

Evaluation of Hydroxyethyl diclofenac as Possible Prodrug for Topical Application

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Abstract

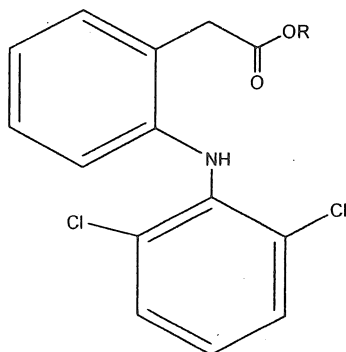
In vitro permeability of 2-hydroxyethyl diclofenac (a possible diclofenac prodrug), diclofenac sodium and diclofenac diethylamine through rat skin were studied using Franz diffusion cells. The partition coefficients of 2-hydroxyethyl diclofenac and diclofenac diethylamine between 1-octanol and acetate buffer at pH 7.4 were 100.6 and 30.97, respectively while the solubilities of 2-hydroxyethyl diclofenac, diclofenac sodium and diclofenac diethylamine were 0.08, 21.97, and 17.73 mg/ml respectively. The prodrug was chemically stable in the donor solution (5% propylene glycol and methanol) and in the receptor solution (20% propylene glycol and acetate buffer pH 7.4) throughout the time of the experiment. On the other hand, it hydrolyzed totally in the skin and only the parent drug was detected in the receptor solution. Diffusion experiments through rat skin showed a greater steady state flux for 2-hydroxyethyl diclofenac by 30% and 12% higher than diclofenac sodium and diclofenac diethylamine respectively. Addition of the penetration enhancers, dimethylsulfoxide and dimethylformamide, increased the steady state flux of 2-hydroxyethyl diclofenac about by 4 fold, while the addition of polysorbate 60 had no effect on the penetration of the prodrug.

Key words: Diclofenac; prodrug; NSAID; topical application; penetration enhancer.

Introduction

Diclofenac (I) is a nonsteroidal anti-inflammatory drug (NSAID) used for the treatment of pain and inflammation. Given orally, gastrointestinal (GI) side effects constitute the most frequent of all the adverse reaction of NSAIDs (Peter *et al.*, 1988; Katzung, 1989; Price *et al.*, 1990). Therefore, percutaneous administration of NSAIDs has been studied as a possible means to minimize the GI side effects. Also, a topically administered drug would be more suitable for treatment of local inflammation (Vanden *et al.*, 1989; Baixauli *et al.*, 1990). One of the approaches that are used to improve the efficiency of drugs for the dermal route is the prodrug approach (Samir *et al.*, 1994; Jarkko *et al.* 2000). Because the epidermis is relatively rich in nonspecific esterase and other enzymatic activities, the prodrug approach has been increasingly used to improve delivery of a drug through the skin and / or to localize drug action within the skin (Brian, 1983; Jarkko *et al.*, 1988). 2-Hydroxyethyl diclofenac (HED, II) was synthesized and evaluated as possible prodrug. It shows $t_{1/2}$'s of 36.66, 22.07 and 1.123 hours in solutions of pH 7.4, 1 and plasma respectively (Jilani *et al.*, 1997). These results and the expected higher partition coefficient of HED, when compared to diclofenac, encouraged us to study the transdermal behavior of HED as topical diclofenac prodrug.

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Diclofenac (I) R=H
 HED (II) R=CH₂CH₂OH

In the present study, HED has been evaluated for topical drug application. Its aqueous solubility, partition coefficient and hydrolysis through rat skin have been studied. The permeability of HED in comparison with diclofenac sodium and diclofenac diethylamine with and without penetration enhancers will be presented.

Materials and Methods

Chemicals: HED was supplied by Dr. J. A. Jilani, Jordan University of Science and Technology, Department of Pharmacognosy and Medicinal Chemistry. Diclofenac sodium and diclofenac diethylamine were supplied by APM (Salt-Jordan).

The reagents used: Methanol, acetonitrile, propylene glycol, dimethyl sulfoxide and N,N-dimethyl formamide were HPLC grade (LAB-Scan). Polysorbate 60 was analytical grade (Aldrich). Methanol and acetonitrile were used for HPLC (Lab-Scan). Dihydrogen sodium acetate and glacial acetic acid analytical grade (Fluka). Propylene glycol, dimethylsulfoxide and N, N-dimethylformamide analytical grade Lab scan analytical science (Dublin, Ireland). Polysorbate 60 analytical grade was supplied by Aldrich (Germany). All chemicals were used as supplied and water used in all experiment was deionized after distillation.

