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Transdermal Delivery of Imipramine Hydrochloride: Development and Evaluation (*in vitro* and *in vivo*) of Reservoir Gel Formulation

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ABSTRACT: The *in vitro* permeation studies of imipramine hydrochloride (IMH) reported earlier from our laboratory showed that a combination of menthol (2.5% w/v) and oleic acid (2.5% w/v) worked well in terms of safety and efficacy. The main objective of this study was to evaluate the *in vivo* performance of this combination; in order to do that, penetration enhancers were incorporated in a hydro-alcoholic gel of hydroxypropylmethyl cellulose along with IMH and used as the drug matrix in a reservoir transdermal patch. A stability study of IMH gel was performed at 40°C/75% RH for 2 months. The results of this study indicate that gels of IMH stored at 40°C/75% RH turned yellow brown in 2 months and the small change in viscosity of gel at 40°C/75% RH had an insignificant effect on the release rate of IMH from the gel ($p>0.05$). The *in vivo* performance of the gel was tested in rats using a reservoir transdermal patch, which consisted of a backing membrane, drug matrix and retaining membrane with an area of 12.5 cm^2. Plasma concentrations of 3 μg/ml of IMH were achieved and in a histopathological study 24 h occlusion was found to be safe. Copyright © 2004 John Wiley & Sons, Ltd.

Key words: tricyclic antidepressants; reservoir patch; pharmacokinetics; menthol and oleic acid

Introduction

Tricyclic antidepressants (TCAs) are widely used in the treatment of unipolar depression characterized by extreme sadness, despair and anhedonia [1]. It has been reported that up to 70% of patients who are prescribed oral antidepressants fail to take 25% to 50% of their prescribed dose [2]. The main reason for this non-compliance is the disbelief in the value of the medication due to the long lag time of 2–3 weeks before therapeutic benefit can be realized [1]. At the same time adverse effects may appear within a few hours of administration of TCAs; these include anticholinergic states, insomnia, sedation and postural hypotension [1]. Hence, development of a suitable Transdermal Drug Delivery System (TDDS) offers a possible approach to overcome the drawbacks of systemic TCA therapy via the oral route. The use of TDDS offers inherent advantages such as: (a) avoidance of first pass metabolism; (b) control of input rates; (c) avoidance of problems associated with delayed stomach emptying, pH effects and enzymatic deactivation within the gastrointestinal tract. A constant and stable plasma concentration, achievable through transdermal delivery, makes this system suitable for drugs such as TCAs. Furthermore, the probability of success in therapy is enhanced

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due to better patient compliance and improved patient convenience; more importantly, its use requires a lesser degree of motivation.

The TCAs are good candidates for transdermal delivery because of their pharmacokinetic and pharmacodynamic properties. However, their poor permeability and the high doses that are required present obstacles to delivery across the skin. Panchagnula (1996) and Stott *et al*. (1996) have suggested the use of solvents, e.g. dimethylsulfoxide and dimethylacetamide and the coacervation technique, respectively, for enhancement of permeation by TCAs through the skin [3,4]. Previously in our laboratory different penetration enhancers (terpenes and fatty acids) were screened for their ability to enhance the permeation of TCAs. Among the terpenes, the maximum flux of imipramine hydrochloride (IMH) was found with 5% w/v menthol [5], whereas in the case of fatty acids (5% w/v) there was no significant difference in IMH flux between saturated and mono- and poly-unsaturated fatty acids with either the *trans* or *cis* conformation [6]. Subsequently, a combination of menthol and oleic acid at a concentration of 2.5% w/v of each was found to be both safe and as effective as 5% w/v menthol and 5% w/v oleic acid when used individually [7]. In this investigation, therefore, this combination was selected for formulation development and for both *in vitro* (drug release rate and permeation of IMH across excised skin) and *in vivo* (plasma concentration profile) evaluation. Since the chosen solvent system consisted of ethanol and water along with menthol and oleic acid as penetration enhancers, the non-ionic cellulose polymer derivative hydroxypropylmethyl cellulose (HPMC, Methocel) was selected as the gelling or viscosity building agent. HPMC is less hydrophilic than methylcellulose (Methocel K4 M); therefore it could be easily solubilized in the chosen solvent system, which contained a high volume fraction of ethanol (i.e. 66% v/v) [8].

