion and occlusion, and at different time intervals.

For the MN application paper filter disks (18mm, Epitest Ltd. Oy (Tuusula, Finland) were used. The disks were saturated with a 0.005M aqueous MN solution and applied on the application sites under investigation for 30 seconds. After removal of the paper disk, excess of MN was removed by using a tissue paper. In the experiments where DF application was assessed, skin application sites were marked on the volar part of the forearm arm in a randomized order. At different time intervals post DF application, the 100% MN induced erythema response and the
inhibited responses at different post DF application sites were evaluated with the Chromameter and/or laser Doppler flowmetry. The number of selected skin sites enabled the application of only one treatment modality per skin site.

### 3.2 Corticosteroid induced blanching

Blanching profiles can provide information concerning the onset of vasoconstriction activity, which indicates the bioavailability of corticosteroid in the skin. Evaluation at different time intervals of the blanching profiles allows evaluation on the effectiveness of topical drug delivery modes. A comparative evaluation on the effectiveness between a pre and a simultaneous sonophoretic treatment was performed using the initial part of the physiological blanching response as an indicator for the penetration process of halcinonide through the SC. The halcinonide treatment consisted of the application of 12 mg of a commercial available 1% corticosteroid cream (Betacorton, Spirig Egerkingen, Switzerland).

After the sonophoretic delivery of halcinonide (1 MHz frequency at an intensity of 1.0 W/cm² for 5 minutes) all treated skin areas were occluded with a plastic film and sealed with self adhesive tape ((6x7cm) 3M™ Tegaderm) for 2 hours. Before treatment and after removal of the occlusive film, skin colour was measured at 6-minute intervals up to 60 minutes post halcinonide application. Corticosteroids induced blanching was evaluated by Chromametry, operating in the L* a* b* modus. For the ultrasound treated skin areas, TEWL was measured directly before and after the ultrasound treatment.
3.3 Structure and chemical properties of the used indicator molecules

In the iontophoresis, sonophoresis and reservoir experiments 12mg of diethylammonii diclofenac (Voltaren Emulgel®) or 12 mg of corticosteroid cream (Betacorton®) was applied on circular demarcated skin areas of 7cm². In a pilot study, we tested the optimal amount of compound for application on the demarcated skin areas. To suit the criteria of fast application time and a sufficient covering of the designated area, 12 mg of cream was found to be optimal.

Voltaren Emulgel® is cream-like oil-in-water topical emulsion, each 100 g of gel contains as active substance 1.16 g of diclofenac diethylammonium, which is equivalent to 1 g diclofenac sodium. Diclofenac diethylammonii (C₁₈H₂₂Cl₂N₂O₂) has a negative charge of 1 a MW of 396.29 g/mol and a partition coefficient (N-octanol/aq. buffer) of 13.4.

Fig.9. Structure of diclofenac
The Betacorton® crème used in the sonophoretic study contains 1 mg halcinonide and 50 mg urea. Halcinonide is designated chemically as 21-Chloro-9-fluoro-11β,16α,17-trihydroxypregn-4-ene-3,20-dione cyclic 16,17-acetal with acetone. Halcinonide (C_{24}H_{32}ClFO_{5}) is insoluble in water and has a MW of 454.96 g/mol.

Fig. 10. Structure of halcinonide.

To assess the bioavailability of DF we used 0.005 M aquatic solution of MN. MN (C_{7}H_{7}NO_{2}) is the methyl ester of nicotinic acid and has a MW of 137.14 g/mol.

Fig. 9. Structure of methyl nicotinate
4. RESULTS
4.1

CHANGES OF SKIN CHARACTERISTICS AND AFTER LOCAL PARAPHANGO THERAPY AS USED IN PHYSIOTHERAPY

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Changes of skin characteristics during and after local Parafango therapy as used in physiotherapy

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Background/aims: In physiotherapy, fango (mud) application is a frequently used heat therapy. The main therapeutic effects are due to the elevated temperature of the different tissues with a significant redistribution of blood towards the heated area. This may influence several cardiovascular parameters. There is only limited information on the effect of fango application on skin characteristics. It was the aim of the present study to evaluate the effects of fango application on skin temperature, perfusion of the microcirculation and skin colour. At the same time, cardiovascular parameters such as heart rate, systolic blood pressure (SBP) and diastolic blood pressure (DBP) were recorded.

Method: Eighteen healthy subjects (age 23.7 ± 3.8 years) entered the study. The skin characteristics and cardiovascular parameters were measured before, during and after a 21-min fango application at 44.5 °C.

Results: Skin temperature and perfusion of the microcirculation increased significantly during fango application: from 35.5 ± 0.4 °C to 44.3 ± 1.2 °C for skin temperature and from 23.2 ± 8.8 to 197 ± 41 p.u. for the skin microcirculation. These two parameters remained elevated during the fango application and decreased slowly to baseline values within 21 min after fango removal. Skin colour (CIELAB, a* parameter) increased from 11.0 ± 2.5 to 17.9 ± 1.9 when comparing pre- with post-treatment values. At the end of the measuring period, the a* parameter did not return to baseline values (15.8 ± 2.1). Heart rate increased with 8 bpm during the fango therapy and returned to baseline within 3 min after removal of the fango. SBP and DBP varied slightly during the fango application. They returned to baseline values within 21 min after fango removal.

Conclusion: The skin parameters indicate a transient temperature effect with an increased perfusion of the microcirculation and a flooding of the superficial capacitance system. The cardiovascular parameters were only slightly influenced and remained in the physiological range. Fango application seems not to be too demanding for the cardiovascular system in healthy subjects.

Key words: fango treatment – physiotherapy – local heat – skin temperature – skin colour – perfusion of the microcirculation

Several therapeutic effects are attributed to local fango treatment as used in physical therapy. Most of the effects are assumptions based on longstanding empirical experience, mainly ascribed to local temperature elevation, with fango ion exchanges through the skin only playing a secondary role (1, 2). However, there are only a few publications describing the influence of local heat on the human body. Application of local heat to the skin leads to an increase in the perfusion of the microcirculation and heat is carried away by the blood flow. Heat is equally conducted towards deeper underlying tissues. Therefore, heat applied externally increases the skin and deep tissue temperature, which will stimulate the thermoreceptors (3, 4). This afferent thermal signal inhibits the transmission of nociceptive signals through the spinal cord to higher centres, leading to pain reduction (5). There is some evidence for antiphlogistic effects (6, 7), muscle tone reduction as a result of the decreased α-motor activity from the dorsal horn of the spinal cord (8), increased flexibility of connective tissue (9), decreased viscosity of the synovia (10), vasodilatation and nitric oxide generation (11), phagocytosis stimulation and white blood cell activation (12) and for synergetic effects with oral ibuprofen therapy (13). Cardiovascular diseases are often considered as a contraindication for local heat application in physical therapy because the vascular effects may be too demanding for heart patients.

Fango (mud) and humolites (humus) are peatbogs, consisting of humus and minerals with...
traces of organic substances (blue-green algae). Peloids are formed in a natural way in water and soil by the activity of microorganisms (microbial colonies and algae). Peloids have an oxido-reduction potential corresponding to that of ascorbic acid. They may contain sulphur, in its elementary form. Minerals from the soil and the environment enter the peloid. Trace elements found in peloids include boron, cobalt, copper, iodine and manganese. In some naturally formed peloids, hormones have been found while little is known about the vitamin, alkaloid and protein content of mud (14). Up to now, there are no studies indicating penetration of active compounds from the mud packs into the human skin (2). In balneotherapy, heat retentivity is an indication for the temperature dissipation of the fango, expressed in °C/cm³. It is the reciprocal of the coefficient of heat conduction (14).

The thermophysical and hygienic conditions of fango were improved using a mixture of paraffin and peloids from the volcanic crater lake of Battaglia (Italy). This so-called parafango, with improved physical properties, ensured a slow heat conduction, allowing application of the parafango heated up to 50 °C without causing any damage to the underlying tissues. The ‘Parafango di Battaglia’ is the fango most commonly used in physical therapy (15).

Aim of the Study
As for the assumed therapeutic effects, few studies describe the temperature effects of parafango application. It was our aim to evaluate and quantify the temperature changes on the skin during parafango therapy. We equally tried to evaluate the local effects on the skin microcirculation on the arterial level (Laser–Doppler) and the venous level (Chromametry). In order to evaluate the impact of blood redistribution towards the covered skin area, we evaluated the effects of parafango therapy on several cardiovascular parameters. The latter may give more information concerning eventual contraindications for parafango application.

Methods
Eighteen healthy subjects (11 women, 7 men) without diabetes, vasoactive pharmacotherapy, inflammatory joint diseases or skin diseases volunteered to participate in this study (age 23.7 ± 3.8 years). All volunteers respected a 20-min acclimatization period (room conditions 20 ± 2 °C; relative humidity 45 ± 5%) before any experimental procedure was started. The first part of the experiment consisted of a relaxation period during which the subjects remained in a supine position for 21 min. During the entire period of this experimental procedure, subjects were covered by a cotton and a woollen blanket as during standard parafango treatment. In order to ensure a relaxing atmosphere, lights were dimmed and noise was avoided in the laboratory. After this relaxation period, fango was applied. A 1-cm-thick parafango mudpack (‘Parafango di Battaglia’, Padua, Italy) with a surface of 2500 cm² at 44.5 °C was applied for 21 min. Subjects were lying on the fango with direct physical contact between the parafango and the skin of their back. After removal of the parafango, the subjects stayed in a supine position for another 21 min.

At a 3-min interval before (relaxation period), during and after the parafango therapy, skin surface temperature (ST) (thermo-couple thermometry Testoterm 9010; Testoterm GmbH & Co., Lenzkirch, Germany) and microcirculation of the skin (PeriFlux PF3 Laser–Doppler; Perimed, Sweden) were measured. The thermometer and Laser–Doppler probes remained in contact with the skin during fango application. The thermal probe was sealed on the skin under the parafango. The Laser–Doppler probe holder was inserted into the parafango, allowing direct contact between the probe and the skin. Owing to the size of the parafango and the treated area (2500 cm²), covering the entire back, measurements on an untreated control side were impossible during the parafango application. Moreover, for the evaluation of the skin redness (as indicated by the $a^*$ parameter from the $L^* a^* b^*$ colour system, Chromameter; Minolta, Japan), the dimension of the measuring probe did not allow data collection during parafango application. As a consequence, information on skin redness was collected only pre- and post-parafango treatment. Before, during and after the parafango treatment, the heart rate, systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured every 3 min using an oscillometric method. An automatic monitor at the left wrist was used (Omron ; RX Classic, Kyoto, Japan). Statistical analysis was performed using the SPSS 13.0. Data were tested on normality by the Kolmogorov Goodness of fit test. Variations in skin parameters (temperature,
perfusion and redness) and in cardiovascular parameters (heart rate, SBP and DBP) were compared using the ANOVA procedure taking into account the entire measurement period (pre, during and post treatment). If significant changes over the entire period were observed, analysis of the separate intervals (pre, during and post-treatment) was carried out. In case of stable (non-significant) pre-treatment values, these values can be used as a reference for the treatment and post-treatment measures (MANOVA procedure). Significance was set at $P < 0.05$.

**Results**

The skin ST baseline value was 35.5 ± 0.4 °C. After parafango application with physical contact to the skin surface, the skin temperature increased up to 44.3 ± 1.2 °C. At the end of the parafango therapy, skin temperature was still elevated (40.3 ± 0.6 °C). After removal of the parafango, skin temperature decreased steadily and reached baseline values within 21 min (36.1 ± 0.4 °C) (Fig. 1). The ANOVA procedure indicated a significant effect over time for the skin temperature values ($P < 0.05$). Analysis of the different intervals revealed that the pre-treatment values remained constant ($P > 0.05$). Comparison with the latter values indicated significant differences for the treatment and post-treatment values ($P < 0.05$).

Baseline perfusion of the microcirculation of the skin (MC) as measured by the Laser–Doppler method was 23.2 ± 8.8 p.u. During parafango application, the perfusion increased immediately and peaked after 6 min (197 ± 49 p.u.). At the end of the parafango therapy, perfusion of the microcirculation was still elevated (159 ± 52 p.u.). After removal of the fango, perfusion of the microcirculation decreased, reaching a value of 48 ± 39 AU at the end of the recording period (Fig. 2). The ANOVA procedure indicated a significant effect over time for the perfusion of the skin microcirculation ($P < 0.05$). Again, the pre-treatment values remained constant ($P > 0.05$). Comparison with these values indicated significant differences in the treatment and post-treatment values ($P < 0.05$).

Skin redness (SR), as quantified by the $a^*$ colour parameter, increased significantly during parafango application (from 11.0 ± 2.5 before up to 17.9 ± 1.9 immediately after removal of the parafango). The $a^*$ parameter was still elevated 21 min after removal of the parafango (15.8 ± 2.2), (Fig. 3). Analysis of the $a^*$ parameter for the pre- and post-treatment period over time indicated significant variations ($P < 0.05$). The pre-treatment skin colour values over time remained stable ($P > 0.05$). Comparison of pre- with post-treatment values indicated significant differences,
with higher values over the entire post-treatment period ($P < 0.05$).

The resting heart rate was around 70.5 ± 9.9 bpm. During heat application, the heart rate increased, and reached the highest values within 6 min (78.8 ± 10.5 bpm). At the end of the parafango therapy, the heart rate was still 75.5 ± 10.5 bpm. Within 3 min after cessation of the parafango application, the heart rate decreased to 71.1 ± 10.0 bpm and remained constant for the rest of the recording period (Fig. 4).

The ANOVA procedure revealed significant variations ($P < 0.05$) over time. The pre-treatment heart rate remained constant ($P > 0.05$). Significant differences were detected when comparing, respectively, the treatment and post-treatment values with the pre-treatment reference values ($P < 0.05$).

SBP varied around 102.9 ± 8.9 mmHg during the pre-treatment period and decreased to 98.7 ± 8.2 mmHg during the parafango application. At the end of the post-treatment period, SBP increased to the baseline level (101.5 ± 9.8 mmHg). DBP slightly increased within 3 min after parafango application (from 59.8 ± 6.8 mmHg to 61.2 ± 6.5 mmHg). Three minutes later, DBP decreased again up to (57.4 ± 6.5 mmHg) and at the end of the 21-min parafango therapy period DBP was 54.3 ± 6.0 mmHg (Fig. 5).

Statistical analysis indicated variations over time for SBP and DBP over the entire measurement period ($P < 0.05$). For both parameters, the pre-treatment values were unstable ($P < 0.05$). As a consequence, these values could not be used as reference values. However, on analysing the during and post-treatment SBP and DBP values we equally detected unstable values ($P < 0.05$).

**Discussion**

Our data showed that parafango therapy ('Parafango di Battaglia'), commonly used as a local heat treatment in physical therapy, resulted in significant changes of skin characteristics at the treatment site. As observed in other experiments (16), these changes are characterized by (i) an increase of the skin ST, (ii) an increase of the superficial microcirculation and (iii) an increase of skin redness indicating flooding of the deeper venous system.

Laser–Doppler flowmetry and skin surface thermo-couple thermometry are methods for evaluating the more superficial skin properties. Both the volume and mean velocity of the erythrocytes circulating in the superficial skin vessels will influence the Laser–Doppler signal. This method is very sensitive for evaluating changes in the microcirculation of the superficial skin layers and reflects cardiac, respiratory and arteriolar vasomotion fluctuations. The latter are the main sources of skin blood flow modification over time (17).