Methods and equipments: The HPLC was performed using Beckman pump (Beckman Instruments Inc., Berkeley, USA), equipped with 50 µl loop injection valve, and with a variable wavelengths detector; Jasco 875-UV Intelligent UV/VIS Detector (Jasco corporation, Japan). In this study a reversed-phase HPLC columns, 250 x 4mm packed with Purosphere, RB-18 5 µm particles (Merck, Germany) was used to analyze diclofenac salts and HED. The mobile phase consists of acetonitrile: sodium acetate 0.006M buffers (140:90). The pH adjusted with glacial acetic acid to be 5.64. The flow rate of the mobile phase was 1ml/min and the detector wavelength was set at 280 nm at a sensitivity of 0.01AUFS. The column effluent was monitored using Thermo Separation Product Chromjet Integrator. Readings of the pH values were done using pH meter, model 3310 Jenway, (UK). The wavelength was determined using UV-VIS record spectrophotometric scanner, Model Opl- 2Kyoto, (Shimadzu Inc, Japan). Diffusion experiments were done using glass Franz diffusion cells. Skins were obtained from white Sprague-Dawley rats.

Determination of the partition coefficient and the saturated solubility of HED: The apparent partition coefficients (P_{app}) of the compounds under investigations were determined at room temperature in 1-octanol/acetate buffer pH 7.4. Presaturated solutions of the drugs in the buffer were stirred vigorously with equivolumes of octanol for 60 minutes using a vortex apparatus to

achieve the equilibrium; the phases were then separated by centrifugation at 4000 rpm for 10 minutes. Each experiment was repeated six times and the concentrations of the compounds in the buffer and octanol were determined by the HPLC method described earlier.

The solubility of HED in the buffer medium was determined at room temperature by placing excess amount of the compound in the buffer system at pH 7.4. The slurries in acetate buffer were placed in an ultrasonic bath for 3 minutes and stirred in a water bath for 4 hours (preliminary studied showed there no hydrolysis of the prodrug over this time). The slurries were then filtered through a Millipore 0.22 μm film. The first ml of the solution was discarded to assure the saturation of the membrane. The concentration of the compound in the saturated solution was determined by the HPLC method described earlier. The solubility of the prodrug was determined in mg/ml.

Determination of the saturated solubility of HED in the chemical enhancers solutions: The saturated solubilities of HED in the acetate buffer containing either 20% dimethylsulfoxide or 20% dimethylformamide or 2% of polysorbate 80 all media being adjusted to pH 7.4, were determined by sonicating an excess amount of HED for three minutes in each penetration enhancer solution at room temperature. Then the slurries were placed in the 25 °C water bath for up to 3 hours. The slurries were then filtered through 0.22 μm Millipore filters and proper dilution was done to reach an acceptable absorbance. The prodrug concentration in the filtrate was measured using the HPLC method mention earlier. The hydrolysis of HED was negligible during the solubility studies.

Skin permeation studies Skin from white Sprague-Dawley adult male rats weighing about 250 gm were used in the permeation studies. The animals were first anaesthetized with ether and then sacrificed by cervical dislocation. Hair was removed with electrical hair clipper. One or two circular pieces of the skin were taken from the mid dorsal area of each rat using surgical blades. Subcutaneous fatty tissue was removed from each membrane with forceps, and the full thickness of the skin was used. Each skin section was washed with normal saline and fixed on Franz diffusion cell with surface area of 1.766 cm^2 . The compounds under investigation, namely, HED, Diclofenac Na, and diclofenac diethylamine, were dissolved in the donor solution (5% w/v propylene glycol in methanol) at level of 1mg/ml concentration. The composition of the selected donor solution assure suffecient stability and solubility of HED through out the time of experiment in addition of its week delipidizing activity on the skin (Bhatt *et al.*, 1991) .1 ml of this solution was placed on the top of the skin mounted in the diffusion cell and incubated at 37 °C. Samples were collected at three hours intervals for 48 hours and analyzed using HPLC system. Each experiment was repeated 3 times.