Materials and Methods

IMH and oleic acid were procured from Sigma Chemicals Co., USA. ^{3}H-IMH (specific activity, 48.00 Ci/mmol) was obtained from Du Pont, Willington, DE, USA. Menthol and ethanol were purchased from Merck, Germany. Scintillation cocktail (BCS 104) was purchased from Amersham International plc, UK. All other chemicals used were of reagent grade. Polymeric membranes, e.g. Scotchpak^{TM} 1009, CoTran^{TM} 9711 and Scotchpak^{TM} 1022, used in the fabrication of the patch were received as gratis samples from 3 M (USA). Methocel K4 M was also received as a gift from Colorcon Asia Pvt Ltd, India.

Formulation of gels

All gels were prepared in 10 ml glass vials (Borosil, India) by the soaking method. Penetration enhancers (menthol and oleic acid 2.5% w/w of each) along with 7.5% w/w of IMH were solubilized in 75% of the required volume of ethanol:water (2:1) system. Then the polymer was added slowly into the prepared solution of penetration enhancers and IMH with constant stirring and the weight was made up to 100% by addition of a sufficient quantity of ethanol:water (2:1). This was kept overnight to allow complete swelling of the polymer. The gel was homogenized to break up any lumps of swollen polymer and was then stored at 2°–8°C in a refrigerator until required for *in vitro* evaluation and fabrication of the patch.

In the preparation of gels containing radioactive IMH, ^{3}H-IMH was added to ethanol:water (2:1) prior to addition of polymer.

Analysis of IMH

In gel formulation. Reverse phase high performance chromatography was used to determine the IMH content of the gel formulation. A HPLC system with a pump (Water^{TM}, USA), autosampler (Water^{TM}, USA) and variable UV absorbance detector (Water^{TM}, USA) was used. The chromatography system was interfaced to Millenium Chromatography Manager software (Water^{TM}, USA) for instrument control, data acquisition and analysis. The mobile phase reported by Gandhi *et al*. [9] was modified by the addition of TEA (ACN:phosphate buffer pH 3.0, 50 mM in 40:60 proportion along with 0.5% v/v of TEA) and used in the analysis of IMH. A 50 µl sample was injected into a C-18 column (Symmetry®

5 μm, 4.6 × 250 mm, 100 Å, Water™, USA) attached to a C-18 guard column (Nova-pack, Water™, USA). The flow rate of the mobile phase was 1 ml/min and samples were analysed at room temperature. IMH was monitored at 250 nm [9]. The limit of detection and quantification of this modified HPLC method were 50 ng/ml and 400 ng/ml, respectively.

Sample preparation. IMH standards for use in constructing calibration curves were prepared in the mobile phase along with menthol and oleic acid to rule out any possible interference by penetration enhancers. The IMH content of the gel was quantified using a linear calibration curve prepared over the range 0.4 μg/ml to 24.0 μg/ml. During sample processing IMH from the gel was completely extracted in ethanol:water (2:1) with the aid of sonication for 30 min. Solutions of appropriate concentrations were made by dilution from a stock solution with mobile phase followed by centrifugation at 5900 × **g** for 10 min (Heraeus, Germany) and then IMH was quantified by HPLC.

In vitro diffusion study. UV-spectroscopy was used to quantify IMH present in *in vitro* diffusion samples. In order to prepare the calibration curve for IMH, an accurately weighed amount of drug was solubilized in water (Elga, USA). The drug solution was sonicated for 10 min in a bath sonicator (Branson, USA) and after cooling, an appropriate volume of water was added to obtain a concentration of 1.0 mg/ml. From this stock solution a linear calibration curve for IMH was generated over the concentration range 5 μg/ml to 30 μg/ml by measuring absorbance at 250 nm.