Using a similar experimental protocol but applying a 15-mm and a 30-mm thick ‘La Léchère’ mud pack (a mixture of clay and La Léchère mineral water) at 50 °C, Poensin et al. (17) found that skin temperature (+2 °C) and superficial skin flow (+600%) increased at the treated side and that these properties remained significantly above the baseline value for at least 5 min after pack removal ($P = 0.004$). In our study, the skin
ST increased by 8 °C while superficial skin blood flow increased 900%. Because methods of evaluating thermometry and flowmetry in both studies were comparable, it seems that the effects of parafango therapy (despite the thinner pack and the lower application temperature) were more pronounced compared with the application of the ‘La Léchère’ mud pack. However, the differences may be explained partially by the use of longer application times or differences in response between the experimental groups: Poensin et al. (17) used female volunteers between 28 and 67 years (median age 51 years) while we worked on younger male and female volunteers (11 women, 7 men; mean age 23.7 ± 3.8 years).

Skin colour as assessed by the Chromameter reflects changes in the superficial and deeper dermis. Our findings indicate that there is a vasomotion of the capacitance vessels of the deeper layers of the skin, increasing the blood circulation and the temperature. This may explain some of the therapeutic effects of fango therapy (e.g. better diffusion of catabolic waste products). However, vasomotion of the superficial and deeper blood vessels may also be a part of the thermoregulatory system. Poensin et al. (17) used the coefficient of variation (standard deviation/mean) of the Laser–Doppler flowmetry signal for superficial skin flow as an indirect evaluation method of vasomotion. They recorded very low-frequency vasomotion waves at the treated side. Together with their finding of the blood flow increasing disproportionally compared with the skin ST increase at the treated side, and the observation that no changes occurred at the non-treated side in combination with a constant core temperature, they concluded that central mechanisms related to thermoregulation were not involved.

Our results indicate that the overall systemic cardiovascular effects (heart rate and blood pressure) were rather weak. An increase of the heart rate (around 10 bpm) was measured immediately after fango application. In our opinion, this may be caused by a redistribution of the blood towards the more superficial veins as part of the thermoregulation response. SBP and DBP varied already during the pre-treatment period, but also during the treatment and post-treatment periods. The pre-treatment values decreased as a function of time. This may be an indication of the decreased stress and that the volunteers became used to the different measurement procedures. Again, the measured variations of SBP and DBP are very weak and of no clinical significance.

We assume that the therapeutic effects are mainly due to the temperature changes and the increased blood flow. Similar therapeutic effects are ascribed to rubeficiant massage products containing nicotinates as active ingredients. Earlier findings indicated a nicotinate concentration-dependent increase in skin temperature (up to 2 °C) and perfusion of the microcirculation (up to 880%) (16). These results are completely comparable with the results of the parafango therapy by Poensin et al. (17).

We conclude that during and after parafango therapy, there are strong local effects on skin properties as measured with thermo-couple thermometry, Laser–Doppler flowmetry and the Chromameter for assessing skin erythema. However, the systemic effects are rather weak. Therefore, there is evidence that parafango therapy may be safe for patients with venous insufficiency.

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4. RESULTS
4.2

The Effects of Iontophoresis in the Treatment of Musculoskeletal Disorders

A Systematic Review and Meta-Analysis

R. Clijser1,2,*, J. Taeymans3, J.P. Baeyens1, A.O. Barel1 and P. Clarys1
The Effects of Iontophoresis in the Treatment of Musculoskeletal Disorders - A Systematic Review and Meta-Analysis

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Abstract: This systematic review and meta-analysis is focusing on the evidences related to iontophoresis used to enhance the topical drug delivery through the skin in the treatment of inflammatory dysfunctions, acute soft tissue injuries and pain. A literature search in the databases, MEDLINE (PubMed), Pedro, and the Cochrane Database of Systematic Reviews was conducted. The methodological quality of the obtained studies was independently rated by two reviewers. Data were pooled from those studies in which the effect of iontophoresis treatment on pain was compared with a control or sham (placebo) intervention. Twenty four experimental studies evaluating the effectiveness of iontophoretic treatment were included. Based on comparable statistical outcome for pain, the results of ten studies could be pooled for meta-analysis. Although several clinical studies claimed an advanced healing process after iontophoresis, controversy on the healing efficacy of iontophoresis remains. The overall summary estimate of the pooled post treatment values for pain was -0.672 [95% CI: -0.970 to -0.375] favouring iontophoresis treatment (p < 0.0001). The observed trend between studies heterogeneity for the post treatment pain values was low to moderate (I² = 39.9%; p = 0.092). The results of the meta-analysis indicate quantitative evidence that iontophoresis is effective in the treatment of pain, however, the lack of solid research design in studies on iontophoresis makes it difficult to ensure that the improvements observed can be explained by the iontophoresis technique in se.

Keywords: Iontophoresis, physiotherapy, transdermal drug delivery, soft tissue injuries, efficacy of iontophoresis.

INTRODUCTION

Inflammatory disorders of the musculoskeletal system and acute soft tissues injuries are often treated topically. However, the poor permeability of the skin, with the stratum corneum as the main barrier, allows only small quantities of drugs to enter the body. Several techniques, such as iontophoresis, have been developed to increase the permeability of the stratum corneum in order to enhance penetration in deeper tissues such as muscles, tendons or synovial fluid [1]. To enhance the delivery of (pro-) drugs through the skin, iontophoresis applies a direct current of low amperage up to 0.5 mA/cm² by means of bipolar electrodes [2,3]. Ionized molecules are hypothesized to migrate along the lines of the applied electric field with positive ions (cations) repelled if applied under the anode and attracted by the opposite electrode while negative ions (anions) are repulsed by the cathode and attracted by the anode. The penetration of unionized moieties is believed to occur by convective flow due to an electrochemical NaCl gradient, which drags these molecules along with water from the anode to the cathode [4].

In recent years transdermal drug delivery has gained increasing interest as the emergence of new physical and chemical techniques expanded the number of drugs that can be delivered transdermally. The introduction of iontophoretic transdermal controlled drug delivery systems improved the effectiveness of therapeutic treatment regimens and appears to be a promising technique for the delivery of a variety of compounds in a controlled and preprogrammed manner [5].

The aim of this systematic review was to establish an overview of the available evidence for the use of iontophoresis in the treatment of musculoskeletal disorders. Furthermore, this study aimed to provide more conclusive results on the effectiveness of iontophoresis in pain reduction, analysing the studies with comparable outcome in a meta-analysis.

METHODS

Search Strategies and Data Sources

The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate health care interventions was followed for this review [6]. An electronic search of the following databases up until February 2011 was conducted: MEDLINE (PubMed), Pedro, and the Cochrane Database of Systematic Reviews. The reference list of the retrieved articles was manually searched for relevant literature.

Study Selection and Research Question

To establish the research question the recommendations from the PICO-model were used (Population: patients with musculoskeletal problems; Intervention: iontophoresis; Comparator: sham iontophoresis or alternative therapy; Outcomes: pain reduction and functional improvement). Hence,
this paper reviews the evidences related to the use of iontophoresis in patient with musculoskeletal problems.

The following keywords were used for formulating the search strategy for this review: iontophoresis, physical therapy modalities, musculoskeletal disorders and pain.

Two independent reviewers (RC, JT) screened the titles and abstracts for eligibility. Randomized controlled trials (RCT) and clinical trials were included as sources of primary research data while systematic reviews, meta-analyses, and pilot studies were accepted as they can provide valuable insights. To narrow the extensive search for findings down to a core of relevant literature of the last twenty years for the research questions of this study, a number of exclusion criteria were established: retrospective studies, case studies, no research question of this study, a number of exclusion criteria.

The methodological quality of the studies was assessed using the Pedro rating scale. For studies retrieved from the Pedro data base the mentioned Pedro scores were accepted. The Pedro rating tool comprised eleven items from which one is not rated. Each item was scored with “+” if the criteria were fulfilled, with “-” if the criteria were not fulfilled or if the provided information was unclear. In addition the quality of all studies was assessed using the Oxford Centre for Evidence-based Medicine Levels of Evidence. In case of disagreement a third researcher (PC) rated the questionable study and agreement was sought by consensus.

General characteristics of the studies were extracted by two researchers independently (RC, JT): design and sample, pathology, type of intervention, outcome measures (e.g. pain, functional improvement), conclusion of the studies and statistical significance.

Data Synthesis

In the retrieved articles the outcomes of interest (pain, functional improvement) were assessed using a variety of scaling tools and the results were presented as means and standard deviations or as medians and ranges. The observed lack of methodological homogeneity made a pooling of the functional improvement data questionable. Therefore, the authors decided only to perform a meta-analysis on pain.

Meta-Analysis

Data were pooled from those studies in which the effect of iontophoresis treatment on pain was compared with a control or sham (placebo) intervention. Out of the 24 studies retrieved for the systematic review, the authors selected ten studies that were comparable, based on a statistical outcome for pain allowing an effect size calculation and meta-analysis. Furthermore 2 subgroup analyses were made comparing the outcome of pain after iontophoretic treatment versus control or sham intervention in the treatment of tendinopathy and rheumatic diseases respectively. Based on the observed methodological heterogeneity between the studies, the authors decided a priori the use of a random effects model. The effect sizes were expressed as Hedges’s g to correct for potential overestimation of effects in small studies. The Comprehensive Meta-Analysis 2 software (CMA – Version 2 Professional, Biostat Inc., Englewood, USA) was used for the calculations of the overall estimates and the 95% confidence intervals as well as for the preparations of the different forest and funnel plots.

RESULTS

Study Characteristics

The literature search revealed a total of 371 possibly eligible studies. After reading tite and abstract the search was reduced to 284 studies. As presented in (Fig. 1), most of the papers had to be excluded (n = 253) because they did not refer to the afore-mentioned search and exclusion criteria.

Five studies were added after reading the reference lists. From the resulting 36 relevant studies, 24 were experimental in vivo studies while 12 were review studies. The latter were used within the discussion section only.

Study Quality and Characteristics of Population

The quality spectrum of the study designs ranged from Pedro-score 3 to 10. Studies with Pedro-score 7/10 were rated as “high”, those with a Pedro-score 5/10 or 6/10 were rated as “moderate” and those with Pedro-score 4/10 or <4/10 as low quality studies. The mean Pedro-score for the studies reporting positive effects of iontophoresis treatment was 6.75 ± 2.12 SD while studies with negative treatment outcome had a Pedro-score of 6.45 ± 2.16 SD, indicating a moderate quality of research in this topic area. Studies reporting positive effects of iontophoresis treatment tend to have a lower level of evidence as 50% was rated as level 1b, 17% as 2b, 8% as 2c, 8% as 3b and 17 % as level 4 studies versus 55 % level 1b and 45 % level 2b from the studies with a negative outcome. A high degree of heterogeneity within the application of iontophoresis therapy was observed. The major characteristics of the 24 selected experimental studies are presented in annex 1 (Synopsis of studies on iontophoresis). Studies were conducted in USA (n = 4), Turkey (n = 4), Canada (n = 3), Germany (n = 2), Nigeria (n = 2), Sweden (n = 2), the Netherlands (n = 2), Australia (n = 1), Hong Kong (n = 1), Italy (n = 1), Poland (n = 1), United Kingdom (n = 1).

Study Characteristics

The Treatment Protocols

In iontophoresis the electrode surface ranges from 7 to 44 cm² [7,8] and the current varies from 4 to 11 mA applied during 10 up to 20 minutes [3,9-11]. The electrodes can be placed on both sides [8] or the active electrode can be placed above the treated area with the return electrode placed at the opposite site [7,11].

Used Drugs for Iontophoretic Delivery

The iontophoretic deliveries of corticosteroids and NSAIDs are commonly used as a treatment in patients with musculoskeletal inflammatory disorders.
The following applications have been reported: acetic acid (4 studies), sodium diclofenac (3 studies), sodium salicylate (2 studies), ketorolac, benzydamine, ketamine, lidocaine and naproxen (1 study each), dexamethasone (11 studies), hydrocortisone (2 studies) (overview of the drugs used for iontophoresis annex 2).

**Treated Disorders**

Iontophoretic delivery of medications is reported on the following disorders: carpal tunnel syndrome and epicondylitis (4 studies each), tendinopathy, plantar fasciitis, calcifying disorders (3 studies each), osteoarthritis knee, pain (2 studies each), temporomandibular joint disorders, adhesive capsulitis, rheumatic disease (1 study each).

**The role of Iontophoresis in Modulating Pain**

A number of studies concerning the treatment of various orthopaedic, musculoskeletal and neurological disorders compared the effectiveness of iontophoresis in modulating pain to different other physical therapy modalities (such as transcutaneous electrical nerve stimulation TENS). In a prospective study Bremerich and Wiegel [12] evaluated the combined use of TENS and iontophoresis with benzydamine (anode) and diclofenac (cathode) in the treatment of neuralgiform facial pain. The evaluation of average pain intensity showed better results (81%) for the combined application of iontophoresis and TENS compared to (63%) TENS alone.
Controversial results were found in the randomized placebo controlled study of Vranken et al. [13], testing the efficacy of 50 and 75 mg S(+)-ketamine iontophoresis against placebo (isotonic saline) iontophoresis, in patients with intractable neuropathic pain. The patches were attached on the most painful area and medication was delivered during 24 hours at a current of approximately 0.05 – 0.16 mA. The primary parameter was pain intensity, evaluated using a visual analogue scale at the beginning of the treatment, at day 5 and at day 7. Secondly, health status and quality of life (QoL) questionnaires were completed before starting the treatment and one week after. The outcome of the study was, that iontophoresic delivery of S(+)-ketamine was no more effective than placebo treatment, in reducing pain scores in patients with severe central neuropathic pain. However, iontophoresic administration of 75 mg S(+)-ketamine improved the health status and QoL in these patients.

The Effect of Iontophoresis on Pain

In the selected trials (n = 10) for the meta-analysis the number of subjects totalled 451 (n = 239 iontophoresis / n = 212 controls).

The overall summary estimate of the pooled post treatment values for pain was -0.672 [95% CI: -0.970 to -0.375] favouring iontophoresis treatment (p < 0.0001). The observed trend between studies heterogeneity for the post treatment pain values was low to moderate (I² = 39.9%; p = 0.092).

(Fig. 3) depicts the funnel plot to assess publication bias. Using the trim and fill iterative method, no missing studies were imputed. Therefore, no change of the overall estimate was observed (black diamond). The fail 'n safe model analysis showed that 88 negative studies would be needed before alpha increases above the 5% level.

Treatment of Carpal Tunnel Syndrome

Concerning the conservative treatment of carpal tunnel syndrome (CTS) with iontophoresis, four studies were detected. Banta [14], conducted a non-randomized study on the effectivity of wrist splinting, anti-inflammatory medication and iontophoresis with dexamethasone sodium phosphate in patients with early-mild carpal tunnel syndrome. After a six month follow-up, four out of 23 cases (17%) were successfully treated with splints and the use of anti-inflammatory medication alone, 11 out of 19 (58%) successfully with dexamethasone iontophoresis and 15 out of 23 (65%) with steroid injections in the carpal region.

Dakowicz and Latosiewicz [15] performed a controlled clinical trial (CCT), to evaluate the effectiveness of a conservative treatment of CTS, using hydrocortisone iontophoresis in combination with ultrasound therapy, on three groups (n = 40) of patients differing in severity of clinical symptoms. The study confirmed that physiotherapeutic methods are effective in the treatment of non-advanced forms of CTS Gr. I (n = 12), Gr. II (n = 24). Patients Gr. III (n = 4) with advanced stage of CTS should be referred to operative intervention.

In a prospective randomized non-blinded clinical trial on 48 medial nerves in 30 patients with clinical and electro-physiological evidence of CTS, Gökkoğlu et al. [16] compared the effectivity of local corticosteroid injection versus iontophoresis of corticosteroids, in the treatment of CTS evaluated with a functional status scale (FSS), a symptom severity scale (SSS) and a visual analog scale (VAS) on pain. Short term outcome indicated a relief in both clinical and objective measures for both application methods. Although significantly better mean SSS and FSS scores were found after two and eight weeks, in favor of the injection group, the authors concluded that the application of a steroid by iontophoresis as an alternative to injection is a safer method without complications or side effects.

