Impact of Ibuprofen at Skin Level – an in vitro and in vivo Study

LAVINIA LIA VLAIA1, IOANA VIORICA OLARIU1, GEORGETA HERMINA CONEAC1, VICENTIU VLAIA2, DORINA CORICOVAC3, SIMONA ARDELEAN4*, SORINA CIURLEA1

1“Victor Babes” University of Medicine and Pharmacy of Timisoara, Faculty of Pharmacy, Department of Pharmaceutical Technology, 2 Eftimie Murgu Sq., 300041, Timisoara, Romania
2“Victor Babes” University of Medicine and Pharmacy of Timisoara, Faculty of Pharmacy, Department of Organic Chemistry, 2 Eftimie Murgu Sq., 300041, Timisoara, Romania
3“Victor Babes” University of Medicine and Pharmacy of Timisoara, Faculty of Pharmacy, Department of Toxicology, 2 Eftimie Murgu Sq., 300041, Timisoara, Romania
4University Vasile Goldis Arad, 94 Revolutiei Blv., 310025, Arad, Romania

Ibuprofen is a known antiinflammatory agent from the class of NSAIDs, widely used for the treatment of systemic and local pain and as therapy in musculoskeletal disorders. Due to its gastro-intestinal side effects, its formulation for topical application with increased skin permeability gained an increased interest in the last period. In this study we showed that in vitro release of ibuprofen from marketed Nurofen® hydrogel across synthetic hydrophilic membrane was higher than across the excised mouse skin. Moreover, the topical administration of ibuprofen in vivo in a mouse model of UVB-induced inflammation improved the physiological skin parameters and attenuated inflammation.

Keywords: ibuprofen, Nurofen® gel, in vitro skin permeation

* email: simonaardelean@yahoo.com

Fig. 1. Chemical structure of Ibuprofen, ((RS)-2-(4-(2-methylpropyl)phenyl) propanoic acid – figure 1), is a potent nonsteroidal antiinflammatory (AINS) drug, with low molecular weight (206 Da) and log P of around 4, widely used in systemic and local pain and in inflammatory therapy of musculoskeletal disorders (acute and chronic rheumatoid osteoarthritis) [1]. This compound is a nonselective inhibitor of cyclooxygenases 1 and 2 and it was described to possess analgesic, antiinflamatory and antipyretic effects [2]. After oral administration, ibuprofen can cause gastro-intestinal mucosal irritation which may result in ulceration and/or bleeding [3]. Therefore, it is an ideal candidate for percutaneous application, which explains the growing interest for developing topical dosage forms of ibuprofen to avoid oral side effects and first-pass hepatic effect. However, the ibuprofen penetration across the skin is low because its lipophilic nature and consequently, it is difficult to achieve relatively high drug levels at the application site for prolonged periods [4]. In order to improve the ibuprofen penetration through the skin, several approaches have been investigated [5-8] and some optimized formulations are marketed in present, such as the commercial product Nurofen® gel.

It was reported that ibuprofen was able to prevent or reduce the pain and inflammation related to UVB irradiation of the skin [9]. Ultraviolet radiation was described to induce a biphasic reaction in the mammalian skin characterized by an immediate phase which appears within few minutes and a late phase that lasts 2-3 days after acute exposure [10]. UVB radiation is considered the most effective type of ultraviolet radiation to determine cutaneous inflammatory reactions described by erythema, oedema and pain in the exposed area of the skin [10]. The model of inflammation and pain related to UVB-induced sunburn was first described by Bickel et al., [11] as a method of evaluation of the effectiveness of NSAIDs [12].

The aims of this study were: (i) to investigate the in vitro ibuprofen release and skin permeation through synthetic membrane and excised mouse skin from Nurofen® gel (5% ibuprofen), a marketed hydrogel and (ii) to evaluate the impact of ibuprofen on skin parameters (transpidermal waterloss, skin moisture, erythema and pH) after exposure to UVB radiation.

Experimental part

Materials and methods

Reagents

Tuffryn HT synthetic hydrophilic membranes of polysulfone (0.45μm, 25 mm) were supplied by Pall Corporation (USA). Double distilled water was used throughout the study. All chemicals and reagents were of pharmaceutical or analytical grade and were used without further purification. In this study it was used Nurofen® gel (5% ibuprofen) produced by Farmasierra S.A. (Madrid, Spain).
**In vitro skin permeation studies**

Preparation of the skin

In vitro skin permeation studies were carried out using full thickness mouse skin with a surface area of 1.767 cm². The skin was excised from SKH1 mice. The integrity of the skin was examined, the thickness of each sheet was measured with a micrometer and then squares of skin of 2 to 2.2 cm² were cut from the sheet.

