Cutaneous Drug Delivery of Capsaicin after in vitro Administration of the 8% Capsaicin Dermal Patch System

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Introduction

Pharmacology of Capsaicin

Capsaicin (8-methyl-N-vanillyl-6-nonenamide) is a lipophilic substance that is virtually insoluble in water, with a molar mass of 305.4 g/mol. It is naturally present in plants of the genus \textit{Capsicum} (family of Solanaceae) and has been known since ancient times. Thus, it has been used in traditional medicine of South American and Asian peoples [1, 2]. Capsaicin binds to the membrane transient receptor potential vanilloid 1 (TRPV1) receptors, and activation triggers a calcium influx into the target cell [3–5]. In the skin, TRPV1 is expressed in small- to medium-diameter sensory neurons (C and A\textdagger) fibres) as

Key Words

Capsaicin · Cutaneous drug delivery · Dermal patch

Abstract

Objective: Epicutaneous application of capsaicin causes a long-lasting analgesic effect by binding to the membrane transient receptor potential vanilloid 1 (TRPV1) on mechanoheat-sensitive C and A\textdagger fibres, changing axonal integrity and inhibiting neurogenic inflammatory processes. To date, no information is available regarding the cutaneous drug delivery of capsaicin following patch application.

Methods: Using a Franz diffusion cell, the cutaneous concentration-time profiles 30, 60 and 90 min after application of a patch containing 8% capsaicin (640 μg/cm\textsuperscript{2}) on ex vivo thin (mamma) and thick (plantar) human skin were investigated at 32 °C, and additionally at 42 °C for thin skin and 10 °C for thick skin. An HPLC-MS method was used for the analytic detection of capsaicin.

Results: The results show that already after a 30-min application of the 8% capsaicin patch, an equilibrium reservoir can be found in the stratum corneum in both thick and thin skin. Under physiological temperature conditions, a sufficient bioavailability of capsaicin in the cutaneous target compartments can be found. Raising the temperature to 42 °C has no relevant impact on the concentration-time profile, while reducing the temperature to 10 °C leads to a significantly lower bioavailability. Conclusion: After 30 min of application, a sufficient cutaneous bioavailability of capsaicin is reached in thick as well as thin skin. Whether shorter application times may suffice to achieve therapeutic effectiveness requires further investigation.

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well as in other cells, especially keratinocytes, fibroblasts, endothelial cells and mastocytes [6–12]. Its physiological functions are considered to be thermosensory perception and the integration of neuroimmunological response patterns [13]. TRPV1 can also be detected in different extracutaneous organs (i.e. central nervous system, bronchial and kidney epithelia) as well as in effector cells of the immune system [14]. Apart from capsaicinoids, physical triggers like heat (≥43°C), an acidic environment (pH <6) or changes in the membrane potential can activate the receptor (fig. 1) [12, 15–17]. Additionally, it has been proven that capsaicin can lead to a reversible reduction in intraepidermal nerve fibres and therefore causes a nociceptive defunctionalization [18, 19].

With regard to its cutaneous pharmacology, preliminary investigations have shown that a rapid elimination of capsaicin via CYP2E1 directly in the skin cannot be expected [20]. Although the systemic bioavailability via transdermal processes quantitatively depends on the duration and area of application, it is generally so low that it has no relevance either pharmacodynamically or toxicologically [21, 22]. This is further reinforced by the rapid hepatic elimination of the amount of active substance that has reached the systemic circulation [23].

**Therapeutic Use**

The medicinal use of capsaicin in preparations for epicutaneous application targets nociceptive neurons in the epidermis and dermis, i.e. afferent neurons that will be activated by stimulation of specialized free nerve endings due to damage or intense mechanical, thermal or chemical stimuli [24, 25]. The reactive hyperaemia and specific neurogenic effects generated hereby are used to treat rheumatic and neuropathic pain [26]. These effects can be divided into medium- and long-term effects. Immediately after application, a painful burning sensation and hyperaemia may occur [27, 28]. When higher doses are used for the treatment of neuropathic pain, a significant reduction of discomfort can be observed that cannot solely be explained by the direct pharmacological effects of capsaicin [29, 30]. The analgesic and antipruritic effects that last for weeks are ascribed to the inhibition of neurogenic inflammatory processes and to alterations in axonal integrity [31–34].

In recent years, different capsaicin derivatives (capsaicinoids) have been identified and are currently investigated for their therapeutic potential [35]. Topical application of capsaicin containing semisolid preparations is very challenging in practice because even small residual quantities cause unwanted effects on accidentally contaminated skin or mucosa. Additionally, an open applica-
tion results in a very short period of contact between substance and skin that restricts the diffusion process of the lipophilic capsaicin and therefore limits the cutaneous bioavailability.