Controversial results are reported from the randomized double-blind placebo controlled study (n = 17) of Amirjani et al. [17], evaluating the effectiveness of a two week intervention with six sessions of dexamethasone iontophoresis to treat CTS. On nerve conduction, the Levine Self-Assessment Questionnaire (severity of CTS symptoms), and the Semmes-Weinstein Monofilaments (determination sensation threshold), were done monthly during six months after intervention. The active, (cathode) electrode was placed over the
The Effects of Iontophoresis in the Treatment of Musculoskeletal Disorders

Iontophoresis has been reported on the following tendinopathies: tendonitis, epicondylitis (lateral and medial), plantar fasciitis, calcifying tendonitis, and acute Achilles tendon pain. Hendricks et al. [18] conducted a RCT to explore the short-term effects of anti-inflammatory drug administration by dexamethasone sodium phosphate (DSP) iontophoresis, compared to placebo iontophoresis in patients with different forms of “primary” tendonitis. The patients (n = 22) became three treatments a week, during four weeks. “Activity of daily living”, pain using the visual analog scale (VAS) and a clinical assessment of local tenderness by pressure and by stretch and resistance were evaluated. The authors concluded that the treatment with (DSP) was superior (p < 0.05) to placebo iontophoresis in all outcome variables.

Desmirtas et al. [8] conducted a RCT on the effectiveness of iontophoresis with salicylate versus diclofenac iontophoresis in the treatment of epicondylitis. Patients (n = 40) were randomly assigned to group I, receiving iontophoresis with sodium diclofenac and infrared radiation or group II receiving iontophoresis with sodium salicylate and infrared radiation. Electrodes (4.5 x 8 cm) were transversally applied and the cathode was used as active electrode. Application duration was 20 minutes and treatment frequency was once a day, five days a week, up to 18 days, using intensities between 6 and 11 mA. Before and 7 days after treatment, pain by pressure at resisting wrist extension, during function and at rest was evaluated. A significant reduction in pain scores (p < 0.05) was found in both groups. When pain scores after treatment were compared for both groups, a stronger significant decrease (p < 0.05) was observed in pain produced on resisting wrist extension and by pressure on the lateral epicondyle in the group treated with sodium diclofenac iontophoresis.

Controversial results were reported on the treatment of lateral epicondylalgia with dexamethasone iontophoresis. Runeson and Haker [11] evaluated in a RCT the short- and long-term pain-relieving effect of corticosteroid iontophoresis versus placebo iontophoresis in patients with lateral epicondylalgia. No significant difference in pain-relief could be observed between the corticosteroid group (n = 33) and the placebo group (n = 31).

Treating lateral epicondylitis, Baškurt et al. [19] compared the effect of phonophoresis and iontophoresis with naproxen, sixty-one patients were randomly divided into a group treated with naproxen phonophoresis (1 mHz, 1 W/cm²) and a group treated with iontophoresis (anode, 0.08-
Accompanying Neeter et al. [20] conducted a double-blind randomized study to evaluate the effects of iontophoresis with dexamethasone versus placebo iontophoresis on patients (n = 25) with acute (less than 3 months) Achilles tendon pain. Several significant improvements were seen in the range of motion test, pain during and after physical activity, pain during walking and walking up and down stairs, morning stiffness and tendon swelling in the experimental group (p < 0.05) and not in the control group (p > 0.05). Poor reliability was found for pain on the palpation test. After a 1-year follow-up period, the authors concluded that the use of iontophoresis with dexamethasone had positive effects on the treatment of patients with acute Achilles tendon pain.

In patients (n = 199) with medial and lateral epicondyli-tis, Nirschl et al. [21] performed a double-blinded randomized placebo-controlled study on the effectiveness of pain control by means of transdermal administration of dexamethasone sodium phosphate. Within fifteen days six iontophoresis treatments were applied at 1 to 3 day intervals. This short time intervention presented a significant pain reduction (p < 0.05).

In another RCT, Taskaynatan et al. [22] compared the effects of steroid iontophoresis (SI) and electrotherapy (ET) on bicipital tendinitis. Patients (n = 47) were randomly divided into two experimental groups. One group received SI (0.5% hydrocortisone acetate under the cathode, stimulated with 3-4 mA galvanic current). The second group was treated with ET (interferential current, 0-100 Hz, for 15 min.). Patients were evaluated pre-, post-, and one month after treatment with a numeric scale for pain at rest, pain during normal activities and under strenuous activities, for range of motion with goniometry and with the Constant’s Shoulder Scale, with a numeric scale for patient satisfaction and disability by using the function section of the Pennsylvania Shoulder Scale. All of the assessments significantly improved in the SI group directly and one month after the treatment. The ET group experienced less immediate improvement and the durability of the benefit was less than with SI. The authors suggested that additional to conventional physical therapy, application of SI provides a better and more prolonged clinical and functional improvement in patients with biceps tendinitis.

Treatment of Plantar Fasciitis

Three controlled studies on the treatment of plantar fasciitis with iontophoresis were identified. Gudeman et al. [2] performed a randomized double-blind placebo-controlled study to determine whether iontophoresis of dexamethasone in conjunction with other traditional therapy modalities (n = 20) was more effective in reducing pain than traditional modalities alone (n = 20). Although the use of iontophoresis with dexamethasone was recommended for patients who need an immediate pain reduction, the end result of function was the same with or without iontophoresis (p = 0.434). Hammer et al. [23] evaluated patients with chronically painful proximal plantar fasciitis, (n = 47, 49 feet) randomly allocated to a group 1, treated with three sessions of extracorporeal shock wave therapy (ESWT) at weekly intervals against a group 2, treated with diclofenac iontophoresis and an oral NSAID for 12 weeks. After this period they were treated using the protocol of group 1. After 12 weeks of iontophoresis treatment the authors concluded, without giving an exact α level that no significant differences for pain (VAS scale for activities of daily living) and comfortable walking time were found in group 2. At 12 weeks after ESWT, a significant reduction (p < 0.01) of pain estimation and increased comfortable walking time was seen for both groups. Osborne and Allison [24] performed a randomized double blind placebo controlled clinical trial on the treatment of plantar fasciitis with dexamethasone iontophoresis, in combination with taping and exercise therapy. During two weeks the patients (n = 31) received six treatments of iontophoresis to the site of maximum tenderness on the plantar side of the foot. The experimental group (n = 11) received 0.4% dexamethasone, whilst the placebo group (n = 10) was treated with 0.9% NaCl or 5% acetic acid (n = 10). Stiffness and pain were recorded at the initial session, the end of six treatments, and after four weeks follow-up. It was concluded that six treatments of acetic acid iontophoresis, combined with taping was the preferred treatment option compared to taping combined with dexamethasone or saline iontophoresis.

Treatment of Calcifying Disorders

Three studies were found concerning the treatment of calcifying disorders by means of acetic acid application. In a RCT, Perron and Malouin [25] combined iontophoresis with acetic acid and ultrasound in the treatment of calcifying tendinitis of the shoulder. They reported a reduction in the size (p = 0.01) and density (p = 0.03) of the calcium deposit but did not detect a difference between the experimental (n = 11) group and the control (n = 10) group (p = 0.05). A randomized controlled trial by Leduc et al. [3] compared the treatment of calcifying tendinitis of the shoulder by 5% acetic acid iontophoresis with physiotherapy without iontophoresis. Pain, shoulder range of motion (ROM), size and density of the tendinious calcifications were assessed before and after the end of the 10 treatment sessions but did not reveal differences between the treatments.

Shetty et al. [26] conducted a pilot study (non-RCT) to investigate whether iontophoresis of acetic acid, followed by ultrasound therapy, was a safe and effective treatment for systemic sclerosis-related calcinosis. Patients (n = 3) received iontophoresis (100 µA) with 5% acetic acid and 8 minutes pulsed ultrasound therapy (1.5 W/cm²) at a 50% duty cycle. After nine therapy sessions none of the patients experienced significant adverse effects but there was no clinically apparent treatment benefit.

Subgroup Analysis

A total of 5 studies were selected for a subgroup analysis, comparing the outcome of pain after iontophoretic treatment.
The Effects of Iontophoresis in the Treatment of Musculoskeletal Disorders

**Results**


versus control or sham (placebo) intervention in the treatment of tendinopathy. The overall summary estimate of the pooled standard deviation for post treatment values on pain was -0.578 [95%CI: -0.930 to -0.226] favouring iontophoresis (p = 0.001).

**Treatment of Degenerative Joint Disease**

Three studies on the treatment of degenerative joint disease were included. Schiffman et al. [10] evaluated in a double blind RCT the short-time effects of iontophoretic delivery of dexamethasone on mandibular range of motion and subjective pain in temporomandibular disorders, in 27 patients who had concurrent temporomandibular joint disc displacement without reduction and capsulitis. In a first group, patients (n = 9) received dexamethasone sodium phosphate and lidocaine hydrochloride iontophoresis, the second group (n = 9) received only lidocaine and the third group (n = 9) was treated with buffered saline. Treatment was administered every other day for a total of three treatments. Patients treated with dexamethasone and lidocaine showed a significant increase in mandibular opening (6 mm) and lateral excursion (1.2 mm) and a decrease in mandibular dysfunction compared to patients of the other two groups. No significant decrease in pain symptoms was reported. (Gr. I p = 0.09, Gr. II p = 0.07, Gr. III p = 0.8).

Aiyejusunle et al. [27] randomly compared the effects of transcutaneous electrical nerve stimulation (TENS) and sodium salicylate iontophoresis, on functional disability and pain in 20 patients with osteoarthritis of the knee. After 6 weeks of treatment the subjects were assessed using the VAS scale of pain and the Disability Index Questionnaire for patient with osteoarthritis of the knee. Although a significant reduction of pain (p = 0.01) and functional disability (p = 0.06) was found in both groups, patients treated with sodium salicylate had a significant higher reduction of pain (p < 0.01) and functional disability (p < 0.06) compared to the patients treated with TENS only. Akinbo et al. [28] evaluated phonophoresis and iontophoresis using dexamethasone sodium phosphate, in 50 randomly assigned patients with knee osteoarthritis on the Western Ontario and McMaster University Osteoarthritis Index (WOMAC), 20 meter ambulatory time and ROM. Patients received ten therapy sessions within two weeks. After two weeks, all outcome measures improved significantly in both groups with no significant between group differences.

**Treatment of Rheumatic Disease**

Two studies were detected concerning the use of iontophoresis in rheumatic disease. Li et al. [29] randomly assigned ten subjects with rheumatoid arthritis in a dexamethasone iontophoresis experimental group and a placebo (sterile water) group. Pain at rest, on movement and on pressure, active joint count, and ROM were evaluated. Statistical differences in favor of dexamethasone iontophoresis were found for pain during movement and pain at rest. These results are in line with the study of Saggini et al. [7] who assessed double-blinded, the efficacy of ketorolac iontophoresis compared with placebo iontophoresis (saline) in 60 patients with pain from rheumatic disease (epicondylitis (12), scapulohumeral periarthritis (30), gonalgia (10) and metatarsalgia (8). Before and after five treatment sessions and seven days after the treatment, the degree of pain and judgement of efficacy were evaluated on categoric scales by patient and physician. Immediately after treatment, the pain scores were similarly reduced in both the placebo and experimental group. Seven days after the treatment, a further decrease in pain was observed in the group treated with ketorolac but not in the placebo group resulting in a significant group difference.

Fig. (5) depicts the forest plot of a subgroup analysis, comparing the effect of iontophoretic treatment on pain in rheumatic disease versus control or (sham) placebo treatment of the above mentioned studies. The summary estimate was -0.644 [95%CI: -1.115 to -0.173] favouring iontophoresis (p = 0.007).

**Discussion**

This review aimed at evidences related to the clinical effectiveness of iontophoresis. There are an ample number of
The skin as a barrier in physiotherapy

4. RESULTS


Studies concerned. The most frequently used drugs in iontophoresis are corticosteroids and NSAIDs.

Skin irritation provoked by either acidic or basic reactions build up at the respective electrodes, are best avoided by keeping current intensity low (<0.5 mA/cm²). The literature presents a lack of consensus on the used application time and treatment intensities. Gudeman et al. [2] administered 0.4% dexamethasone sodium phosphate using the Phoresor II iontophoretic drug delivery system with a dosage of 40 mA over 20 minutes.

In the study of Vranken et al. [13] a self powered disposable patch (Active Area: 15.5 cm², dosage 80 mA-min) delivered medication (ketamine) during 24 hours. Consequently, it is impossible to discriminate between the different factors influencing the percutaneous uptake.

Controversial results are reported for the use of dexamethasone iontophoresis in the treatment of tendinopathy. The studies from Hendricks et al. [18] and Neeter et al. [20] compared the use of dexamethasone iontophoresis with the treatment of placebo iontophoresis in patients with acute tendinopathy and concluded that the use of dexamethasone iontophoresis was superior to placebo treatment. These results should be interpreted carefully, as the use of an inactive placebo substance, is not a valid method to prove the effectiveness of dexamethasone iontophoresis. During iontophoresis the treated skin area is covered with a water humidified electrode provoking an occlusion. It is known that occlusion of the skin causes a more hydrated skin and improves the percutaneous absorption. The claimed effect of pain relief can also be provoked by the increased percutaneous absorption (passive diffusion) of dexamethasone and is therefore no proof for the potency of current.

A recent systematic review [30] concerning treatment options for tendinopathy (k=177) reported about conflicting results. It was concluded that there is little evidence to support the use of most physical modalities including the use of iontophoresis with corticosteroid or NSAIDs.

Controversial results are reported on the effectuality of dexamethasone iontophoresis in the treatment of epicondylalgia. The Pedro-scores of the studies reporting positive effects range between 3 and 7, while studies who found no effect of iontophoresis on the reduction of pain, had a Pedro-score between 3 and 6.

Desmirtas et al. [8] compared the effectiveness of iontophoresis with two different medical compounds, salicylate versus diclofenac in the treatment of lateral epicondylitis. Without the comparison of a control group testing the effect of passive diffusion and occlusion, it is impossible to draw conclusions on the transdermal enhancement effect of current and the potency of iontophoresis as a treatment on itself.

The review study from Andres and Murrell [30] already concluded that high powered studies to determine effective treatment strategies on the treatment of tendinopathies are needed.

Reviewing the treatment of lateral epicondylitis, Johnson et al. [31] reported ample evidence that iontophoresis with NSAIDs may be effective in reducing pain and that there is insufficient evidence supporting the use of corticosteroid iontophoresis. Also the systematic review (k = 28) of Bisset et al. [32] concerning the effectiveness of physical interventions for lateral epicondylalgia, reported about contradictions in results and heterogeneity of the interventions making it difficult to draw conclusion.

No consensus on the potency of iontophoresis with acetic acid in treatment of calcifying disorders was found. Leduc et al. [3] found no clinical or radiological differences between physiotherapy treatment with and without acetic acid iontophoresis in the treatment of calcifying tendinitis of the shoulder. Studies claiming to have a positive effect on size and density of the calcification [25,26] combined the use of iontophoresis with ultrasound therapy or other therapy modalities.

A number of studies used multiple treatments or evaluated the effects of iontophoresis on multiple diagnoses [7,12,15,18,19,22,24], making it impossible to make an interpretation on the outcome of iontophoresis as a treatment on its own.

Osborn and Allison, [24] evaluated if iontophoresis of acetic acid and dexamethasone combined with low-Dye taping was effective in reducing the symptoms of plantar fasciitis.