**In vitro drug release studies**

The in vitro release of ibuprofen from commercial hydrogel Nurofen® was determined to evaluate the preparation performance. The release experiments were performed on a system of 6 Franz diffusion cells (Microette-Hanson system, 57-6AS9 model, Hanson, USA) using synthetic hydrophilic membranes of polysulfone (HT Tuffryn membrane, Pall Corporation, USA). Franz diffusion cells presented an effective diffusional area of 1.767 cm² and 6.5 mL of receptor cell capacity. The receptor chambers were filled with freshly prepared isotonic saline solution (0.9% sodium chloride solution) to mimic the physiological conditions. The synthetic membranes were mounted between donor and receptor compartments of Franz diffusion cells and were put in previous contact with isotonic saline solution 30 min prior placing the samples. The tested formulation (300 mg) was placed into each donor compartment. The receptor compartment was constantly stirred at 600 rpm and the diffusion cells were maintained at 32±1°C throughout the experiment. A volume of 0.5 mL sample of the receptor medium was withdrawn at predetermined time (15, 30, 45, 60, 90, 120, 150, 180, 210 and 240 min) and replaced with an equal volume of fresh receiver medium to maintain a constant volume. Collected samples were analysed for ibuprofen content by UV spectrophotometric method, at 273 nm. Three replicates of each experiment were performed.

**Data analysis of in vitro drug release studies**

Cumulative amount of ibuprofen permeated through the membrane (μg/cm²) was plotted as a function of time (t, min). The permeation rate of drug at steady-state (flux, J, μg/cm²/min) and the lag time (tL, min) were calculated from the slope and the x intercept of the linear portion of the plots of cumulative amount of drug permeated versus time in steady state conditions, respectively. Permeability coefficient (Kp, cm/min) was calculated by dividing the flux with initial concentration of drug in donor compartment. The release rate (k) values were calculated using the pseudo steady-state slopes from plots of cumulative amount of ibuprofen permeated through membrane (μg/cm²) vs. square root of time. Diffusion coefficient (D) values were calculated from the release rate values.

**Design of the in vivo study**

The SKH1 mice were divided in 2 groups (n=5 mice/group): group 0 – control group exposed to UBV radiation and group 1 – treated group – mice received topical treatment with Nurofen® hydrogel (5% ibuprofen) 30 min prior exposure to UBV radiation. The exposure protocol was the following: irradiation 3 times/week/3 min for 16 days. The cage surfaces were cleaned in an automatically time-switched irradiation setup. In the experiment, VL-6.M/6W (312 nm wavelength and 680 μW/cm² intensity at 15 cm) tubes (Vilber Lourmat, France) were used.

**Animals**

SKH1 mice (18 – 20 g, 8-10 weeks) were purchased from Charles River (Sulzfeld, Germany). All experimental procedures were conducted in accordance with the Guide for the Care and Use of Laboratory Animals (1996, published by National Academy Press) regarding the protection of animals used for scientific purposes. Animals were kept on a 12 h/12 h light/dark cycle, at a normal (24 °C) animal house temperature, humidity above 55%, fed ad libitum and they had free access to water. The experiments were approved by the Bioethical Committee of “Victor Babes” University of Medicine and Pharmacy Timisoara and, also respected the international regulations.

**In vivo permeation study**

The evaluation of the in vitro ibuprofen permeation was performed on Franz diffusion cells (Microette-Hanson system, 57-6AS9 model, Hanson, USA) with an effective diffusional area of 1,767 cm² and 6.5 mL of receptor cell capacity. Sink conditions were achieved in the receiver compartment with 6.5 mL freshly prepared isotonic saline solution as receptor fluid. The skin pieces were mounted carefully on the Franz diffusion cells, between the donor and receptor compartments, with stratum corneum facing donor chamber. After that, the skin pieces mounted in the cells were allowed to rest in contact with isotonic saline solution 1 h prior the application of the formulations. The tested formulation (300 mg) was placed into each donor compartment. The receiver fluid was constantly stirred at 600 rpm and the diffusion cells were maintained at 32±1°C throughout the experiment. A volume of 0.5 mL sample of the receptor medium was withdrawn at predetermined intervals (15, 30, 45, 60, 90, 120, 150, 180, 210 and 240 min) and replaced with an equal volume of fresh receiver medium to maintain a constant volume. Collected samples were analysed for ibuprofen content by UV spectrophotometric method, at 273 nm. Three replicates of each experiment were performed.