In vitro evaluation of gel

Permeation studies. To establish the rate-limiting step in transdermal permeation of IMH from gel, *in vitro* permeation across the rat skin was studied and compared with *in vitro* permeation of IMH in solution. Rat skin was prepared as described elsewhere [3] and frozen at −20°C until required. IMH permeation across the skin was determined ($n = 4$) using unjacketed Franz cells with a diffusion surface area of 0.785 cm^2. The cells were placed on a dry heater and stirrer block (Permegear, USA). Phosphate buffer (pH 7.4) containing 0.01% w/v sodium azide as a preservative was used as the receptor medium. The receptor fluid was sonicated to remove dissolved gases and equilibrated at 37°C before placing in the receptor compartment. Cells were filled with receptor fluid and thawed rat skin was mounted in the diffusion cell with the dermal side in contact with the receptor medium and allowed to equilibrate at 37°C for 8–10 h. 200 μl of gel or solution containing 75 mg/ml of IMH along with or without penetration enhancers was applied to the *stratum corneum* (SC) side in the donor compartment of the diffusion cell. Since the analytical method used in this study was radiochemical analysis, the solution applied in the donor compartment was spiked with tritium labelled IMH (1 μCi/ml). After application of the drug solution, samples (200 μl) were withdrawn from the receptor compartment at predefined time intervals for 12 h. The sample volume was immediately replaced with fresh receptor medium after each sampling. The collected samples were analysed by radiochemical analysis. Biodegradable scintillation cocktail (Amersham, UK) was mixed with the aqueous *in vitro* samples (200 μl) in 8 ml diffuse scintillation vials (Tarson, India) and vortexed for 30 s. Radioactivity was measured (Wallac, Finland) after 12 h to minimize chemiluminescence. During measurement of each sample batch, three blanks (200 μl of buffer, as used in the receptor compartment) were included to determine background radioactivity.

Stability studies. To establish the structural integrity and chemical stability under accelerated storage conditions, the developed gel was studied over a period of 2 months in accordance with regulatory guidelines. The gel containing IMH was sandwiched between the backing membranes and heat-sealed (Sevana, India) on all sides. Samples were stored in a refrigerator (2°–8°C, controls) and at 40°C/75% RH for periods of up to 2 months. The gel formulation was evaluated for appearance and drug content at time intervals of 1, 2, 4 and 8 weeks. In order to study the effect of storage conditions on the release rate of IMH from the gel due to possible

changes in gel structure, *in vitro* drug release studies were conducted at these same times [10]. The release profile of IMH from the gel was determined over 12 h with the same experimental set-up and conditions as outlined above, except that a polyethylene microporous membrane (CoTran^{TM} 9711, 3 M, USA) was sandwiched between the donor and receptor compartments in place of the rat skin and water was used as the receptor medium in place of the phosphate buffer. The concentration of IMH in samples collected during the diffusion studies was determined by UV-spectroscopy (Perkin Elmer, Germany) as described above.

Rheological measurements were performed separately using blank gel (containing penetration enhancers without drug) to study possible changes in gel structure during the course of stability testing. Drug-free gel was prepared and subjected to accelerated stability studies as described above. Viscosity measurements were performed at 32°C using a Brookfield Viscometer (Brookfield Engg., USA). The gel viscosity was measured at 20 rpm over a period of 2 min using a SC4-21 spindle; the rheogram was interfaced to a Rheocalc V2-1 (Brookfield Engg., USA) for instrument control, data acquisition and analysis.

Fabrication of patch

Transdermal patches of IMH were prepared manually by the heat-sealing method. Patches consisted of a backing membrane (Scotchpak^{TM} 1009, 3 M, USA), a polyethylene microporous membrane (CoTran^{TM} 9711, 3 M, USA), peripheral adhesive and a release liner (Scotchpak^{TM} 1022, 3 M, USA). In order to fabricate the transdermal patch, the gel was sandwiched between the backing and the polyethylene microporous membranes. In order to avoid the evaporation of ethanol and to protect the site of drug release from unwanted contacts a release liner was used.

In vitro and in vivo evaluation of patch

Fabricated patches were characterized for *in vitro* permeation across the rat skin using a similar experimental set-up to that described above except that in place of the gel an experimental transdermal patch of IMH was applied to the skin in the donor compartment.

The pharmacokinetic profile of IMH following intravenous administration was investigated in anaesthetized Sprague-Dawley rats (200–300 g, $n = 6$) of either sex. The animals were housed at 25°C, with alternating dark–light cycles of 12 h each, and were provided with free access to food and water. IMH (2 mg/kg) in saline spiked with 2 μCi/ml of ^{3}H-IMH was administered to each rat over a period of 10 s through the jugular vein. Blood samples of 0.4–0.5 ml were withdrawn from the retroorbital plexus and collected in a heparinized (7.5 IU) microcentrifuge tube (Tarsons, India) at time intervals of 20, 40, 60, 120, 300, 480, 720 and 1440 min. Saline (0.3–0.5 ml) was given to the rat intraperitoneally to minimize any change in the volume of the central compartment. Samples were centrifuged (Heraeus, Germany) at 5900 × **g** for 10 min. The IMH concentration in plasma was determined by radiochemical analysis as described above.