Fig. (5). Forest plot of 2 studies comparing the effect of iontophoretic treatment on pain versus control or (sham) placebo intervention in the treatment of rheumatic disease. The analysis reports Hedges’s g transformed post intervention pain values on x-axis.
The efficacy of iontophoresis in this study remains unclear, as low-Dye taping itself is reported to be effective for the short-term treatment of the common symptom of 'first-step' pain in patients with plantar heel pain [33].

In the treatment of pain, there is evidence for the iontophoretic administration of corticosteroids and NSAID’s providing an alternative to injection and oral therapy [14,16,34]. The non invasive and safe method, the low incidence of side effects as well as the well tolerated therapy are the advantages of current assisted transdermal delivery [7,13].

The outcome of the ten trials selected for the present meta-analyses on the effect of iontophoresis on pain compared to control or (sham) placebo treatment, revealed a larger effect size in favour of the iontophoretic treatment. Nevertheless pain reduction after iontophoretic treatment compared to placebo iontophoresis or iontophoresis with different substances without a study arm testing passive diffusion should be interpreted carefully.

Analgesic therapy effects can falsly be attributed to assisted penetration with an active substance whereas in fact it is the result of the electric current or ultrasound itself or the passive diffusion of the active substance. An analgesic effect of the current was revealed by Saggini et al. [7] who found an immediate analgesic activity of placebo iontophoresis.

There is insufficient evidence that iontophoresis with corticosteroids is effective in the treatment of musculoskeletal disorders [17,30,35,36]. Furthermore, most of the in vivo studies have methodological weaknesses (PEDRo-scale ranging between 3 and 10). Studies reporting positive effects from iontophoresis treatment often involved small sample sizes, using iontophoresis in combination with other treatment modalities [14,15,18,22,24,27]. Actually, the support for the use of iontophoresis is the weakest in well designed studies, using iontophoresis as a single treatment [3,11,13].

The BlueCross BlueShield Association Technology Evaluation Center (TEC) assessment of iontophoresis for medical indications (BCBSA, 2003), concluded that iontophoretic administration of NSAIDs, or corticosteroids for musculoskeletal inflammatory disorders not consistently found better outcomes from corticosteroids delivered by iontophoresis compared to placebo iontophoresis. They found no randomized controlled clinical studies comparing the iontophoretic delivery of NSAIDs and corticosteroids by other ways of transdermal delivery. To explore the validity of iontophoresis, it should be questioned whether the effects of iontophoresis exceed placebo effects and how iontophoretic drug delivery compares with alternative drug administration (e.g., topical, oral, injection) [37].

Jewell et al. [38], examined the short-term improvement in patients with adhesive capsulitis of the shoulder by physical therapy interventions. Data from 2,370 patients who received outpatient physical therapy were evaluated. A logistic regression model was used to identify intervention categories that predicted a 50% or greater change in Physical Component Summary-12 (PCS-12) evaluating physical function, bodily pain, and hybrid function scores. The results of this study demonstrated the effectiveness of joint mobilisation and exercise therapy, while the use of iontophoresis could not be supported. The use of iontophoresis, sonophoresis, massage or ultrasound reduced the likelihood of improvement by 19% to 32%.

Murphy, [39] evaluated in his review study the use of different physical therapy modalities in the management of temporomandibular disorders (TMD): trigger point injections, high voltage electrogalvanic stimulation, TENS, iontophoresis, ultrasound and manipulative therapy. Murphy (1999) concluded that the use of iontophoresis to deliver local anesthetics agents was valuable to maintain medication concentrations in the temporomandibular joint in the treatment of TMD.

CONCLUSION

The calculated summary estimate for the 10 studies in the present meta-analysis showed a significant effect of iontophoresis compared to sham (placebo) iontophoresis.

The use of iontophoresis, to enhance topical drug delivery through the skin, provides advantages of improved efficacy with reduced side effects compared to oral or parenteral administration [7,13,14,16,34].

However, the number of studies describing the effect of iontophoresis in realistic (physiotherapeutic) conditions is rather limited. A number of studies used multiple treatments, and as a consequence it is impossible to discriminate between the different factors influencing the percutaneous uptake. It can be stated that in most human in vivo studies, the basic principles of percutaneous penetration are not always taken into account. The effectiveness of iontophoretic delivery of an active substance is usually compared with placebo iontophoresis without an active substance. Since passive penetration can be substantial during delivery it is important to control this. Most studies on the effectiveness of iontophoresis did not use objective outcome measures or control groups. Another limitation of this systematic review may have been the use of summary scores (such as the Pedro rating scale) to identify trials of high quality [40]. The authors are well aware that the use of a more research-specific item based tool (such as GRADE) [41] including relevant methodological aspects a priori to assess the quality of the studies may have influenced the overall effect estimate. To date most physiotherapy-related reviews, however, are based on the Pedro rating scale for quality assessment of the included studies. To avoid inclusion bias, the authors of the present review and meta-analysis additionally assessed the quality of the studies using the Oxford Centre for Evidence-based Medicine Levels of Evidence but the lack of well-performed and adequately sized trials cannot be remedied by meta-analyses of small trials of questionable quality. Therefore, future research in this context should emphasize on standardisation of the treatment methods, the outcome measures and the inclusion of several controls.
### Annex 1. Synopsis of Studies on Iontophoresis

<table>
<thead>
<tr>
<th>Study / Country</th>
<th>Study Design</th>
<th>Publication Year</th>
<th>Disorders of Interest</th>
<th>Sample Size (n)</th>
<th>Experimental vs. Control</th>
<th>Outcome Measures</th>
<th>Main Results</th>
<th>Pedro score</th>
<th>Level of Evidence</th>
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</thead>
<tbody>
<tr>
<td>Hendricks et al. NED</td>
<td>RCT</td>
<td>1992</td>
<td>Tendonitis</td>
<td>n = 22 exp. n = 14 contr.</td>
<td>dexamethasone iontophoresis vs. Placebo treatment</td>
<td>VAS - score, ADL-scale</td>
<td>Sign. % reduction of median VAS-score after 2 weeks: exp. VAS -69 % (-41 - -89) / contr. VAS -4% (-10 - 1) (p &lt; 0.05)</td>
<td>10/10</td>
<td>1b</td>
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<tr>
<td>Demirtas and Oner TUR</td>
<td>RCT</td>
<td>1998</td>
<td>Epicondylitis</td>
<td>n = 40 exp. n = 20 contr.</td>
<td>Sodium di-</td>
<td>Pain; by pressure, by resisting wrist extension, during function and at rest</td>
<td>Reduction in pain by resisting wrist extension (p &lt; 0.01) and pain by pressure on lat. epicondyle (p &lt; 0.05) was statistically more significant in the group treated with diclofenac</td>
<td>3/10</td>
<td>2b</td>
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<tr>
<td>Neeter et al. SWE</td>
<td>RCT</td>
<td>2003</td>
<td>Achilles tendon pain</td>
<td>n = 25 exp. n = 14 contr.</td>
<td>Dexamethasone iontophoresis vs. with placebo iontophoresis</td>
<td>Toe -raise test, Range of motion test, Pain</td>
<td>After 6 weeks, exp. group had significant reduction (p &lt; 0.05) of pain during activity and during walking</td>
<td>8/10</td>
<td>1b</td>
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<tr>
<td>Runseson and Haker SWE</td>
<td>double blinded pros. random. Study</td>
<td>2002</td>
<td>Epicondylalgia</td>
<td>n = 64 exp. n = 33 contr.</td>
<td>Dexamethasone iontophoresis vs. placebo (saline) iontophoresis</td>
<td>Pain; by palpation, by resisted wrist extension, by dig. III test, by vigorimeter test, threshold when gripping (KPa), threshold when gripping (VAS)</td>
<td>No sign. differences (p &lt; 0.05) between both treatment groups, both groups improved throughout the study. Posttreatment median values pain threshold when gripping higher for placebo group (VAS) Exp. 9 vs. contr. 9 (p = 0.04)</td>
<td>6/10</td>
<td>2b</td>
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<tr>
<td>Nirschl et al. USA</td>
<td>RCT</td>
<td>2003</td>
<td>Epicondylitis</td>
<td>n = 199 exp. n = 99 contr.</td>
<td>Dexamethasone iontophoresis vs. placebo (saline) iontophoresis</td>
<td>Efficacy based on comparison of baseline and 2-day follow-up after treatment: patient pain evaluation (VAS) Investigator's pain evaluation (VAS)</td>
<td>Patient's* and investigator's pain evaluation, VAS Mean improvement (in mm) Exp. 23* vs. Contr. 14* (p = 0.012), Exp. 27 vs. Contr. 19 (p = 0.019)</td>
<td>7/10</td>
<td>1b</td>
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<td>Başkurt et al. TUR</td>
<td>RCT</td>
<td>2003</td>
<td>Epicondylitis</td>
<td>n = 61 exp. n = 32 ionto. n = 29 phono.</td>
<td>Naproxen iontophoresis vs. Naproxen iontophoresis</td>
<td>VAS-score, Grip-strength, functional status (Nirschl-Petterone Score)</td>
<td>Sign. decrease in VAS-score and sign. increased grip strength and Nirschl-Petterone Grading in both groups after treatment, but no sign. differences between groups before or after treatment (p &gt; 0.05)</td>
<td>3/10</td>
<td>2b</td>
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<td>Taskaynatan et al. TUR</td>
<td>RCT</td>
<td>2007</td>
<td>Bicipital tendinitis</td>
<td>n = 47 exp. n = 26 contr.</td>
<td>Hydrocortisone acetate iontophoresis vs. Interferential current</td>
<td>Pain, function, ROM and strength Constant's Shoulder Scale (CSS), patient's report of pain, function and satisfaction Pennsylvania Shoulder Scale (PSS)</td>
<td>Pennsylvania total pain before treatment / final assessment, ionto.* / curr. (mean ± SD): 13.2* ± 4.2 / 17.3* ± 1 (p = 0.009) / curr. 13.2 ± 4.1 / 14.6 ± 2.6 (p = 0.027)</td>
<td>7/10</td>
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<td>Study / Country</td>
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<td>Perron and Malouin CAN</td>
<td>RCT</td>
<td>1997</td>
<td>calcifying tendinitis</td>
<td>n = 22 exp. n = 11 contr.</td>
<td>acetic acid iontophoresis and ultrasound vs. no treatment</td>
<td>radiological evaluations, ROM, Pain (Present Pain Index Scale)</td>
<td>The mean percent reduction over time (post-pre) in the area of the calcium deposit was 20% (± 29 %) and 36% (±43 %), respectively, for the exp. and contr. group.</td>
<td>6/10</td>
<td>2b</td>
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<tr>
<td>Leduc et al. CAN</td>
<td>RCT</td>
<td>2003</td>
<td>calcifying tendinitis shoulder</td>
<td>n = 27 exp. n = 17 contr.</td>
<td>acetic acid iontophoresis vs. sham acetic acid iontophoresis</td>
<td>Shoulder Pain Disability index (SPADI), ROM shoulder and radiologic evaluation of shoulder calcifications</td>
<td>SPADI score, ROM and number and size of calcifications improved significantly in both groups no sign. differences between the exp. and contr. Group. SPADI Score before and after treatment exp. Group* / contr. Group (mean ± SD); 38* ± 19 / 23* ± 15 / 45 ± 17 / 40 ± 17</td>
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<td>Shetty et al. GBR</td>
<td>pros. pilot study</td>
<td>2005</td>
<td>calcinosis</td>
<td>n = 3 n = 3 exp. n = 0 contr.</td>
<td>acetic acid iontophoresis combined with ultrasound therapy</td>
<td>degree of radiographic calcinosis</td>
<td>The mean (SD) grey-scale intensity values for patients 1, 2, and 3 were 9.3 (± 3.6), 27.3 (± 0.8) and 85.6 (± 2.6) respectively pre-treatment and 7.6 (±0.3), 24.9 (±0.8) and 78.3 (± 0.4) respectively post-treatment</td>
<td>N/A</td>
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<td>Gudeman et al. USA</td>
<td>RCT</td>
<td>1997</td>
<td>plantar fascitis symptoms</td>
<td>n = 36 (40 feet) n = 20 exp. n = 20 contr.</td>
<td>dexamethasone iontophoresis vs. placebo (saline) iontophoresis</td>
<td>Maryland Foot Score (MFS)</td>
<td>There was a greater, immediately improvement in the MFS after 6 treatments with dexamethasone, (mean ± SD) (Exp. +6.8 ± 5.6 / Contr. +3.1 ± 4.1) but no difference after the 1-month follow-up</td>
<td>6/10</td>
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<td>Hammer et al. GER</td>
<td>pros. clinical study</td>
<td>2003</td>
<td>chronic plantar fascitis</td>
<td>n = 47 (feet 49) n = 24 exp. n = 23 exp.</td>
<td>extracorporeal shock wave therapy vs. diclofenac iontophoresis and oral NSAID</td>
<td>Pain VAS, comfortable walking time</td>
<td>No sign. treatment between the both treatment modalities. Both groups improved on all the assessed parameter. Pain at rest VAS (mean ± SD) before* and after treatment ESWT 34.0° ± 27.1 / 12.0 ± 25.9, into 43.1° ± 26.9 / 5.0 ± 20.4</td>
<td>5/10</td>
<td>2b</td>
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<tr>
<td>Osborne and Allison AUS</td>
<td>RCT</td>
<td>2006</td>
<td>plantar fascitis symptoms</td>
<td>n = 31 (42 feet) n = 21 exp. n = 10 contr.</td>
<td>dexamethasone or acetic acid iontophoresis vs. Placebo iontophoresis (NaCl)</td>
<td>pain (VAS), Stiffness (VAS)</td>
<td>Taping combined with acetic acid iontophoresis is the preferred treatment option compared with taping combined with dexamethasone or saline iontophoresis</td>
<td>8/10</td>
<td>1b</td>
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<tr>
<td>Gökoğlu et al. TUR</td>
<td>RCT</td>
<td>2005</td>
<td>mild carpal tunnel symptoms</td>
<td>n = 30 (48 nerves) n = 15 exp. n = 15 contr.</td>
<td>local methyl-prednisolone acetic acid injection vs. dexamethasone iontophoresis</td>
<td>Symptom Severity Scale (SSS), Functional Status Scale (FSS), Pain (VAS).</td>
<td>Injection therapy results in significantly better outcomes; however, the application of steroid by iontophoresis is a safer method and has no complications or side effects</td>
<td>5/10</td>
<td>1b</td>
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<tr>
<td>Study / Country</td>
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<td>Amirjani et al. CAN</td>
<td>RCT</td>
<td>2009</td>
<td>carpal tunnel symptoms</td>
<td>n = 17, n = 9 exp. n = 8 contr.</td>
<td>dexamethasone iontophoresis vs. distilled water iontophoresis</td>
<td>Levine Self-Assessment Questionnaire, nerve conduction test and the Semmes-Weinstein Monofilaments test</td>
<td>No sign. differences between the two groups: subjective severity score at baseline (median) 38 in exp. and 36 in the placebo group, after treatment 26 in the exp. and 31 in the placebo group</td>
<td>10/10</td>
<td>1b</td>
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<tr>
<td>Dakowicz and Latos-</td>
<td>CT</td>
<td>2005</td>
<td>carpal tunnel symptoms</td>
<td>n = 40, Gr.I n = 12, Gr.II n = 24, Gr.III n = 4</td>
<td>hydrocortisone iontophoresis combined with ultrasound therapy</td>
<td>subjective complaints before and after treatment: pain VAS, Phalen test, Tunnel Test</td>
<td>Sign. decrease ($p &lt; 0.05$) in VAS-score (mean ± SD) before* and after treatment in Gr.I (7.4* ± 0.5 / 1.8 ± 1.9) and Gr.II (8.1* ± 1.2 / 1.8 ± 1.5)</td>
<td>5/10</td>
<td>2b</td>
</tr>
<tr>
<td>Banta USA</td>
<td>pros. CT</td>
<td>1994</td>
<td>carpal tunnel syndrome</td>
<td>n = 18 (23 hands) n = 11 hands exp. n = 4 hands contr.</td>
<td>wrist splinting and nonsteroidal anti-inflammatory medications and additional dexamethasone / lidocaine iontophoresis</td>
<td>symptoms of early mild carpal tunnel syndrome</td>
<td>11 of the 19 hands treated with iontophoresis had a positive response. Iontophrosis may become an alternative to steroid injection to the carpal tunnel region</td>
<td>N/A</td>
<td>4</td>
</tr>
<tr>
<td>Schiffman et al. USA</td>
<td>RCT</td>
<td>1996</td>
<td>temporomandibular disorders</td>
<td>n = 27, n = 9 exp. n = 9 contr. n = 9 placebo</td>
<td>dexamethasone iontophoresis and lidocaine hydrochloride vs. Contr. lidocaine hydrochloride vs. placebo (saline)</td>
<td>Helkimo's clinical Dysfunction index (DI), Symptom Severity Index (SSI) and CranioMANDIBular Index (CMI)</td>
<td>No statistical change ($p &gt; 0.05$) in total SSI from pretreatment to post treatment (mean post treatment values ± SD) ionto. 0.47 (0.2) / contr. 0.40 (0.1) / placebo 0.50 (0.2)</td>
<td>8/10</td>
<td>1b</td>
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<tr>
<td>Aiyegusunle et al.</td>
<td>CT</td>
<td>2007</td>
<td>osteoarthritis knee</td>
<td>n = 20, n = 10 exp. n = 10 contr.</td>
<td>sodium salicylate iontophoresis vs. TENS</td>
<td>Pain (VAS), functional disability (D.I)</td>
<td>Significant reduction in pain and functional disability in both groups: VAS (mean ± SD) TENS group pre/post-treatment 7.3 ± 0.76 / 6.0 ± 0.47 ($p = 0.01$) ionto. group pre/post treatment 7.2 ± 0.63 / 4.0 ± 0.94 ($p &lt; 0.01$)</td>
<td>N/A</td>
<td>3b</td>
</tr>
<tr>
<td>Akinbo et al. NGA</td>
<td>pros.</td>
<td>2007</td>
<td>osteoarthritis knee</td>
<td>n = 50, n = 25 exp. n = 25 contr.</td>
<td>Dexamethasone phenoxipheosis vs. dexamethasone iontophoresis</td>
<td>WOMAC-score, 20 meter ambulatory time and ROM knee</td>
<td>No sign. differences ($p &lt; 0.05$) between both treatment groups; pain (likert scale, Mean ± SD) ionto / phono 2.4 ± 1.9 / 2.9 ± 2.3 ($p = 0.350$), Stiffness ionto / phono 1.0 ± 1.4 / 1.1 ± 1.5 ($p = 0.215$)</td>
<td>5/10</td>
<td>2b</td>
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<tr>
<td>Saggini et al. ITA</td>
<td>RCT</td>
<td>1996</td>
<td>rheumatic pain</td>
<td>n = 60, n = 30 exp. n = 39 contr.</td>
<td>ketorolac iontophoetic vs placebo iontophoresis (saline)</td>
<td>pain (VAS), efficacy of treatment</td>
<td>No sign. difference in pain after treatment between the exp. and placebo group, (VAS mean ± SD) 4.22 ± 2.51 / 3.88 ± 2.12 ($p &lt; 0.05$). Pain increase the placebo group (not sign.) 7 days after end of treatment 4.12 ± 2.45</td>
<td>6/10</td>
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</table>
The Effects of Iontophoresis in the Treatment of Musculoskeletal Disorders