**Fig. 2. In vitro ibuprofen permeation profiles through synthetic membrane and excised mouse skin from Nurofen® hydrogel (mean ± SD, n = 3)**
### Table 1
PERMEATION AND RELEASE PARAMETERS OF THE IBUPROFEN COMMERCIAL HYDROGEL (NUROFEN®) THROUGH SYNTHETIC MEMBRANE AND EXCISED MOUSE SKIN

<table>
<thead>
<tr>
<th>Nurofen®</th>
<th>Permeation parameters</th>
<th>Release parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>J</td>
<td>Kₚ x 10⁻⁶</td>
</tr>
<tr>
<td>through synthetic membrane</td>
<td>9.16±0.39</td>
<td>1.83</td>
</tr>
<tr>
<td>through excised mouse skin</td>
<td>4.55±0.62</td>
<td>0.91</td>
</tr>
</tbody>
</table>

Non-invasive skin measurements

In order to evaluate skin physiological parameters (transepidermal water loss – TEWL, skin moisture, erythema and skin pH) it was used a Multiprobe Adapter System (MPA5) from Courage-Khazaka, Germany. The measurements of erythema were obtained by the means of MPA5 Mexameter® MX 18 probe, as quantitative results regarding erythema (haemoglobin) subject to modifications by the inflammatory reaction, TEWL measurements were carried out with Tewameter® TM300 and the skin pH with Skin-pHmeter® PH905.

Results and discussions

In vitro drug release and skin permeation studies

The results of the in vitro ibuprofen permeation and release through synthetic membrane are illustrated in figure 2 and listed in table 1.

According to our results the total amount of ibuprofen released after 240 min from the commercial hydrogel through synthetic membrane was slightly higher than that observed through mouse skin, but in both cases the values of this parameter did not exceed 26% (w/w) (fig. 2).

The plots of cumulative amount of ibuprofen released per surface area of membrane vs. time and the permeation profiles of drug through excised mouse skin over 240 min period showed one linear portion, during the steady state (from 15 to 90 min and from 15 to 180 min respectively). It can be observed a 2 fold higher and faster drug transfer of ibuprofen through synthetic membrane than through mouse skin. The calculated release rates of drug during the steady state for both types of membranes were found to be considerably higher than that of the corresponding fluxes, but the value obtained for synthetic membrane was only 1.4 fold higher than that corresponding to mouse skin, as it can be observed (table 1). Taking all these into consideration, it is evident that the delivery of ibuprofen from Nurofen® hydrogel through synthetic hydrophilic membrane and through mouse skin is dependent on the rate of its release from the formulation. The differences in ibuprofen transfer from Nurofen® hydrogel through

Fig. 3. Skin hydration evaluations (Transepidermal water loss - TEWL and moisture of stratum corneum) : Group 0-SKH1 mice exposed to UVB and used as control group; Group 1 - SKH1 mice exposed to UVB and received ibuprofen topically 30 min prior exposure; *, ** and *** indicate p<0.05, p<0.01 and p<0.001 compared with control group
In vivo effects of ibuprofen treatment

In order to verify the effects of ibuprofen topical treatment in vivo, we used an animal model of UVB-induced inflammation developed on SKH1 mice. The mice were exposed to UVB radiation according to the protocol detailed in Materials and Methods section and treated with ibuprofen hydrogel prior UVB exposure. For the characterization of ibuprofen effects at skin level in inflammatory conditions, we carried out some non-invasive skin measurements of the most important skin parameters (TEWL, skin moisture, erythema and skin pH).

TEWL and skin moisture measurements

The values recorded for TEWL and skin moisture can be used to evaluate the skin hydration state and also its integrity as barrier function. During the 16 days of experiment when the mice were exposed to UVB radiation, the TEWL increased approx. 1 g·m⁻²·h⁻¹ (average values from 2.1-2.2 to 3.0-3.1) for the control group (group 0 – fig. 3); it is important to mention that this is not a very large change probably due to a not very high doses of radiation. The level of TEWL decreased to 2.5 g·m⁻²·h⁻¹ for those mice treated with ibuprofen (Group 1) as compared to control group (fig. 3).

Regarding the evolution of moisture of stratum corneum, our results indicated that the values of this parameter decreased around 2 arbitrary units in the first week (average values from 8.9-9.2 to 7.0-7.1 arbitrary units) in both groups, but after the first week, the values measured for the treated group (group 1) were significantly higher than the ones for the control group (fig. 3), which indicate the protective effect of ibuprofen.