To test *in vivo* performance, IMH transdermal patches of approximately 12.5 cm^2 containing 500–900 mg of gel were applied to the previously shaven abdominal skin of anaesthetized (urethane, 1.2 g/kg) Sprague-Dawley rats (200–250 g, $n = 6$) of either sex. Blood samples were collected and processed in a similar manner to that described for the case of i.v. administration of IMH at time intervals of 2, 5, 8, 11, 15, 24 and 30 h. The IMH in the plasma samples was quantified by radiochemical analysis as discussed for the i.v. administration of the IMH.

Following the administration of IMH by the transdermal patch, the dose absorbed was estimated by subtracting the dose remaining in the patch from the amount applied. The amount of drug present in the skin was determined as follows: the rat skin over which the transdermal patch had been applied was removed surgically and cut into small pieces. Each piece was solubilized in 500 μl of tissue solubilizer (tissue solubilizer was custom-made in the NIPER transdermal drug delivery laboratory, consisting of toluene, t-butyl ammonium hydroxide, TritonX-100 and water). After addition of scintillation cocktail (5 ml) and 100 μl of glacial acetic acid to each glass scintillation vial [11], the samples were counted for radioactivity in a liquid

scintillation counter (Wallac, Finland). In order to do a mass balance calculation, the remaining amount of IMH was determined in the patch after application for 24 h. IMH was extracted from the patch or gel using the ethanol:water (2:1) system in a screw cap specimen jar (Jain Scientific, India) with constant shaking at 37°C (Julabo, Germany). After 24 h the gel was completely solubilized in EtOH:W (2:1). 100 µl of solubilized gel was mixed with 5 ml of biodegradable scintillation cocktail (Amersham, UK) and vortexed for 30 s and the radioactivity was measured after 12 h.

Histopathological study

Skin occluded by the patch for 24 h was fixed with 10% v/v formalin solution in saline for at least 48–72 h before routine processing and then cut, vertically to the skin surface at the central region, in 4 mm sections [12]. Each section was dehydrated using a graded series of ethanol solutions and embedded in paraffin wax. Tissues were sliced (4–7 µm thick) and stained with haematoxylin and eosin. All sections were then examined using a light microscope (Leica, Germany) at x250 magnification.

Data analysis

Flux values ($\mu g.cm^{-2}.h^{-1}$) were estimated from the slopes of the steady-state portions of cartesian plots of the cumulative amount of drug present in the receptor compartment versus time.

Equation (1), which represents an open two-compartment model, was fitted (PCNonlin, Version 4.0, SCI software, Scientific Consulting Inc., USA) to the plasma concentration–time data obtained following a single i.v. administration of IMH

$$C_t = Ae^{-\alpha t} + Be^{-\beta t} \quad (1)$$

where C_t is the drug concentration at time t; α, β are distribution and terminal elimination rate constants, respectively, and A and B are the biexponential equation constants.

In order to analyse the plasma concentration–time profile observed after the single application of a transdermal patch, Equation (2), which assumes a constant rate of absorption of drug (R_p) following a lag time (t_{lag}), was used and the plasma concentrations (C_t) of IMH after transdermal application were predicted by the following equation [12]. R_p is the input rate (mg/h) based on the *in vitro* flux of IMH and the surface area used in the the *in vivo* study and t_{lag} is the lag time during *in vitro* permeation study across the rat skin

$$C_t = \frac{R_p}{V_{10}k_{10}} \times \left\{1 + \frac{\beta - k_{10}}{\alpha - \beta}(e^{-\alpha(t-t_{lag})}) + \frac{k_{10} - \alpha}{\alpha - \beta}(e^{-\beta(t-t_{lag})})\right\} \quad (2)$$

The areas under the plasma concentration–time profiles (AUC_{0-24}) after i.v. and transdermal administration were calculated by the trapezoidal method using all the experimental points. The absolute bioavailability (F) of IMH was calculated using Equation (3)

$$F = \frac{\text{Dose applied} - \text{Dose remaining in patch}}{\text{Dose applied}} \times 100 \quad (3)$$

Results and Discussion

Preliminary studies indicated that gels of 0.5% and 1.0% w/w were 'thin' and would be likely to give problems of leakage following heat-sealing of the backing and retaining membranes. Gel of 2.5% w/w was found to be thicker but difficult to handle in the preparation of transdermal systems containing IMH. It was found that the consistency of the 2.0% w/w gel was optimum and easy to handle. Hence, a 2% w/w concentration of polymer was selected for further formulation development.