4. RESULTS

<table>
<thead>
<tr>
<th>Study / Country</th>
<th>Study Design</th>
<th>Publication Year</th>
<th>Disorders of Interest</th>
<th>Sample Size (n)</th>
<th>Experimental vs. Control</th>
<th>Outcome Measures</th>
<th>Main Results</th>
<th>Pedro score</th>
<th>Level of Evidence</th>
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</thead>
<tbody>
<tr>
<td>Li et al. HKG</td>
<td>RCT (pilot)</td>
<td>1996</td>
<td>rheumatoid arthritic knee</td>
<td>n = 10, n = 5 exp., n = 5 contr.</td>
<td>dexamethasone iontophoresis vs. placebo (sodium chloride) iontophoresis</td>
<td>pain on movement, at rest, pressure-pain threshold, active joint count (AROM), patient's global assessment (PGA)</td>
<td>Sign. difference over time (day 1 / day 20) only for pain on movement within the exp. Group, (VAS mean rank) 3.00 / 1.60 ($p = 0.0224$)</td>
<td>N/A</td>
<td>4</td>
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<tr>
<td>Vranken et al. NED</td>
<td>RCT</td>
<td>2005</td>
<td>neuropathic pain</td>
<td>n = 33, n = 22 exp., n = 11 contr.</td>
<td>ketamine iontophoresis 50 and 75 mg $S^+$ vs placebo iontophoresis saline</td>
<td>pain (VAS), Health status and quality of life (QQL), Pain Disability Index (PDI)</td>
<td>Iontophoretic administration of $S^+$ ketamine is not more effective than placebo treatment in reducing pain. However compared to placebo, ionotophoretic delivery of 75 mg $S^+$-ketamine improved the (QQL) sign. ($p &lt; 0.025$)</td>
<td>10/10</td>
<td>1b</td>
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<tr>
<td>Bremerich and Wiegel GER</td>
<td>pros. CT</td>
<td>1992</td>
<td>facial pain</td>
<td>n = 46, n = 46 exp., n = 46 contr.</td>
<td>TENS and the combined use of TENS and benzydamine and diclofenac iontophoresis</td>
<td>Pain (VAS)</td>
<td>81% pain relief with the combined therapy of TENS and iontophoresis to 63% by TENS only</td>
<td>N/A</td>
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</table>

Annex 2. Overview of the Drugs Used for Iontophoresis

<table>
<thead>
<tr>
<th>Author</th>
<th>Diclofenac</th>
<th>Acetic Acid</th>
<th>Salicylate</th>
<th>Ketorolac</th>
<th>Benzydamine</th>
<th>Lidocaine</th>
<th>Ketamine</th>
<th>Naproxen</th>
<th>Dexamethasone</th>
<th>Hydrocortisone</th>
<th>Corticosteroids</th>
<th>NSAID</th>
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CONFLICT OF INTEREST

The author(s) confirm that this article content has no conflicts of interest.

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REFERENCES


The Effects of Iontophoresis in the Treatment of Musculoskeletal Disorders


4. RESULTS

The skin as a barrier in fysiotherapy

DETERMINATION OF THE IN VIVO BIOAVAILABILITY OF IONTOPHORETICALLY DELIVERED DICLOFENAC USING AMETHYL NICOTINATE SKIN INFLAMMATION ASSAY

Renzo Lambrecht1, Peter Clarys1, Ron Clijsen2 and Andre´ O. Barel1
Determination of the in vivo bioavailability of iontophoretically delivered diclofenac using a methyl nicotinate skin inflammation assay

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Background/aims: In this study, we investigated the bioavailability of iontophoretically delivered diclofenac with the methylnicotinate (MN) test. The inhibition of an erythema provoked by MN is proportional to the bioavailability of diclofenac in the skin. It was our aim to use this procedure in the determination of the contribution of, respectively, passive diffusion, occlusion and electrically assisted delivery during an iontophoretic procedure as used in physiotherapy.

Method: A total of six application sites were marked on the volar forearms of each volunteer (n = 12), for the following treatment and/or control modes: A = cathodal iontophoresis of 12 mg/cm² Voltaren Emulgel (diethylammonii diclofenac 1%) for 20 min; B = passive diffusion under a contact sponge; C = passive diffusion without any covering; D = current alone; E = standard MN response; and F = blanco site. Tristimulus surface colorimetry and Laser Doppler flowmetry were used to measure, respectively, the skin color and the perfusion of the microcirculation. Bioavailability was assessed by quantification of an MN-induced erythema under the different conditions.

Results: A significant reduction of the MN-induced erythema was observed with the Chromameter and Laser Doppler measurements for the following treatment modalities: (1) electrically assisted delivery: respectively, 65% and 100%, (2) application under a contact sponge: 66% and 97% and (3) passive diffusion without any covering: 32% and 65%. A significant reduction was equally observed for the site treated with the current alone: 19% and 42%. There was no significant difference between the response after iontophoretic-delivered diclofenac (mode A) and application of diclofenac under a contact sponge (mode B).

Conclusion: The procedure used enabled us to evaluate the bioavailability of a non-steroidal anti-inflammatory drug in the skin. Under the conditions used, we did not find an increased bioavailability after electrically assisted delivery of diclofenac compared with the passive percutaneous penetration under the contact sponge.

Key words: iontophoresis – methylnicotinate – occlusion – passive diffusion

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In physiotherapy, iontophoresis is used to enhance the penetration of drugs in the topical treatment of muscles, tendons and joints. In practice, the drug is applied under a wet cellulose sponge, which covers the electrode. The current intensity and treatment time used are rather limited: up to maximum 0.5 mA/cm² for 20 min.

When screening the literature on iontophoretic delivery as used in physiotherapy, we observed that in most human in vivo studies, some basic principles of percutaneous penetration were not always taken into account (1–5). Indeed, iontophoretic delivery is seldom evaluated vs. passive diffusion. Iontophoretic delivery is usually compared with a placebo without an active ingredient. As the passive penetration can be substantial during the delivery, it is important to include this control to estimate the enhancement factor.

The methylnicotinate (MN) assay in vivo has already been proposed to assess the bioavailability of corticosteroids and non-steroidal anti-inflammatory drugs (NSAIDs) (6, 7). It has been demonstrated that the reduction of the MN-induced erythema is related to the NSAIDs concentration in the skin and the potency of the drug (6, 8). It was also possible to discriminate between different formulations (9). This method can be useful for predicting the clinical performance of a
formulation or to test bioequivalence in the development of a generic product (7). The MN response can easily be determined with colorimetric evaluation of the skin and determination of the perfusion of the microcirculation (10).

In this experiment, we tried to estimate the contribution of the passive percutaneous penetration during electrically assisted delivery of diclofenac.

Therefore, we used the method as proposed by Duteil et al. (6) and Treffel and Gabard (7) to estimate the bioavailability of diethylammonium diclofenac (Voltaren Emulgel*) in the skin after iontophoretic delivery vs. several controls on the same subject.

Hence, the contribution of different factors such as passive diffusion, semi-occlusion and electrical assistance can be estimated. Therefore, the results obtained may better reflect the real contribution of the iontophoretic stimulation.

**Method**

Twelve volunteers (six males and six females, mean age = 22.3 ± 3.4 years) participated in this study. A total of six application sites (from A to F, surface 7 cm²) were marked on both volar forearms.

An acclimatization period of 30 min prior to the measurements was followed. Temperature (20 ± 2 °C) and relative humidity (45 ± 5%) were kept constant during the experiment.

Skin color was evaluated with the Minolta Chromameter-CR200 (Minolta Camera Benelux BV, Kontich, Belgium). The $a^*$ parameter, expressed in arbitrary units, which typically measures the redness of the skin, is a good indicator of skin erythema. In addition, local blood flow, expressed in dimensionless perfusion units, was evaluated using Laser Doppler flowmetry (Laser Doppler, Järfalla, Sweden) (Periflux PF3).

**Experiment part 1: diclofenac application**

After baseline measurements, the following applications were performed:

- **Spot A (iontophoretic delivery):** application of 12 mg/cm² diethylammonium diclofenac 1% (Voltaren Emulgel, Novartis, Novartis Consumer Health, Brussels, Belgium), iontophoretically stimulated by a direct current: 0.2 mA/cm² at the cathode, for 20 min. Current was generated with Sonopuls 992 (Enraf Nonius NV, Aartselaar, Belgium) using a pen electrode covered with a wet circular cellulose sponge of a 7 cm² surface. The anodal sponge of 42 cm² was placed perpendicularly under the application site at the counterpart of the forearm (Fig. 1). The sponges and electrode were obtained from Gymna (Bilzen, Belgium).

- **Spot B (passive diffusion under contact sponge):** application of 12 mg/cm² diethylammonium diclofenac 1%, covered with a wet circular cellulose contact sponge on the same surface of 7 cm² for 20 min.

- **Spot C (passive diffusion without any covering):** application of 12 mg/cm² diethylammonium diclofenac 1% for 20 min.

- **Spot D (current effect on barrier):** direct current: 0.2 mA/cm², cathode, for 20 min.

- **Spot E (standard MN response):** no treatment with diclofenac.

- **Spot F: (blanco):** no treatment.

Sponges were saturated with distilled water before application.

A randomization schedule was followed in order to exclude regional effects.

After the 20 min treatment period, all skin sites were cleansed with distilled water using a wet soft tissue.

**Experiment part 2: MN application**

Because the current application induced a skin erythema, a resting period of 90 min was allowed after current application. At that time, a nicotine test was performed using paper filter disks (18 mm, Epitest Ltd Oy, Tuusula, Finland) saturated with MN solution (0.005 M) and kept 212 Lambrecht et al. Belgium)
for 30 s on all skin sites, except the blanco site (spot F). Bioengineering measurements were carried out every 5 min post-MN application for 65 min.

**Statistical analysis**

All data were tested on normality using the Kolmogorov–Smirnov goodness-of-fit test.

Kinetics were compared using the MANOVA procedure, while areas under the response curve were compared using an ANOVA test followed by Scheffé test.

Inhibition, corrected for blanco response, was expressed in percentage of the standard MN inflammation assay. Significance level was set at 5%.

**Results**

During iontophoresis, a mild prickling sensation was experienced on the skin at the cathode side when current was turned on but the sensation diminished during the treatment. A mild erythema was visible after iontophoretic stimulation but this was no longer detectable with color or microcirculation evaluation 90 min after current cessation.

After a resting period of 90 min, the MN test was performed.

For the colorimetric measurements, we observed a significant inhibition of the MN response on the three skin sites that were pre-treated with diclofenac. The inhibition after iontophoretic delivery was significant compared with the standard MN protocol (A vs. E, \( P = 0.001 \)), with delivery under a contact sponge (B vs. E, \( P = 0.001 \)) and with passive diffusion alone (C vs. E, \( P = 0.04 \)) (Fig. 2). The response after iontophoretic delivery did not differ from the response under contact sponge occlusion (A vs. B, \( P = 0.13 \)). Also, after placebo iontophoresis, the erythema was inhibited compared with the standard MN-induced erythema (D vs. E, \( P = 0.04 \)).

With the data presented as areas under the curve, we calculated the percentage of inhibition compared with the standard MN response (Fig. 3). Total inhibition was 65% and 66% for iontophoretic and passive penetration under the contact sponge, respectively, while passive diffusion without any occlusion resulted in a 32% inhibition compared with the standard MN response.

Inhibition, corrected for blanco response, was expressed in percentage of the standard MN inflammation assay. Significance level was set at 5%.

The iontophoretically delivered diclofenac compared with delivery under the contact sponge (\( P = 0.79 \)). After placebo iontophoresis we observed a significant inhibition of the MN-induced redness (19%) compared with the standard MN response.

For the perfusion of the microcirculation (Laser Doppler), we observed similar results (Fig. 4). Inhibition of the MN response was significant for all diclofenac pre-treated sites: inhibition after iontophoresis (A vs. E, \( P = 0.0001 \)), passive penetration under a contact sponge (B vs. E, \( P = 0.0001 \)) and passive diffusion without any covering (C vs. E, \( P = 0.002 \)).