Data analysis : Skin flux was determined from Ficks first law of diffusion $J = (dM/dt)/A$, where dM/dt is the amount of the drugs in μg penetrated per hour, while A is the effective diffusion area 1.766 cm^2 . Steady state flux (J_{ss}) was determined from the slope of the linear portion of a comulative penetration- time curve, and lag time (t_{lag}) was obtained by extrapolating the linear portion curve to the abscissa. The permeation coefficient P cm/hr was then calculated from the equation:

$$J_{ss} = PC \dots\dots\dots (1)$$

Stability of HED The stability of HED in the donor system, receiver system, penetration enhancers solutions and through rat skin were evaluated as follows.

Evaluation of stability through rat skin Two diffusion cells were used to evaluate the hydrolysis of HED in the skin. Cellulose membrane was placed in one cell, while rat skin was mounted in the second cell. 1ml of the drug solution (1 mg/ml in 5% w/v propylene glycol in methanol) was placed at the top of the each cell, samples were withdrawn at regular time (each 8 hours) interval for 48 hour at 37 °C, then the samples were analyzed using HPLC. The above experiment was repeated three times.

Evaluation of the stability of HED in the donor and the receptor systems: To assess the stability of HED in the donor system, three diffusion cells containing cellulose membrane were set up, one ml of the donor solution (5% w/v propylene glycol in methanol) containing 1mg/ml of HED was placed at the top of the membranes. Samples from the donor solution were taken initially at zero time then at defined time intervals (each 8 hours) for 48 hr at 37 °C. The samples were analyzed after proper dilution to assess the stability of the drug using HPLC.

On the other hand, the stability of HED in the receiver system was done as follows: Three diffusion cell each containing a known amount of HED dissolved in the receptor medium (20% w/v propylene glycol in acetate buffer at pH 7.4) were incubated for 6 hours at 37 °C. Samples from the receptor medium were taken at (0,1,2,3,4,5,6 hours) and analyzed for the HED and diclofenac using HPLC.

Effect of the chemical enhancers on the skin permeation of HED: To study the effect of the different chemical enhancers on the skin penetration of HED, each one of the penetration enhancers was dissolved separately in the donor system containing HED yielding three different solutions: 1mg/ml HED, 20% w/v DMSO; 1mg/ml HED, 20% w/v DMF and 1mg/ml HED, 2% w/v polysorbate 60. 1ml of the above solutions was placed on the top of the skin of each diffusion cell which was incubated at 37°C. Samples were collected from each solution at 3 hours intervals for 48 hours and analyzed using HPLC system.

Results and Discussion

The stability studies conducted showed that HED is sufficiently stable in the donor and the receiver systems throughout the time of experiment. On the other hand, only diclofenac and not HED was detected in the receiver system in the skin diffusion studies with lag time of 14 hours. This result indicates rapid and complete hydrolysis of HED by skin esterases. One of the main aims in transdermal prodrug delivery is to obtain a prodrug that does not only exhibit increased lipid solubility but also maintains significant water solubility compared with the parent drug (Marriane *et al.*, 1986; Suresh *et al.*, 1991). The solubility of the HED in acetate buffer was compared with the solubility of the diclofenac sodium and diclofenac diethylamine, Table 1. HED, as expected has relatively less water solubility than diclofenac and its salts at pH 7.4 due to ionization.

Table 1. Physicochemical drug properties at pH 7.4 in acetate buffer.

Drug	M.W. (g/mol)	M.P. °C	Partition Coefficient	Solubility (mg/ml)
Diclofenac acid	296.14	171.6	64.04±1.0	11.74
Diclofenac sodium	318.12	294.3	25.99±5.3	21.97
Diclofenac diethylamine	367.26	155.5	30.97±1.0	17.73
Hydroxyethyl-diclofenac	340.19	84.12	100.64±2	0.08

The solubility of diclofenac salts and its prodrug, HED, in the receiving medium (20% propylene glycol in acetate buffer at pH 7.4) were 39.2 mg/ml for diclofenac sodium, 27.33 mg/ml for diclofenac diethylamine and 0.2698 mg/ml for HED. These solubility values satisfy the sink condition in the receiving medium for the compounds under investigation.

The solubility of HED in the donor system increases in the presence of penetration enhancers as shown in Table 2. Both DMSO and DMF are polar organic solvents capable to solubilize both hydrophilic and hydrophobic drugs. Concerning polysorbate 60, the solubility was relatively very high, possibly due to the formation of micelles.