Prepared gel was then evaluated for appearance, drug content, *in vitro* release and permeation. The gel was transparent in appearance. Since IMH is light sensitive, the gel containing IMH was immediately packed in sachets of backing membrane (Scotchpak™ 1009, 3 M, USA), capable of protecting the drug from light exposure. The IMH content of gels was found to be 97%. *In vitro* release studies through polyethylene microporous membrane (CoTran™ 9711, 3 M, USA) were performed to characterize the delivery of IMH from the reservoir gel. A first order release kinetic model was fitted to the

in vitro release data and the rate constant was determined by semi-logarithmic linearization of the drug release profile; this is related to the diffusion properties of IMH through the polymeric gel and membrane. The first order rate constant for IMH release from the gel through the polyethylene microporous membrane was determined to be $0.19\,h^{-1}$ ($r^2 = 0.9861$). Rapid drug release from the gel suggests that neither gel nor the polyethylene microporous membrane imposed any resistance to the diffusion of IMH; this implied that skin and not the gel would act as a rate controlling barrier in IMH permeation from the patch. This was also found to be true in the *in vitro* permeation studies of IMH from gel and solution where no significant difference in the lag time and flux values of IMH were observed (Figure 1). The gel was subjected to an 8-week physical and chemical accelerated stability study. The appearance of the gel kept in a refrigerator at 2°–8°C was unchanged during the study period. However, the colour of the gel stored at 40°C/75% RH had turned to a yellowish brown by week 8, though the transparency remained unchanged (Table 1). The first order

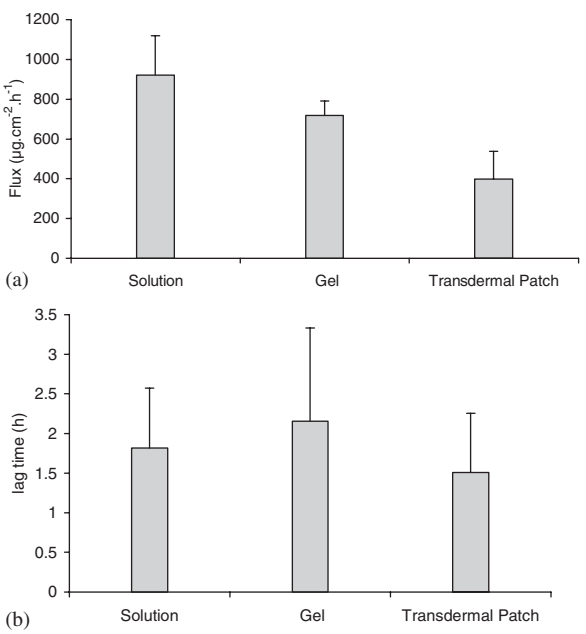

Figure 1. Comparison of flux and lag time of IMH. (a) *in vitro* flux across the skin in solution, gel and transdermal patch; (b) *in vitro* lag time of IMH in solution, gel and transdermal patch

Table 1. Stability studies of IMH gel: appearance, drug content and release rate [mean (SD), n=3]

	Week 0	Week 1		Week 2		Week 4		Week 8	
Parameter		2–8°C	40°C/75% RH	2–8°C	40°C/75% RH	2–8°C	40°C/75% RH	2-8°C	40°C/75% RH
Appearance	T	T	T	T	T	T	T	T	TYB
Drug content (%)	97.62 (3.57)	102.02 (7.79)	101.22 (16.22)	102.37 (3.65)	106.85 (20.05)	105.97 (3.36)	106.82 (0.99)	92.87 (16.39)	93.01 (13.94)
Release rate (h^{-1})	0.19[a]	0.17 (0.08)	0.14 (0.02)	0.12 (0.01)	0.16 (0.05)	0.24 (0.04)	0.20 (0.07)	0.21 (0.13)	0.22 (0.02)