Again, there was no significant difference between the response obtained after iontophoretic delivery and passive penetration under the contact sponge (A vs. B, \( P = 0.440 \)).

Presenting the data, corrected for blanco va-
that the inhibition of the perfusion of the microcirculation was complete after iontophoretic delivery (100%) and did not differ significantly from the response after passive penetration under the contact sponge, which resulted in a 97% reduction of the erythema response ($P < 0.13$). The perfusion of the microcirculation after passive penetration without covering was inhibited (65%) compared with the standard MN response. Again, after placebo iontophoresis, we observed, using the Laser Doppler, a significant inhibition of the MN-induced response (42%) compared with the standard MN response.

**Discussion**

The anti-inflammatory properties of diclofenac cyclooxygenase (COX), also called prostaglandin endoperoxide H synthetase. Our interest lies in COX-2 because this enzyme is responsible for the formation of inflammatory prostaglandins. It has already been demonstrated that diclofenac inhibits this COX-2, resulting in a reduction of the inflammation (11). We used this property to evaluate the tissue bio-availability of diclofenac, which is proportional to the inhibition of an MN-induced erythema. It can be assumed that the reduction is a function of the amount of NSAIDs available at the site of inflammation. It has already been demonstrated that there is a good correlation between the *in vitro* uptake of NSAID in the skin and the *in vivo* inhibition of the provoked inflammation (12).

The iontophoretically delivered diclofenac was compared with the passive diffusion when the application site was left uncovered or occluded with the contact sponge electrode. This allowed us to compare the iontophoretic delivery with the passive diffusion and to estimate the occlusive effects.

For the Laser Doppler measurements, we observed a complete inhibition of the signal when the test sites were pre-treated with diclofenac. It seems that there is a strong inhibition of the microcirculation at a measuring depth of 1 mm. The Laser Doppler data represent the result from the blood flow in the superficial dermal plexus and capillary loops, and do not reflect the blood flow in the deeper regions.

The results therefore differ from the color evaluation. Treffel et al. (12) hypothesized that the redness measured by the Chromameter was mainly because of the blood increase in the capillary loops and in the arteriovenous shunts of the subpapillary plexus. Skin redness gives information from different depths and is therefore more appropriate for this type of measurements.

Diclofenac inhibits the induced inflammation redness, resulting in lower $a^*$ values obtained with the Chromameter. Hence, we may assume that lower values represent higher diclofenac concentrations in deeper tissue layers. The inhibition of the erythema was stronger after iontophoresis and passive diffusion under contact sponges compared with the passive diffusion alone, pointing to an enhanced delivery under iontophoretic and occlusive conditions. We observed no significant difference between the MN
compared with passive diffusion under the contact sponge. As a consequence, the enhanced delivery compared with the passive diffusion seems to be explained by an occlusive effect and not by a current-induced mechanism. We were not able to demonstrate any advantage of the current for a single application of diclofenac. The major factor influencing the penetration seems to be the occlusion. During iontophoresis, the treated area is covered with a water-humidified sponge electrode and this occlusion causes an increase in the hydration of the skin. It is well known that occlusion of the treated area improves the percutaneous absorption (13).

A current pre-treatment seems to have an effect on the passive penetration of MN. In a previous study, we demonstrated that a current pre-treatment influences the passive uptake of MN, resulting in shorter time-to-peak values and lag times (14). An increased passive uptake after a current pre-treatment was also observed in other studies (15, 16). The increased uptake may be explained by a significant decrease of the skin impedance, which persists several hours after the current removal (15, 17). Lee et al. (18) suggested that an increased hydration after a current treatment is responsible for the increased passive uptake. An increased hydration is responsible for a drop in resistance, explaining the increased permeability. With electron microscopy, they found changes in the structure of the stratum corneum intracellular lipids after iontophoresis. Moreover, they found similar phenomena at the control site, suggesting that the changes in the stratum corneum intercellular structure may be a result of hydration and occlusion and not a current-induced mechanism. This could explain the increased uptake of MN after current pre-treatment as observed in our study, but equally corroborate our findings of a similar inhibition after iontophoretically and passively delivered diclofenac under occlusion. Our results suggest that the electromotive forces may be overestimated during iontophoretic delivery in experimental designs where the passive uptake and occlusion effects are not evaluated.

Conclusion

Objective quantification of the bioavailability of NSAID can be performed with the MN assay. It has been used to discriminate between different NSAIDs, as well as to distinguish between different formulations. Moreover, it has the potentials to evaluate the bioavailability after iontophoretic delivery of NSAIDs. Colorimetric evaluation seemed to be appropriate for evaluating the inhibition, whereas evaluation of the microcirculation gives additional information that can support the colorimetric data. In our in vivo experiment, we demonstrated that for a single application, the presumed enhancing effect of a current is negligible compared with passive penetration under a contact sponge. In fact, the wet occlusion seemed to be the most important factor explaining the enhanced percutaneous penetration.

We recommend that in future experiments where iontophoresis is investigated, the inclusion of several controls needs to be considered, because the passive penetration and the effect of the occlusion or other manipulation effects may be the dominant factors compared with the current-induced delivery.

References


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4. RESULTS
4.4

IN VIVO DETERMINATION OF THE DICLOFENAC SKIN RESERVOIR: COMPARISON BETWEEN PASSIVE, OCCLUSIVE AND IONTOPHORETIC APPLICATION

R. Clijsen1,2,3, J.P. Baeyens 1,3, A.O. Barel1, P. Clarys 1
In vivo determination of the diclofenac skin reservoir: comparison between passive, occlusive and iontophoretic application

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Abstract

There is scarce information concerning the pharmacodynamic behavior of topical substances used in the physiotherapy setting. The aim of the present study was to estimate the formation and emptying of the diclofenac (DF) skin reservoir after passive, semi occlusive and electrically assisted applications of DF.

Five different groups of healthy volunteers (n_total= 60; 23 male and 37 female), participated in this study. A 1% DF (Voltaren Emulgel®, Novartis) formulation (12mg) was applied on the volar forearms on randomized defined circular skin areas of 7cm². DF was applied for 20 minutes under 3 different conditions at the same time. Presence of DF in the skin results in a reduction of the methyl nicotinate (MN) response. To estimate the bioavailability of DF in the skin, MN responses at different times following initial DF application (respectively 1.5, 6, 24, 32, 48, 72, 96, 120 hours) were analysed.

At 1.5 hours after the initial DF application a significant decrease in the MN response was detected for the occluded and iontophoresetical delivery. Passive application resulted in a decrease of the MN response from 6 hours post DF application. The inhibition remained up to 32 hours post DF application for the iontophoresetic delivery; 48 hours for the occluded application; and 72 for the passive delivery. At 96 and 120 hours post DF application none of MN responses were inhibited.
The formation and emptying of a DF skin reservoir was found to be dependent of the DF application mode. Penetration enhanced delivery resulted in a faster emptying of the reservoir.

**Keywords:** skin reservoir – diclofenac – passive diffusion – occlusion – iontophoresis

**Introduction**

Transdermal delivery of non steroidal anti-inflammatory drugs (NSAIDs) is a topical treatment routinely used in physiotherapy to reduce pain and inflammation in musculoskeletal disorders. In order to weaken the skin barrier and to achieve a clinical effective drug concentration in the target tissues, various transdermal enhancement techniques, such as sonophoresis, iontophoresis and occlusion are used. However little information is available concerning the efficacy of the treatment protocol and the penetration profiles of the substances used in the physiotherapy setting. Indeed, most clinical studies have complex treatment protocols and are unable to relate clinical outcome with drug penetration. A recent meta-analysis (2012) suggested a better clinical outcome for iontophoretically delivered substances. Despite, due to methodological flaws (e.g. combined therapies, lack of control for passive diffusion and occlusion) definite conclusions regarding the possible penetration enhancement of the electrical assisted delivery cannot be inferred.

The building up of a stratum corneum reservoir (SC) is an important component of the pharmacodynamics of topically applied substances. Rougier et al. (1985) used the reservoir effect of the SC after 30 minutes of application to predict the total amount absorbed. Moreover, the human SC has the capacity to store topically applied substances through a
The existence of a reservoir within the SC has been documented for several topically applied solutes such as corticosteroids, caffeine, nicotine, DF and chemical UV filters. Roberts et al. (2004) emphasised that reservoir properties can also be attributed to the viable epidermis and dermis. Specifically, DF binding capacities in dermal tissue have been reported.

Lambrecht et al. (2006) estimated the DF skin bioavailability by quantifying the reduction of a MN induced vasodilatation using chromametry and Laser-Doppler flowmetry. They detected a significant reduction of the MN response 90 minutes after different application modalities of DF. Open application resulted in a 32% reduction of the MN response, application under occlusion accounted for a reduction of 66%, whilst the iontophoretic application resulted in a 65% reduction. At that time, one could not discriminate between the occlusive and electrically assisted delivered DF. To obtain more insight in the pharmacodynamics and reservoir properties of DF, the aim of the present study was to evaluate the bioavailability in the skin of DF at longer time intervals after DF application using different application modalities. Therefore, three different forms of application (passive, occlusive, iontophoretic) were examined in order to detect the anti-inflammatory effect and thereby getting a better understanding of the skin-penetrating and reservoir-building behavior of DF in function of time. The MN responses were quantified by skin colorimetry at different moments of time (1.5, 6, 24, 32, 48, 72, 96, 120 hours) post initial DF application.

**Methods**

**Subjects**

Five different groups, (group 1, n=13 (1.5h.); group 2, n=12 (6h.); group 3, n=12 (24, 32h.); group 4, n =14; (48, 72h.) group 5, n=9; (96, 120h.) of healthy volunteers (n= 60, 23 male and
37 female), not treated with any drugs participated in this study. The volunteers were Caucasians and had healthy skin. Mean age of the subjects was 21.4 ± 4.1 years. Approval by the ethical committee of the Vrije Universiteit Brussel was obtained for this study. Before testing, all subjects were informed of the research protocol and signed an informed consent. During the duration of the experiments the volunteers were asked to maintain their daily activities but to abstain from swimming and extensive showering.

The measurements were performed under standardized temperature (20 ± 2 °C) and relative humidity (45 ± 5%) conditions. Before every DF application and each measurement session the volunteers participated in a 30 minutes acclimatization period. For each time interval tested, five randomized circular skin areas (7cm²) were demarcated on the subjects’ volar forearms, for the following treatment and/or control modes: (1) open application (without occlusion), (2) passive diffusion under a semi occlusive humid sponge, (3) iontophoretic application (4) standard MN response (5) untreated skin side. This means that either five skin areas for group 1 and 2 or ten skin areas for group 3, 4 and 5 (testing two time intervals) were randomized demarcated on both arms. This procedure enabled a single application of MN on every skin site at different time intervals avoiding the occurrence of tachyphylaxis.

<table>
<thead>
<tr>
<th>Group</th>
<th>Time interval</th>
<th>Number of subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.5 h</td>
<td>13</td>
</tr>
<tr>
<td>2</td>
<td>6h</td>
<td>12</td>
</tr>
<tr>
<td>3</td>
<td>24h; 32h</td>
<td>12</td>
</tr>
<tr>
<td>4</td>
<td>48h;78h</td>
<td>14</td>
</tr>
<tr>
<td>5</td>
<td>96h;120h</td>
<td>9</td>
</tr>
</tbody>
</table>

Table 1. Overview of the different groups, time intervals investigated and number of participants
Bioavailability test

DF skin bioavailability was assessed by quantifying the reduction of the MN response as proposed by Lambrecht et al. (2006)\(^7\). Topical application of MN provokes an increase of local cutaneous blood flow (erythema)\(^25\). When DF is present in the skin the nicotinate response is depressed in a concentration dependent way\(^26\). Treffel et al. (1993) demonstrated a good correlation between the in vivo inhibition of the MN induced inflammation and the vitro determination (chromatographic analysis) of NSAID concentration levels in the SC\(^27\). Lambrecht et al. (2006) evaluated the inhibition of the MN induced erythema with both Laser Doppler Flowmetry and the colorimetric method, which resulted in equivalent conclusions. Therefore it was optioned to use the Chromametric evaluation\(^28\). In the present study, the inhibition of the MN response with sum of the a* parameter (\(\Sigma a^*\)) was evaluated up to 50 minutes post MN application, making the protocol more feasible and bearable for the subjects.

**Instruments**

The skin surface colorimetric measurements were performed with the Minolta Chromameter CR 200 operating in the CIE L*a*b* color space (CIELAB). For the quantification of skin surface colour and erythema the a* parameter is especially useful as it represents the chromaticity between red/magenta and green (a*, negative values indicate green while positive values indicate magenta)\(^29\)\(^30\). Chromameter measurements were carried out before DF application; prior to MN application and every 5 minutes until 50 minutes post MN application for the different reservoir estimation time points. Since different groups of subjects were involved, an untreated skin area was included in order to be able to correct for blank values.
Materials

On application side (1), (2) and (3) a commercially available 1% DF (Voltaren Emulgel®, Novartis) formulation (12mg) was applied. The product was gently rubbed into the skin using a gloved finger. The MN test was applied using paper filter disks which were saturated in a 0.005M aqueous MN solution and applied on the circular demarcated skin areas for 30 seconds. After removal of the filter disk, excess solution was gently wiped away using a tissue paper.

Application modes

DF was applied for 20 minutes under three different conditions; (1) open application (without occlusion), (2) passive diffusion under a semi occlusive humid sponge, (3) an iontophoretic application (direct current, 0.2 mA/cm², cathode). To reproduce physiotherapeutical conditions, skin occlusion was performed using electrode sponges as applied in the clinical therapeutic setting. Two marked skin areas were not treated with DF, the application side for the standard MN-induced response and the blanco untreated skin side.

Reservoir estimation

Bioavailability of DF in the SC under the three conditions was assessed by quantification the MN induced erythema at 1.5, 6, 24, 48, 72, 96, and 120 hours post DF application.
Calculations and Statistics

To estimate the presence of DF in the skin, MN responses at the different time periods following initial DF application (1.5, 6, 24, 32, 48, 72, 96, 120 hours) were compared. The \((\Sigma a^*\)) corrected for blank values (untreated skin) up to 50 minutes post MN application was used as an indicator for the magnitude of the MN response. Normality was evaluated using the Kolmogorov-Smirnov Goodness of Fit Test. At the different time periods following initial DF application the skin response to the different application modalities was compared using an ANOVA procedure with Bonferroni correction for post-hoc tests. Statistical significance between any application mode and the standard MN response was used as an indication for the presence of DF in the skin at that particular time point following initial DF application. The significance level was set at 5%.