**Capsaicin-Containing Patch System**

Taking this into consideration, a matrix patch has been developed that contains a lipogel with the highly concentrated active substance dissolved in glycol ether. Using a silicon adhesive, the patch brings the active substance into contact with the skin surface [36]. This allows for a significantly longer contact period as well as a locally confined application, but it also alters the conditions for penetration. The high initial capsaicin concentration in the lipogel (640 μg/cm²) causes a distinct concentration difference between donor (patch) and acceptor (stratum corneum) and should ensure the formation of a reservoir in the stratum corneum.

Limited information is available regarding the penetration of capsaicin from the patch into the individual skin layers. Based on in vitro studies, it is estimated that during a 1-hour application of the capsaicin 8% cutaneous patch, approximately 1% reaches the epidermis and dermis [37]. During clinical development, the efficacy and safety of the high-concentration capsaicin patch were assessed for application times of 30, 60 and 90 min [38–49]. Based on these data, application times for individual skin types were recommended: 30 min for thick skin (also termed ‘hairless’ or ‘rigid’ skin, which includes the palms of the hands and soles of the feet) and 60 min for the remainder of the integument (thin or ‘hairy’ skin) [37]. Treatment is usually applied at body temperature (32–35 °C). Should patients experience application-related discomfort, application site cooling or the use of analgesics during or after treatment is advised [37]. It has, however, been suggested that the cooling procedure be delayed until after or close to the end of the treatment as it is not clear whether cooling during patch application may impact the activation of the TRPV1 receptor [50]. During a recent interdisciplinary capsaicin workshop, the expert panel recommended further investigation of the skin penetration of capsaicin and the distribution of the compound in the individual skin layers [51].

This paper describes the validation of the cutaneous pharmacokinetic profile in order to evaluate the concentration-time profile of capsaicin in the different target compartments following epicutaneous application to thick and thin skin. Furthermore, the cutaneous pharmacokinetic profile was investigated under different temperature conditions to validate the influence of cooling on thin skin and heating on thick skin application areas, as it is common in practice [52]. The results should allow conclusions about optimizing the practical use of the patch system [51].

**Materials and Methods**

**Diffusion and Permeation Studies**

The investigations were performed using a glass Franz diffusion cell (Crown Glass Company, Somerville, N.Y., USA) on excised human skin from reduction mammoplasty (thin skin) and plantar skin (thick skin) retrieval [53–55]. The tissue sections were postoperatively cleaned with mull pads and isotonic NaCl solution. The subcutaneous adipose tissue was mechanically dissected and discarded. Circular pieces of skin (20 mm in diameter) were punched, hermetically sealed in tin foil and stored in an occlusive polyethylene bag at –20°C for 2–3 weeks. At the time of the investigation, the pieces of skin were completely thawed at room temperature, and the surface was dried using cotton pads.

Subsequently, original 8% capsaicin patches adapted to the size were brought onto the individual skin specimen lying on filter gauze. Isotonic NaCl solution was used as an acceptor (fig. 2). The specimens were stretched onto the preheated diffusion cell, enabling the underside of the skin with the filter gauze to get in direct contact with the acceptor medium, which was continuously stirred to reduce the thickness of the diffusion layer. The following settings were applied: thick skin at 32°C (setting 1), thin skin at 32°C (setting 2), thick skin at 42°C (setting 3) and thin skin at 10°C (setting 4). Skin specimens of 3 donors with 3 pieces of skin each per setting and for each of the application periods of 30, 60 and 90 min were investigated. To determine the amounts of penetrated capsaicin after the respective application period, the skin specimens were taken from the diffusion cell and the residues were carefully removed from the surface using a cleansing gel (containing polyethylene glycol 300, acrylate crosspolymer 1382, sodium hydroxide, sodium-EDTA, butylated hydroxyanisole and water) and a cotton pad. In order to determine the concentration-time profile, the individual skin layers were separated horizontally to the epidermis (table 1). For layer-by-layer removal of the stratum corneum, Sellotape strips were taken from a 3.14-cm² piece of skin using a punch with a circular window of 20 mm in diameter. Two successive pieces each were combined. From the centre of the remaining piece of skin, 3 biopsies of 6 mm (0.2827 cm²) size were punched and dissected horizontally to the epidermis to the given thickness and frozen at –40°C using a refrigeration microtome (fig. 3).

**Investigational Product**

For all investigations, a self-adhesive dermal system containing 640 μg/cm² capsaicin in form of matrix patches (Qutenza; Astellas Pharma Europe B.V., The Netherlands) was used. The patches were cut to size immediately before application using sterile metal punches.