Erythema and skin pH measurements

In this study we also measured the values of erythema and skin pH. According to our results, it was observed that UVB exposure produced an important increase of erythema during the experiment (from approx. 35 to over 60 arbitrary units) in the control group not treated with ibuprofen (fig. 4). A lesser degree of erythema was detected in the treated group (group 1) that received ibuprofen topically 30 min before UVB exposure (fig. 4).

As regards the skin pH values there were not observed important changes of this parameter during the experiment. It is necessary to mention that skin-pH fluctuated between 6.28 and 6.64 arbitrary units with an easier upward trend during the UVB exposure (fig. 4).

The skin represents the largest organ of the body and its major function is to protect the body against the intrusion of environmental factors and substances and from transepidermal water loss [13]. The main skin barrier is represented by stratum corneum which is permeable for a small number of substances that present a moderate oil-water partition coefficients, small molecular weights and low melting points [13]. Stratum corneum is a rate-limiting factor in the penetration of a drug through the skin [7]. The administration of a drug transdermally presents several advantages, including: low systemic side effects, good patient compliance, and avoidance of the hepatic first pass effect what leads to an increased therapeutic effect [14].

The formulation of ibuprofen, a consecrated anti-inflammatory drug, for topical administration in a form that increases its skin permeability and also offers effective concentrations after topical delivery was highly debated in a significant number of studies [7, 13-15].

In this study we tested the in vitro ibuprofen release and skin permeation through synthetic membrane and excised mouse skin from Nurofen® gel (5% ibuprofen), a marketed hydrogel by the means of Franz diffusion cell method and we observed that the total amount of ibuprofen released after 240 min from the commercial hydrogel through synthetic membrane was slightly higher than the amount released through mouse skin, but in both cases the values of this parameter did not exceeded 26% (w/w).

![Fig. 4. Skin quality measurements (Erythema and Skin-pH): Group 0 - SKH1 mice exposed to UVB and used as control group; Group 1 - SKH1 mice exposed to UVB and treated with ibuprofen topically 30 min prior exposure; *, ** and *** indicate p<0.05, p<0.01 and p<0.001 compared with control group.](image-url)
The in vitro diffusion study through an artificial synthetic membrane using the Franz diffusion cell is considered at present the most appropriate method for assessment of drug release from the vehicle, being of great importance as it allows ascertaining that the drug release from the vehicle has occurred and was not the rate-limiting step for penetration and partition into the skin. In vitro drug permeation through skin is an alternative technique to in vivo studies in humans for evaluating the bioavailability of a topical formulation [16].

The permeation profiles of ibuprofen commercial hydrogel through synthetic membrane and through mouse skin was determined in accordance with the Korsmeyer-Pepas model equation \( R^2 > 0.9 \) without lag time.

Exposure to ultraviolet radiation induces a series of biochemical and immunologic changes at skin level that determine inflammation characterized by sunburn cells, dermal edema, depletion of Langerhans cells, endothelial cell enlargement and neutrophilic dermal infiltrate at a later time point [12].

The second objective of our study was to verify the effectiveness of ibuprofen administered topically in a mouse model of UVB-induced inflammation by monitoring the values of the physiological skin parameters (TEWL, skin moisture, erythema ass skin pH) using a non-invasive technique. Transdermal barrier loss (TEWL) is an important indicator of skin functionality and integrity and an increased value of this parameter is associated with damage at skin level [17, 18]. Our results showed that ibuprofen administered topically determined an improvement of this parameter as compared to control group (group 0). In addition, the hydration skin state evaluated by moisture values was higher in the group that received ibuprofen (fig. 3). These data indicate the beneficial effects of ibuprofen at skin level against UVB toxicity.

Another parameter with a key role in skin pathology, diagnosis and surveillance is erythema [18]. In the present study erythema was measured by a Mexameter® MX 18 probe and it was observed that ibuprofen treatment induced a decrease of erythema as compared to control group what indicates the antiinflammatory effect of ibuprofen.

Similar antiinflammatory effects after oral administration of ibuprofen were described in a study designed on C57BL/6j with ulcerative dermatitis [2]. Rother and co-workers [12] showed in a study developed on humans that ibuprofen orally administrated suppressed UVR-induced pain and inflammation.

Conclusions

This study has provided an evaluation of ibuprofen in vitro transport from marketed Nurofen® hydrogel across synthetic hydrophilic membrane and mouse skin. Comparison between in vitro data revealed a 2 fold higher permeability of synthetic membrane than that of mouse skin. Furthermore, the topical administration of ibuprofen in vivo in a mouse model of UVB-induced inflammation improved the physiological skin parameters and attenuated inflammation.

References


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