Results

Analysis of the skin colour (\(a^*\) parameter) after MN application for the different application modes (open application, occlusion, iontophoresis) and at the different reservoir estimation times (1.5, 6, 24, 32, 48, 72, 96, 120 hours) clearly shows changes in MN response as a function of the application mode and reservoir estimation time. Kinetics are represented for the reservoir estimation time 1.5 and 96 hours post DF application only (see figure 1 and 2). At 1.5 hours post DF application a clear inhibition is visible for the application under occlusion and under iontophoresis whilst the open DF application did not provoke an inhibition on the MN response (figure 1).
Fig. 1. $a^*$ Parameter changes over time 1.5 hours after the initial application of DF

(●) MN, (X) open, (●) occlusion, (▲) iontophoresis, (□) blank. Mean ± SD

At 96 hours post DF application, inhibition of the MN response is no longer perceived (figure 2).
Fig. 2. a* Parameter changes over time 96 hours after the initial application of DF

(♦) MN, (X) open, (●) occlusion, (▲) iontophoresis, (□) blank. Mean ± SD

Differences between application modes at the different reservoir estimation times were assessed using the (Σa*) values calculated from the different kinetics (table 1). At 1.5 hours after the initial DF application a significant decreased response was detected for the occluded and iontophoretically delivered DF only. From 6 hours up to 32 hours post DF application a significantly reduced MN response was detected at all DF pre-treated skin sites. At 48 hours post DF application only the passive and occluded applications resulted in a significant reduction. At 72 hours post DF application only the response after the passively delivered DF remained significant. From 96 hours on, no differences between the MN responses were detected (see table 1).
### Table 2. Comparison of the total responses (Σa*) (mean ± SD) corrected for blank over the total MN evaluation period)

<table>
<thead>
<tr>
<th>TIME AFTER INITIAL</th>
<th>DF APPLICATION</th>
<th>MN</th>
<th>OPEN</th>
<th>OCCLUSION</th>
<th>IONTO</th>
<th>ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.5 h. (n=13)</td>
<td></td>
<td>40.11 ± 20.4&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>34.08 ± 22.9</td>
<td>15.82 ± 11.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>17.35 ± 15.4&lt;sup&gt;c&lt;/sup&gt;</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>6 h. (n=12)</td>
<td></td>
<td>33.11 ± 19.5&lt;sup&gt;a,b,c&lt;/sup&gt;</td>
<td>13.4 ± 13.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>15.54 ± 12.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>16.37 ± 16.9&lt;sup&gt;c&lt;/sup&gt;</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>24 h. (n=12)</td>
<td></td>
<td>22.46 ± 13.5&lt;sup&gt;a,b,c&lt;/sup&gt;</td>
<td>8.85 ± 12.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>10.62 ± 11.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>14.06 ± 9.7&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.001</td>
</tr>
<tr>
<td>32 h.</td>
<td></td>
<td>26.81 ± 17.3&lt;sup&gt;a,b,c&lt;/sup&gt;</td>
<td>8.5 ± 10.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.39 ± 10.8&lt;sup&gt;b&lt;/sup&gt;</td>
<td>9.39 ± 15.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>48 h. (n=14)</td>
<td></td>
<td>34.54 ± 19.2&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>16.41 ± 17.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>26.23 ± 18.8&lt;sup&gt;b&lt;/sup&gt;</td>
<td>29.46 ± 17.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>72 h.</td>
<td></td>
<td>39.34 ± 20.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>20.43 ± 17.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>25.65 ± 11.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>25.36 ± 17.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>96 h. (n=9)</td>
<td></td>
<td>36.12 ± 18.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>35.57 ± 19.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>30.24 ± 13.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>34.65 ± 17.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>120 h.</td>
<td></td>
<td>30.03 ± 12.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>29.96 ± 17.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>28.63 ± 17.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>30.06 ± 19.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>&gt; 0.05</td>
</tr>
</tbody>
</table>

Table 2. Comparison of the total responses (Σa*) (mean ± SD) corrected for blank over the total MN evaluation period) for the reference MN test and the different DF applications at 1.5, 6, 24, 32, 48, 72, 96, and 120 hours after the initial DF application. Equal indices (a,b,c) at a particular reservoir estimate time, indicate a significant difference for the post hoc test (p < 0.05).

**Discussion**

The applied method, inhibition of a physiological reaction (MN) due to the presence of the inhibitor (DF) in the skin reservoir, enabled us to estimate penetration kinetics and the DF skin reservoir building and emptying properties of three different application conditions up to 120 hours post initial DF application. In contrast with other reservoir protocols an active substance liberating action such as occlusion or increased hydration was not required<sup>24</sup>. This may be a confirmation of the presence of DF in the viable tissue (dermal compound) since the MN induced vasodilatation is inhibited immediately<sup>24</sup>.

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The skin as a barrier in physiotherapy

4. RESULTS
The application modality influences the formation as well as the emptying of the reservoir. Penetration enhancing factors such as occlusion and current induced a faster formation and emptying of the reservoir as compared to an open (passive) DF application. The presence of the reservoir for the passive DF application after 6 hours only is in contrast with earlier findings from our laboratory. Lambrecht et al. (2006) presented the data of the MN response as calculated areas under the curve, relating the inhibition of MN response throughout a 65 minutes time period. They found a 32% reduction of the MN response 1.5 hours after application, reaching borderline significance (p=0.04). The 25% reduction of the MN response did not reach significance. Consequently, the weaker inhibition potential at 1.5 hours post DF application as noticed in both studies indicates lower bioavailability at that moment, due to a slower percutaneous penetration compared to the other application modes. The literature designates that formation of a skin reservoir for topically applied substances is determined by a variety of factors (e.g. lipid/water solubility, protein binding capacity, percutaneous absorption, compound concentration, clearance, application time and application mode). Our results support the previous findings of Vickers (1972) showing that an increased drug diffusivity in the SC as provoked by penetration enhancers, results in a faster formation of the reservoir. More specifically, Takahashi et al. (1995) suggested an increased diffusivity of DF into the SC from vehicles containing urea compared to vehicles without urea. The latter was explained by the hydration enhancement effect of urea on the SC.

The results concerning the penetration enhancement effect of iontophoresis are in line with the results of Curdy et al. (2001) on the in vivo uptake of Piroxicam after passive, occlusive and iontophoretic administration. Only after iontophoresis, enhanced drug uptakes were found at 30, 60 and 125 minutes following the initial application at different depths in the
In contrast with our results, Curdy et al. found no significant difference between passive delivery and the application under occlusion. A possible explanation could be the low lipophilicity of Piroxiam resulting in a low passive uptake into the stratum corneum. Fang et al. (2000) postulated that the route and mechanism for the iontophoretic delivery of DF through the skin might be different as compared to passive delivery. Therefore, the importance of SC as a rate-limiting barrier is reduced for iontophoretic delivery of DF.

Our results indicate that the emptying of the reservoir is influenced by the application mode. After iontophoretic delivery the DF reservoir was present up to 32 hours following initial application. However, after occlusive and passive delivery of DF, the reservoir was present up to 48 and 72 hours respectively. It is well established that diffusivity influences clearance from the reservoir, with faster emptying as a function of increasing diffusivity. Increasing SC hydration as well as iontophoresis has been shown to be effective methods for enhancing the percutaneous penetration of DF. The present data fit in the model proposed by Roberts et al. (2004), with a shorter lag time resulting in a faster reservoir emptying.

Equally, based on the results of Rougier et al. (1985), one can assume that the application with the fastest reservoir building up conditions may result in a greater delivery of active substances to the viable tissues, which may have its effect on the clinical outcomes of the physiotherapeutic treatment.

Limitations of the present study are the lack of information on absolute DF quantities entering the viable skin and the fact that the methods used did not allow to differentiate between an epidermal reservoir and a dermal reservoir. Further research estimating in vivo tissue concentrations after different modes of application are required for further elaboration of the pharmacodynamics of topical applied substances in the physiotherapeutic practice.
Conclusion

This study measured the penetration kinetics and reservoir properties of DF after a single topical passive, occlusive and electrical assisted application in a realistic physiotherapeutic setting. The results indicate that the contribution of occlusive and passive penetration in the iontophoretic delivery can be substantial. The prompt inhibition of the vasoactive reaction may be an indication for a dermal DF reservoir. The formation and emptying of the reservoir was found to be dependent of the application mode.

References


5. General Discussion

This research combined the disciplines of physiotherapy and bioengineering of the skin. Aspects of physiotherapeutic applications, whereby the skin acts as a limiting barrier, were assessed by means of bioengineering techniques. Investigating the specific research questions provided insight in the underlying mechanisms and effectiveness regarding the physiotherapeutic procedures of paraphango, sonophoresis and iontophoresis. This chapter presents a global discussion on the obtained results.

Paraphango: Research question 1 and 2

1. What are the effects of paraphango therapy on skin temperature, perfusion of the microcirculation and skin colour?
2. What are the systemic effects of local paraphango therapy?

Pharaphango therapy aims to increase skin temperature which stimulates cutaneous afferent fibers, subsequently influencing the modulating properties of the gate control system. With "pain reduction by gate control", stimulation of large diameter, fast conducting afferent fibers excitate the substantia gelatinosa, ultimately inhibiting the transmission of nocisensory stimuli towards the thalamus and cortical regions responsible for the perception of pain [101].

Our pharaphango study revealed that during and 21 minutes after paraphango therapy, there were strong local effects on skin properties in terms of increased temperature, microcircular perfusion and skin erythema (see table 1.)
5. General Discussion

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Table 1. Overview of the local changes in skin characteristics before, during and after local Paraphango therapy.

<table>
<thead>
<tr>
<th>SKIN CHARACTERISTIC</th>
<th>PRE TREATMENT</th>
<th>DURING TREATMENT</th>
<th>POST TREATMENT</th>
</tr>
</thead>
<tbody>
<tr>
<td>skin temperature (°C)</td>
<td>35.5 ± 0.4</td>
<td>44.3 ± 1.2</td>
<td>40.3 ± 0.6</td>
</tr>
<tr>
<td>perfusion microcirculation (a.u)</td>
<td>23.2 ± 8.8</td>
<td>197 ± 49</td>
<td>159 ± 52</td>
</tr>
<tr>
<td>skin redness (a*parameter)</td>
<td>11.0 ± 2.5</td>
<td>17.9 ± 1.9</td>
<td>15.8 ± 2.2</td>
</tr>
</tbody>
</table>

The results are in concordance with those obtained by Poensin et al. (2003) [122]. Poensin et al. used comparable methods (Laser Doppler flowmetry and skin temperature) and also presented increased values for ST (+2 °C), skin blood flow (+600%).

Erasala et al. (2001), demonstrated in healthy volunteers that heating pad treatment, increasing skin temperatures to 38 °C, 40 °C, and 42 °C increased deep tissue blood flow in the trapezius muscle by 27%, 77%, and 144% respectively [53]. Muscle tissue blood flow was not measured in our study. Despite, the measured increases in ST of 8.8°C, related to the findings of Ersala et al. support the hypothesis of increased muscle perfusion.

The study revealed significant but small variations in SBP and DBP. SBP values decreased from pre treatment 102.9 ± 8.9 mmHg to 98.7 ± 8.2 mmHg during treatment and DBP values increased from 59.8 ± 6.8 mmHg pre treatment to 61.2 ± 6.5 mmHg during treatment. These changes have limited clinical relevance. The increased HR values (around 10 bpm) immediately after the phango application may be caused by a redistribution of blood towards the more superficial veins, as part of the thermoregulation response. These findings are also in concordance with Poensin et al. who presented constant values for core temperature [122].
In summary, it can be stated that young healthy subjects during and after paraphango therapy present strong local effects on skin properties with weak systemic effects. Future research should reveal whether these weak systemic effects will also be present in patients with cardiovascular insufficiencies.

**Iontophoresis: Research question 3 and 4**

3. *Is the clinical applicability of iontophoresis in the physiotherapeutic treatment of musculoskeletal disorders evidence based?*

4. *Is the application of iontophoresis effective in reducing pain in patients with musculoskeletal disorders?*

The 3rd research question concerned the evidences for the use of iontophoresis in the physiotherapeutic treatment of musculoskeletal disorders. Our literature exploration revealed insufficient evidences concerning the clinical effectiveness of iontophoresis in the treatment of musculoskeletal disorders [37] [6] [14]. The most frequently used drugs in physiotherapeutic iontophoresis are corticosteroids and NSAIDs. Specifically for iontophoresis with corticosteroids insufficient support is presented for its value in the treatment of musculoskeletal disorders [37] [6] [5].

Furthermore, there is no consensus concerning the potency of iontophoresis with acetic acid in the treatment of calcifying disorders [88]. Studies claiming to have a positive treatment effect on size and density of the calcification combined the use of iontophoresis with ultrasound therapy or other therapy modalities [119] [142]. Controversial results have been reported for the efficiency of dexamethasone iontophoresis in the treatment of tendinopathy and epicondylalgia [44] [148] [110] [112]. These results corroborate with the systematic review (k = 28) of Bisset et al.
(2005) evaluating the effectiveness of physical interventions for lateral epicondyalgia. The authors concluded that there were contradictions in results and heterogeneity of the interventions [13]. Also the systematic review from Andres and Murrell (2008) concerning treatment options for tendinopathy (k=177) reported conflicting results. It was concluded that there is little evidence to support the use of most physical modalities including the use of iontophoresis with corticosteroid or NSAIDs. The authors concluded that high powered studies to determine effective treatment strategies on the treatment of tendinopathies are needed and future research in this context should emphasize on standardisation of the treatment methods and the outcome measures [6].

The literature lacks evidenced consensus concerning application time and treatment intensities. Consequently, it is impossible to discriminate between the different factors influencing the percutaneous uptake. A number of studies used multiple treatments or evaluated the effects of iontophoresis on multiple diagnoses, making it impossible to make an interpretation on the independent outcome of iontophoresis [9] [11] [69] [138] [19] [148] [41] [116].

With regard to the 4th research question, there is evidence for the iontophoretic administration of corticosteroids and NSAID’s providing a non invasive alternative to injection and oral therapy to control pain [9] [138] [162] [61] [38]. Johnson et al. (2007) reported scant evidence that iontophoresis with NSAIDs may be effective in reducing pain and that there is insufficient evidence supporting the use of corticosteroid iontophoresis [76].

Although our meta-analysis on ten trials concerning the effect of iontophoresis on pain as compared to a control or placebo group revealed a larger effect size in favour of the iontophoretic treatment, these results should be interpreted carefully.
Most studies on the effectiveness of iontophoresis did not use objective outcome measures or control groups. Most of the in vivo studies presented methodological weaknesses (PEDro scale ranging between 3 and 10). Studies reporting positive effects from iontophoresis treatment often involved small sample sizes, using iontophoresis in combination with other treatment [2] [9] [41] [69] [116] [148].

It can be stated that in most of the in vivo studies on humans the basic principles of percutaneous penetration were not always taken into account. Since passive penetration can be substantial during delivery it is important to control this. This problem motivated us to conduct a study on the bioavailability of DF after electrical assisted delivery in comparison to passive percutaneous penetration and occlusion.

Research question 5

5. Is there an increased bioavailability of DF in the skin after electrically assisted delivery in comparison to the passive percutaneous penetration and occlusion?

Our 5th research question aimed to answer the question if there is an increased bioavailability after electrically assisted delivery of DF in comparison to the passive percutaneous penetration and occlusion. For that purpose, we estimated the tissue bio-availability of DF by means of its proportional relationship with MN induced erythema. The MN induced erythema was measured with Laser Doppler and Chromameter. Laser Doppler represents the blood flow in the superficial dermal plexus and the capillary loops 1-2 mm below the skin surface and does not reflect the amount of blood in the deeper regions [161]. Wilhelm et al. (1989) stated that erythema depends on the total volume of blood under a given area of skin [167]. As
compared to passive diffusion, the passive diffusion of DF with the application site occluded under the contact sponge presented stronger inhibition of the microcirculation at a measuring depth of ≈1mm.

Treffel et al. (1993) hypothesized that the skin redness measured by the Chromameter was mainly because of the blood increase in the capillary loops and in the arteriovenous shunts of the subpapillary plexus [151]. By means of lower a* parameter values (Chromameter), we could demonstrate the bioavailability of DF in the deeper layers of the skin. As compared to passive diffusion alone, inhibition of the erythema was stronger after iontophoresis and passive diffusion under contact sponges without a significant difference between the protocols of iontophorized DF and passive diffusion of DF under occlusion. Limitations of our study are that we were not able to measure absolute DF tissue values. A strong MN inhibition in the superficial layers of the skin does not implicate that there is no possible enhancing effect of current in the deeper layers of the skin.