**Analytic Method**

An analytic HPLC-MS method for quantification of capsaicin (Sigma-Aldrich, Steinheim, Germany) in skin had been developed [56–58] using an Agilent 1200 HPLC System linked with an Agilent 6120 Single Quadrupole mass spectrometer (Agilent,
Waldbronn, Germany). Mass-spectrometric detection was performed with electrospray ionization in positive ion mode using the parameters listed in Table 2. For validation of the HPLC method, a calibration series and 3 quality control series for very low, low, medium and high concentrations each were measured on 2 different days (interday variability). Intraday variability was assessed using 3 calibration samples and 3 × 3 quality control samples for each concentration. Mean values ± SD, coefficients of variation as measures of imprecision and relative errors as measures of incorrectness were determined. The reproducibility of values was determined by 6-fold processing and measuring. The peak areas that were determined using UV detection were evaluated via weighted linear regression: \( y = mx + n \). Weighting of the values was done with \( 1/C^2 \). The correlation (\( R^2 \)) of nominal concentrations and measured values was calculated using the least-squares method. Recovery determinations for capsaicin from human skin were carried out. Acetonitrile/H\(_2\)O (50/50, v/v) was found to be the extraction agent yielding the highest recovery (85%).

### Table 1. Processing of the cutaneous layers

<table>
<thead>
<tr>
<th>Skin compartment</th>
<th>Medium</th>
<th>Extraction volume, ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-permeated proportion</td>
<td>swab</td>
<td>20</td>
</tr>
<tr>
<td>Stratum corneum</td>
<td>10 μm</td>
<td>0.5</td>
</tr>
<tr>
<td>Vital epidermis 1 (EP1)</td>
<td>2 sections of 20 μm</td>
<td>0.2</td>
</tr>
<tr>
<td>Vital epidermis 2 (EP2)</td>
<td>2 sections of 20 μm</td>
<td>0.2</td>
</tr>
<tr>
<td>Dermis 1 (DR1)</td>
<td>5 sections of 40 μm</td>
<td>0.2</td>
</tr>
<tr>
<td>Dermis 2 (DR2)</td>
<td>5 sections of 40 μm</td>
<td>0.1</td>
</tr>
<tr>
<td>Dermis 3 (DR3)</td>
<td>5 sections of 40 μm</td>
<td>0.1</td>
</tr>
<tr>
<td>Dermis 4 (DR4)</td>
<td>5 sections of 40 μm</td>
<td>0.1</td>
</tr>
<tr>
<td>Dermis 5 (DR5)</td>
<td>5 sections of 40 μm</td>
<td>0.1</td>
</tr>
<tr>
<td>Remaining stump</td>
<td></td>
<td>0.2</td>
</tr>
<tr>
<td>Permeated proportion</td>
<td>filter gauze (membrane)</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>acceptor fluid</td>
<td>–/–</td>
</tr>
</tbody>
</table>

### Processing of the Samples for Analysis

Sections of the same skin layer were stored together in 1.5-ml Eppendorf tubes. All samples, including receptor fluid, had been frozen at –20°C before processing and analysing. The swabs and filter gauze used to wipe off any excess capsaicin were placed in test tubes, mixed with extraction agent and placed on a laboratory shaker (GFL 3006; GFL Gesellschaft für Labortechnik GmbH, Burgwedel, Germany) for 24 h. Also the skin sections were mixed with extraction agent and placed on a laboratory shaker for 24 h. The samples were then centrifuged for 10 min at 13,000 rpm, and the supernatant was analysed. One millilitre acceptor fluid for each specimen was concentrated at 40°C under nitrogen gas flushing and then resolved with 0.5 ml acetonitrile/H\(_2\)O. The concentrated sam-
Fig. 3. Methodology of harvest procedure, conditioning of skin samples, and analytical method for detection of capsaicin amount.

Table 2. Characteristics of the HPLC-MS method for analytical detection of capsaicin