Table 2: solubility of HED in the penetration enhancers

<i>Penetration enhancer</i>	<i>Solubility of HED (mg/ml)</i>
Dimethylsulfoxide	0.49159
Dimethylformamide	0.96648
Polysorbate 60	4.075610

As regards the partition coefficient, diclofenac has relatively high partition coefficient in comparison with its salts, diclofenac sodium and diclofenac diethylamine, while HED, a non-electrolyte, exhibits the highest partition coefficient. Table 1.

As shown in Table 3, HED shows a 30% increase in the steady state flux compared with diclofenac sodium, and a 12% increase compared with diclofenac diethylamine. This increase can be attributed to the increase in the partition coefficient of 2-hydroxyethyl diclofenac. Diclofenac sodium and diclofenac diethylamine exist mainly in the ionized form at the pH of the skin (5.5). They show high water solubility and less lipid solubility than the prodrug. Rate of dissociation of the salt will affect skin flux. The unionized part of the salt is the suggested species that can penetrate the lipid dehydrated stratum corneum. Other investigators showed that ionized diclofenac salts can also penetrate the skin through the hydrophilic pores that exist in the skin but the extent of this penetration through these channels is not clearly defined (Manabe *et al.*, 1996). It was reported that the maximum permeability coefficient of diclofenac was observed at around pH 3.0-4.0, suggesting that the charge on the skin surface was neutralized and that the lipophilicity of the skin might be maximized at these pH values. Further increase in the pH of the donor solution led to significant decrease in the permeability coefficient of diclofenac. The permeability of diclofenac at pH 3 was about 100 fold greater than that at pH 7 (Yasuko *et al.*, 1993). In percutaneous absorption, it is generally accepted that the penetrant must have a well-balanced lipophilic-hydrophilic property, in our experiment diclofenac diethylamine appears to permeate the skin faster rate and higher level than diclofenac sodium due to the difference in their partition coefficient. On the other hand, the relatively high flux of HED can be attributed to its suitable physicochemical properties resulted from masking the carboxylate moiety with the hydroxyethyl group.

Table 3: Effect of enhancers on skin transport parameters

<i>Compound</i>	<i>J_{ss}</i> ($\mu\text{g/hr.cm}^2$)	<i>Permeation coefficient</i> (cm/hr)	<i>Lag Time (hr)</i>
Dimethylsulfoxide	7.33 \pm 0.23	7.33exp-3 \pm 3.4exp-4	10.47 \pm 1.69
Polysorbate 60	1.76 \pm 0.01	1.76exp-3 \pm 2.7exp-4	14.03 \pm 4.65
Dimethylformamide	6.38 \pm 0.361	6.38exp-3 \pm 1exp-4	10.86 \pm 1.47
Diclofenac sodium	1.29 \pm 0.06	1.29exp-3 \pm 1.6exp-4	15.23 \pm 2.60
Diclofenac diethyl-amine	1.502 \pm 0.14	1.502exp-3 \pm 4exp-4	14.02 \pm 1.33
Hydroxyethyl- diclofenac	1.69 \pm 0.198	1.69exp-3 \pm 5.5exp-4	13.25 \pm 1.03

In addition to the prodrug approach, utilizing penetration enhancers represents an alternative way to improve skin penetration. In this study, we attempted to combine the prodrug and the penetration enhancer techniques to maximize the skin permeability. As shown in Table 3, dimethylsulfoxide and dimethylformamide increase the steady state flux of HED by 4 fold as a result of the nature and the mechanism of these penetration enhancers. DMSO can affect the permeability of the skin in different ways depending on their concentration (Harry *et al.*, 1988; Angela *et al.*, 1995). At the concentration applied in our work the increase in the flux may be

attributed to the change in protein conformation from alpha helical to beta-pleated sheet (Angela *et al.*,1995). The chemical similarity of both the dipolar aprotic solvent, DMSO and DMF explains their similar penetration enhancement character. On the other hand, polysorbate 60 did not show significant increase in drug permeability. This can be attributed to the very low ability of polysorbate to extract the membrane component and increase the fluidity (Brian 1983).

From the data generated in this study, it can be concluded that blocking the carboxylate moiety of diclofenac together with the increase in partition coefficient lead to significant increase in the steady flux through skin barriers. Also it can be concluded that utilizing penetration enhancers together with the prodrug may lead to better skin penetration.

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