The in vivo experiments from our research demonstrated that for a single application of DF, the presumed enhancing effect of a current is negligible as compared with passive penetration of DF under a contact sponge. In fact, the wet occlusion was the primary factor for the enhanced percutaneous penetration. The passive penetration and/or the effect of the occlusion and/or other manipulation effects may be the dominant factors compared with the current induced delivery. Consequently, it is recommended that in future experiments with iontophoresis, the inclusion of the abovementioned controls is imperative.

Research question 6

6. Is the formation and emptying of a DF skin reservoir dependent of the DF application mode?

The literature indicates that formation of a skin reservoir for topically applied substances is determined by a variety of factors (e.g. lipid/water solubility, protein binding capacity, percutaneous absorption, compound concentration, clearance, application time and application mode) [121] [160] [118] [32] [149] [132].

In our second bioavailability study it was the aim to estimate the accumulation and emptying of the DF skin reservoir after passive, semi occlusive and electrically assisted applications of DF. The evaluation was done by means of measurement of the inhibitory effect on a physiological reaction (MN) due to the presence of the inhibitor (DF) in the skin reservoir. This enabled us to estimate the penetration kinetics and the DF skin reservoir building properties of three different application conditions up to 120 hours after the initial application.

Our results showed that the building up as well as emptying of the reservoir was influenced by the application modality. Penetration enhancing factors such as occlusion and current induced a faster building up and emptying of the reservoir in comparison to an open (passive) DF application. After iontophoretic delivery the reservoir remained present up to 32 hours following initial application. However, for occlusive and passive delivery the reservoir persisted up to respectively 48 and 72 hours post DF application.

The results concerning the penetration enhancement effect of iontophoresis are in line with the results of Curdy et al. (2001) who investigated the in vivo uptake of Piroxicam after passive, occlusive and iontophoretic administration. They reported for the iontophoresis condition increased Piroxicam concentrations in the horny and...
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deeper layer at 30, 60 and 125 minutes after drug application [40]. In contrast with our results, Curdy et al. found no significant difference between passive delivery and the application under occlusion. A possible explanation for this could be the low lipophilicity of Piroxiam resulting in a low passive uptake into the SC [40].

The data of this study fit in the model proposed by Roberts et al. (2004), with a shorter lag time resulting in a faster reservoir emptying [132]. Our results indicate that the emptying of the DF skin reservoir is influenced by the mode of application. It is well established that diffusivity influences clearance from the reservoir, with faster emptying as a function of increasing diffusivity. Increasing SC hydration as well as iontophoresis has been shown to be effective methods for enhancing the percutaneous penetration of DF [55]. Based on the results of Rougier et al. (1985), it can be assumed that the application with the fastest reservoir building up conditions may result in a greater delivery of active substances to the viable tissues, which may have its effect on the clinical outcomes of the physiotherapeutic treatment [134].

In the study investigating the bioavailability of DF 1.5h after application [87], DF skin bioavailability was estimated by comparing the sum of total colour change (\(\Sigma \Delta a^*\) parameter calculated over the entire measurement period of 65 min) in relation to the standard MN. In this study, a 32% reduction of the MN response 1.5 hours after passive DF application was noticed, reaching borderline significance (p=0.040). In the study investigating the DF skin reservoir (Clijsen et al. 2013), the bioavailability of DF in the skin, the observation period was limited to 50 minutes. There, the 25% reduction of the MN response after passive DF delivery did not reach significance. To cope with this difference in observation time interval between the “bioavailability experiment” and the “reservoir experiment”, the Chromameter values of the
Bioavailability experiment were recalculated over a 50 min observation period (see figure 12a and 12b).

Recalculating the data of the iontophoresis bioavailability study over an observation period of 50 minutes revealed a borderline significant inhibition of the MN response after passive delivery of DF as compared to the 100% MN response ($p=0.046$).

Compared to the 100% MN response, significant inhibitions were found for the responses after iontophoretic DF delivery ($p<0.001$) and the DF delivery under contact sponge occlusion ($p<0.001$). Again, there was no significant difference between the response obtained after iontophoretic delivery and passive penetration under the contact sponge ($p=0.766$). The weaker inhibition at 1.5 hours post DF application as noticed in both studies indicates lower bioavailability at that moment, probably due to a slower percutaneous penetration compared to the other application modes. This direct comparison confirms the repeatability of this experimental design.

**Fig.12a**

**Fig.12.a, b.** Colorimetric evaluation of the microcirculation: response to an MN application; comparison of the different application modes expressed in percentage of the standard MN response ($\sum \Delta a^* =$ sum of total colour change corrected for blank values). * $p<0.05$, **$p<0.001$, compared to the standard MN response. Fig.12a. Recalculation of the Chromameter values of Lambrecht et al. (2006) over an observation period of 50 minutes. Fig.12b. Chromameter values of Clijsten et al. (2013).
Limitations of the present study are the lack of information on absolute DF quantities entering the viable skin and the fact that the methods used did not allow discrimination between an epidermal reservoir and a dermal reservoir. Further research estimating in vivo tissue concentrations after different modes of application are required to expand insight on the pharmacodynamics of topical applied substances in the physiotherapeutic practice.

**Research question 7**

7. Does the timing of sonophoretic application influences the availability and the penetration process of corticosteroids?

With the last research question we evaluated to what extent the timing of sonophoretic application influences the bioavailability and penetration process of halcinonide. For that purpose, a comparative evaluation was performed between a sonophoretic treatment scheduled before or simultaneously with halcinonide application. The initial part of the physiological blanching response was used, as an indicator for the penetration process of halcinonide through the SC. Our research could not reveal a blanching response to topical application of halcinonide of the skin under the condition of simultaneous ultrasound application. On the other hand, a significant blanching response at the skin, which was pretreated with ultrasound, appeared two hours after the initial application of halcinonide as evaluated during 60 minutes.

In other studies, the skin blanching response has been evaluated by the L* and a* coordinates [1] [23]. As such, a limitation of this study might be that the blanching response was evaluated only by means of the a* parameter. A skin blanching effect results in increased L* values and decreased a* values, as skin pallor becomes
lighter and its redness fades [127]. In this context, several authors report changes of the same magnitude for the \( L^* \) and \( a^* \) coordinates in the quantification of the corticosteroid-induced skin blanching effect [127] [23] [33].

A combination of an increased exposure time with increased occlusive dressing enhances the treatment effect of drug application [139] [32] [132]. As such, to unravel the mechanisms behind the timing of ultrasound treatment, a valuation of the reservoir several hours after the initial application may provide additional information on the penetration kinetics. This corroborates our findings of an absence in physiological response for the simultaneous treatment mode. On the other hand, the presence of a blanching response 2 hours after removal of the occlusion indicates fast bioavailability of the corticosteroid in the viable tissues under the condition of pre-treatment ultrasound.

Increased TEWL values after ultrasound treatment are an indication for alterations in barrier function [157]. However, in the present setting no discrimination could be performed between skin surface water loss due to product application (contact medium with or without halcinonide) and increased water flux due to ultrasound induced structural changes (cavities) in the stratum corneum. The short evaluation period of the blanching may be argued as a drawback in this setting.

In conclusion, therapeutically applied ultrasound of 1 MHz before occluded corticosteroid application resulted in a significant blanching response 2 hours after occlusion. Simultaneous treatment of corticosteroid application with US (as commonly used in physiotherapy) with removal of the contact medium immediately after the treatment did not result in a blanching response 2 hours after occlusion. This clinically implies that to physiotherapeutically deliver pharmaceutical substances through the skin, better results may be obtained when US is applied before application. Future research should focus on the effect of dose and time.
6. General Conclusion and suggestions for further research

This doctoral thesis comprises research results on physical applications in physiotherapy in context of the skin as a limiting barrier. Non-invasive evaluation of skin properties enabled to investigate the inherent physiological processes in an in vivo and realistic physiotherapeutic setting.

Our research results on paraphango therapy instigated the conclusion of a transient temperature effect of paraphango on the skin with an increased perfusion of the microcirculation. The systemic (cardiovascular) effects were weak and remained in physiological range. Future research should focus on whether the systemic effects of local heat application in patients with cardiovascular insufficiencies are also weak and in case of clinical significance to define evidence based clinical guidelines.

The experiments on the bioavailability of iontophoretic delivery of DF revealed no enhanced percutaneous penetration of current assisted delivery in comparison to the passive percutaneous penetration under the contact sponge. The contribution of passive penetration and occlusion can be a substantial factor in iontophoretic drug delivery. Consequently, to avoid overestimation of the effect of iontophoretic drug delivery, it is crucial that further investigations related to the penetration enhancement effects of current assisted drug delivery will comprise several controls. In our study, the applied method did not provide the possibility to evaluate absolute drug quantities entering the viable dermis or deeper tissues. Further research with more invasive measurement procedures could focus on absolute drug quantities, e.g. in function of time after iontophoretic drug delivery could reveal information on quantitative drug uptake in the target tissues.

As opposite to an ultrasound treatment before halcinonide application on the skin, simultaneous administration of ultrasound with halcinonide followed by immediate removal of the contact medium did not result in a blanching response 2 h after the occlusion. This clinically implies that to physiotherapeutically deliver pharmaceutical substances through the skin, better results may be obtained when US is applied before application. Future analysis of the therapeutic effectiveness of sonophoresis should focus on the effect of dose and time effects of US stimulation in combination with dose effects of drug delivery.

The literature study and meta-analysis on the treatment effects of iontophoresis in musculoskeletal disorders provided quantitative evidence that iontophoresis might be effective to cope with pain. However, the research designs lack solidity making it difficult to link the presented improvements to the iontophoresis technique per se. Consequently, research in this area should cover standardized and adequately sized RCT’s with inclusion of several controls.
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The study concerning the penetration kinetics and reservoir properties of DF after a single topical passive, occlusive or electrical assisted application indicated dependency of the formation and emptying of the reservoir on the mode of application. The skin reservoir properties of a topically applied substance is an indication for the total amount of the substance which has reached the viable tissues. The application with the fastest condition of reservoir accumulation may
result in a greater delivery of active substances to the viable tissues, consequently may alter the clinical outcome of the physiotherapeutic treatment.

Evidenced based practice in physiotherapy is relatively new, the positive impacts of which are just becoming to be validated. Within the context of evidence based practice, continued research in physiotherapy is essential to improve the effectiveness of treatment strategies. In this doctoral thesis we investigated aspects of physiological processes related to physical applications in physiotherapy from the point of view of the skin as a limiting barrier. Within the paradigm of evidence based practice, the results of this research support the competence of the physiotherapist as a clinical practitioner to optimize the efficacy of his/her treatment strategies.
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8. Dutch summary

Niettegenstaande de beroepsorganisaties en de opleidingen fysiotherapie het paradigma van Evidence Based Practice (EBP) binnen de competenties heeft opgenomen en in gedragsindicatoren heeft gedefinieerd, en niettegenstaande de fysiotherapie het laatste decennium redelijke vooruitgang heeft geboekt in zijn zoektocht naar ondersteunende evidenties, zijn er zeker wat betreft de fysiotherapeutische applicaties nog ettelijke lacunes. In deze doctoraatsthesis onderzochten we vanuit het gezichtspunt van de huid als barrière deelaspecten van fysiologische processen gekoppeld aan fysieke applicaties binnen de fysiotherapie: meer specifiek rond de behandelingen met fango, iontoforese en ultraso.

Parafango

Lokale parafango induceert verhoogde spierweefseldoorbloeding met vermindering van ischaemie en pijnssensaties bij musculoskeletale aandoeningen. Lokale warmteapplicatie wordt bij cardiovasculaire aandoeningen echter als contra-indicatie gesteld. Niettegenstaande deze claims zijn de fysiologische controlemechanismes in verband met vasomotoriek en thermoregulatie bij warmteapplicatie nog onvoldoende gekend en is de informatie rond door warmte geïnduceerde veranderingen in huidparameters beperkt.

Uit de resultaten van onze studie blijkt dat een parafango applicatie sterke lokale effecten op de huidparameters heeft met verhoogde huidtemperatuur, verhoogde perfusie van de microcirculatie en huiderytheem. De systemische (cardiovasculaire) effecten daarentegen waren zwak en bleven binnen normale fysiologische grenzen.
Fysiotherapeutische ondersteuning van topische transdermale applicaties

Topische transdermale applicaties worden standaard gebruikt bij reumatische inflammatoire dysfuncties en acute zachte weefselaandoeningen. In de fysiotherapie worden ter versterking van deze transdermale applicaties sonoforetische, i ontoforetische en occlusietechnieken gebruikt om de huidbarrière te beïnvloeden en zodoende een hogere concentratie van actieve stoffen in de doelweefsels te bereiken.

De literatuurstudie en meta-analyse gericht op de effecten van i ontoforetische behandeling bij musculoskeletale aandoeningen tonen aan dat er kwantitatief bewijs is voor de effectiviteit van i ontoforese bij pijnbestrijding. Niettegenstaande verscheidene klinische studies een versneld genezingsproces na iontoforese onderbouwen blijven de evidenties controversieel, en tonen de studies methodologische tekortkomingen. Zo werd er onvoldoende rekening gehouden met de basisprincipes van percutane penetratie. Vaak werden de effecten van iontoforese vergeleken met die van placebo iontoforese (zonder actieve substantie) wat natuurlijk geen valide bewijs is voor de penetratie versterkende werking van de stroom.

In onze iontoforese studie bepaalden we de in vivo opname van diclofenac door gebruik te maken van een methylnicotinaat geïnduceerd huiderytheem. In deze proefopzet werd tussen de opname van de stroomgeassisteerde toediening van diclofenac en die van passieve diffusie onder semi-occlusieve bedekking geen significant verschil vastgesteld. De resultaten suggereren dat de elektromotorische krachten van iontoforese kunnen worden overschat vooral in die experimentele studies waar de passieve opname en occlusie effecten niet werden geëvalueerd.
Als belangrijke farmacodynamische component heeft het menselijke stratum corneum de eigenschap om topisch aangebrachte substanties voor een bepaalde tijd op te slaan. In deze context richtte een deel van ons onderzoek zich op de farmacodynamische eigenschappen van topisch aangebrachte stoffen binnen de fysiotherapie. In deze experimentele studie werd onderzocht of de **vorming en de lediging van het diclofenac huidreservoir** afhankelijk zijn van de applicatie methode van diclofenac. Drie verschillende applicatie methodes, zijnde (1) iontoforese, (2) onder een semi-occlusieve spons, en (3) een open applicatie werden op verschillende tijdsintervallen met elkaar vergeleken.

De resultaten van dit onderzoek tonen dat zowel de vorming als de lediging van het diclofenac huidreservoir afhankelijk zijn van de applicatie modaliteit. Interpretatie van de resultaten in functie van de bestaande literatuur suggereert dat applicaties met snelle reservoir opbouw en lediging kunnen leiden tot een grotere afgifte van actieve stoffen aan de doelweefsels. Dit kan een bepalend effect hebben op de klinische resultaten van de fysiotherapeutische behandeling.

**Ultrasound** wordt in de fysiotherapie als ondersteunend gebruikt bij topische applicaties van NSAIDs of corticosteroïden. Hierbij kan voor een ‘pre’ applicatiemodus of een ‘simultane’ modus gekozen worden. Niettegenstaande laagfrequente sonoforese (20-100 KHz) in vergelijking met een 1MHz toepassing een drievoudig verhoogde efficiëntie heeft om de huidpermeabiliteit te verhogen, blijft hoogfrequente sonoforese (>= 0.7Hz) het meest gebruikt in het kader van therapeutische behandelingen. In deze studie bepaalden we de invloed van de sonoforetische behandeling op de opname van halcinonide door middel van een verblekingstest en werd de effectiviteit van de ‘pre’ applicatiemodus met die van de ‘simultane’ applicatiemodus vergeleken.