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Specification</th>
</tr>
</thead>
<tbody>
<tr>
<td>HPLC apparatus</td>
<td>Agilent 1,200 series</td>
</tr>
<tr>
<td>Column</td>
<td>Reprosil (Wicom) + precolumn Nucleosil RP 18</td>
</tr>
<tr>
<td>Mobile phase</td>
<td>ACN/H₂O + 0.1% FA, 50/50 (v/v), 8 min</td>
</tr>
<tr>
<td></td>
<td>ACN + 0.1% FA, 8 min</td>
</tr>
<tr>
<td></td>
<td>3 min rinsing time</td>
</tr>
<tr>
<td>Flow rate</td>
<td>0.9 ml/min</td>
</tr>
<tr>
<td>Pressure</td>
<td>104 bar</td>
</tr>
<tr>
<td>Volume of injection</td>
<td>10 μl</td>
</tr>
<tr>
<td>Temperature of column</td>
<td>30°C</td>
</tr>
<tr>
<td>Detection</td>
<td>MS: electrospray ionization positive; m/z = 306.2</td>
</tr>
<tr>
<td>UV</td>
<td>wavelength: 280 nm</td>
</tr>
<tr>
<td>Stock solution</td>
<td>1 mg/ml capsaicain in ACN</td>
</tr>
<tr>
<td>Solvent</td>
<td>ACN/H₂O, 50/50 (v/v)</td>
</tr>
<tr>
<td>Calibration ranges</td>
<td>0.05–5 μg/ml</td>
</tr>
<tr>
<td>Limit of determination</td>
<td>0.05 μg/ml</td>
</tr>
<tr>
<td>Limit of detection</td>
<td>0.025 μg/ml</td>
</tr>
</tbody>
</table>

ACN = Acetonitrile; FA = formic acid.
samples were analysed. Capsaicin concentrations were determined using HPLC-MS. To ensure the correct analysis of the samples, quality controls with known concentrations were determined in parallel.

**Results**

The data show that capsaicin reaches cutaneous bioavailability in both the vital epidermis and the dermis (fig. 4, 5). Already after 30 min, a depot of the active substance can be detected in the stratum corneum, which remains fairly constant in all settings even after longer application times. Only for thin skin at 10°C, a lower bioavailability is detected when directly compared with that at 32°C, particularly after 30 min. Over time, the penetration rate increases slightly but remains below average. The comparison between both skin types shows that under physiological conditions (32°C), thick skin develops a larger reservoir in the stratum corneum and slightly higher concentrations in the vital epidermis than thin skin. By increasing the temperature for thick skin to 42°C, no relevant changes in the concentration-time profile can be observed, while cooling thin skin to 10°C considerably reduces the bioavailability of capsaicin in all skin layers. Table 3 outlines the amount of the applied dose that penetrates in total and into the target compartments.

**Discussion**

A key question when evaluating pharmacokinetic data in the skin relates to the designated microcompartment. It is crucial to know where the active substance is supposed to have the best effect and which concentration ensures both its efficacy and its safety. The distribution of expression of TRPV1 in the skin indicates that both the vital epidermis and the dermis are to be the designated microcompartments for capsaicin [35]. TRPV1 is ex-
pressed by keratinocytes in the epidermis as well as by fibroblasts, endothelial cells and immunocompetent cells in the dermis [7, 14]. However, of main relevance in the context of analgesic effects is the anatomic fitting of the skin layers especially with C and Aδ pain fibres, which are established in the dermis but grow into the vital epidermis [59, 60]. With regard to an optimized bioavailability of capsaicin, the mean effect concentration (EC50) at mechanoheat-sensitive C fibres of approximately 350 nmol/l as well as toxicological in vitro data about apoptosis induction at >5 μmol/l over 6 h for keratinocytes and >5 μmol/l over 24 h for cutaneous fibroblasts need to be

![Fig. 5](http://example.com/fig5.png)

**Fig. 5.** Penetration amounts of capsaicin in the cutaneous compartments 30, 60 and 90 min after application of the investigational product. SC = Stratum corneum; EP1 = upper vital epidermis; EP2 = lower vital epidermis; DR1 = upper dermis; DR2 = upper/middle dermis; DR3 = middle dermis; DR4 = middle/lower dermis; DR5 = lower dermis.
considered [10, 61, 62]. The relevant toxic concentration limit for in vivo conditions with much shorter application periods is likely to be significantly higher but can only be estimated, due to lack of data.

For an evaluation of the study results, it is also important to consider that capsaicin is a lipophilic substance, that the use of a patch system extends the application time compared with semisolid preparations and that, therefore, the galenical conditions are definitely advantageous [44, 47]. The concentration-time profile should be determined by the release rate of capsaicin from the lipogel vehicle [63]. The development of a reservoir of lipophilic substances in the stratum corneum depends on layer thickness and the composition of the acceptor layer. A larger reservoir and a longer application period can therefore be expected for thick skin [64]. The present data confirm this for capsaicin under physiological conditions (32°C). Kinetics therefore causes higher concentrations in the vital epidermis. In deeper layers, however, the differences become aligned again. Nevertheless, the data clearly show that application to both thick and thin skin leads to a sufficient bioavailability of capsaicin, confirming the precondition for efficacy as shown in clinical data [44]. However, to postulate a direct correlation between ex vivo penetration data and results in clinical response would be highly speculative, since, firstly, the Franz diffusion chamber model allows only limited conclusions about the absolute concentration in tissue (non-existent sink conditions) and, secondly, the dose-effect relationship still remains unclear [65]. Remarkably, though, the concentration equilibrium is reached rapidly, and the bioavailability established after 30 min cannot be significantly increased by extending the application period regardless of the skin type. A possible reason for this might be the retention of the transepidermal water flow due to occlusion caused by the patch. The hydration building up in the stratum corneum could act as a hydrophilic barrier for the liberation and penetration of the lipophilic capsaicin. Furthermore, it cannot be completely ruled out that molecules of the active substance that had already penetrated into the stratum corneum rediffuse into the matrix of the patch due to increased hydrophilicity.

Based on these findings, it is likely that a shorter patch application time may suffice to achieve clinically relevant capsaicin concentrations in the target compartments. Further studies investigating the impact of application times <30 min are required. In clinical practice, the temperature is already manipulated to either improve the efficacy by heating or to reduce unwanted side effects by cooling. The present data show that heating up to 42°C would be highly beneficial.

### Table 3. Penetrated amount (percentage and absolute concentration) of applied dose of capsaicin in the different skin compartments

<table>
<thead>
<tr>
<th></th>
<th>30 min</th>
<th>60 min</th>
<th>90 min</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>of the applied dose, %</td>
<td>of the applied dose, %</td>
<td>of the applied dose, %</td>
</tr>
<tr>
<td></td>
<td>capsaicin concentration, μmol/l</td>
<td>capsaicin concentration, μmol/l</td>
<td>capsaicin concentration, μmol/l</td>
</tr>
<tr>
<td>Thick skin 32°C</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EP</td>
<td>0.41±0.20</td>
<td>2,133.8±543.5</td>
<td>0.34±0.24</td>
</tr>
<tr>
<td>DR</td>
<td>0.58±0.07</td>
<td>603.5±177.0</td>
<td>0.94±0.25</td>
</tr>
<tr>
<td>TPA</td>
<td>1.37</td>
<td>2,778.8</td>
<td>1.77</td>
</tr>
<tr>
<td>Thick skin 42°C</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EP</td>
<td>0.41±0.70</td>
<td>563.0±172.6</td>
<td>0.19±0.19</td>
</tr>
<tr>
<td>DR</td>
<td>0.41±0.05</td>
<td>363.9±98.4</td>
<td>0.68±0.19</td>
</tr>
<tr>
<td>TPA</td>
<td>1.10</td>
<td>941.4</td>
<td>1.29</td>
</tr>
<tr>
<td>Thin skin 32°C</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EP</td>
<td>0.11±0.05</td>
<td>2,125.5±833.6</td>
<td>0.21±0.16</td>
</tr>
<tr>
<td>DR</td>
<td>0.35±0.04</td>
<td>425.5±158.6</td>
<td>0.38±0.14</td>
</tr>
<tr>
<td>TPA</td>
<td>0.62</td>
<td>2,609.1</td>
<td>1.15</td>
</tr>
<tr>
<td>Thin skin 10°C</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EP</td>
<td>0.01±0.01</td>
<td>66.5±23.3</td>
<td>0.04±0.03</td>
</tr>
<tr>
<td>DR</td>
<td>0.24±0.07</td>
<td>255.7±188.6</td>
<td>0.12±0.01</td>
</tr>
<tr>
<td>TPA</td>
<td>0.65</td>
<td>364.9</td>
<td>0.48</td>
</tr>
</tbody>
</table>

Values denote means ± SD. EP = Vital epidermis (comprises compartments EP1 + EP2); DR = dermis (comprises compartments DR1–DR5); TPA = total penetrated amount (stratum corneum, epidermis, dermis, remaining stump, acceptor, gauze membrane). N = 9 for each sample.
has no impact on the bioavailability of capsaicin. Although the desired effect cannot be achieved, heating does not seem to have a negative impact. Cooling to 10°C leads to a considerably lower bioavailability of capsaicin in the target compartments. Since this may have immediate consequences on the effectiveness of treatment, it is recommended to omit cooling practices.

In summary, it can be said that neither longer application times nor changes in the temperature improve the bioavailability of capsaicin in the skin target compartments. Cooling the area of application in order to reduce unwanted effects leads to a reduced bioavailability of capsaicin in the skin and is therefore not advisable. Whether a shorter application time can be sufficient to show adequate clinical effects has to be investigated in further pharmacokinetic and clinical studies.

References


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