T, transparent gel; TYB, transparent gel with yellow and light brown tint.[a]
n = 2

release rate constants for IMH from the *in vitro* release studies did not differ significantly throughout the study ($p>0.05$). Although the viscosity of the gel (without drug and penetration enhancers) stored at 40°C/75% RH increased over the first week, no significant change in the release rate of IMH from the gel was observed in the *in vitro* release study (Figure 2, $p>0.05$). The results of the study into the physical and chemical stability of the gel indicated that it was suitable for fabrication of a transdermal patch.

The results from the *in vitro* permeation study with the fabricated transdermal patch showed that the flux value of IMH across the skin from the patch was significantly different from the gel and solution ($p<0.05$) (Figure 1).

The plasma concentration–time profile of IMH after single dose i.v. administration declined in a biexponential fashion as shown in Figure 3. The pharmacokinetic parameters of IMH were calculated by fitting an open two-compartment model to the plasma concentration–time data (by PC Non-Lin software). The mean plasma concentration versus time profile of IMH following application of the transdermal systems to rats is shown in Figure 4. Analysis of the patches upon removal ($n=5$) showed that 38.0% ± 8.1% (mean ± SD) of the applied IMH was released over the initial 24 h period of the experiment. However, at 24 h 45.6% of the released IMH was present in the skin immediately beneath the patch as shown in Figure 5. The mean results of

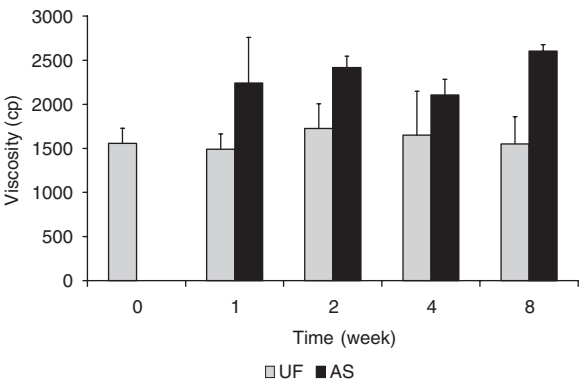

Figure 2. Effect of storage conditions on viscosity of HPMC gels prepared in EtOH:W (2:1) system; UF, at 2–8°C; AS, at 40°C/75% RH

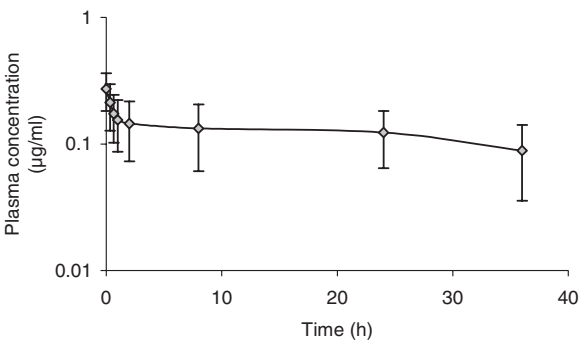

Figure 3. Plasma concentration of IMH vs time profile following single i.v. administration ($n=6$)

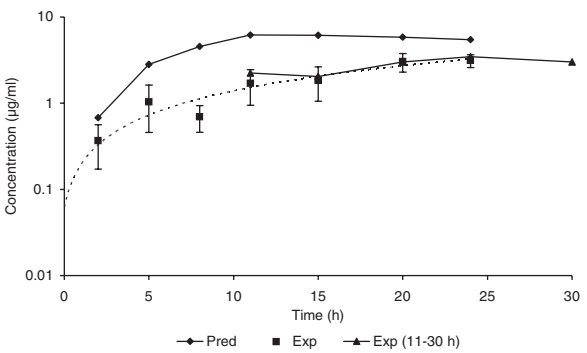

Figure 4. Plasma concentration of IMH vs time profile following the single application of transdermal patch ($n=6$). Inset picture shows magnified view of plasma profile of IMH until 30 h ($n=2$) in comparison with predicted and experimental plasma levels (24 h): Pred, predicted; exp, experimental; exp (11–30 h), experimental (11–30 h)

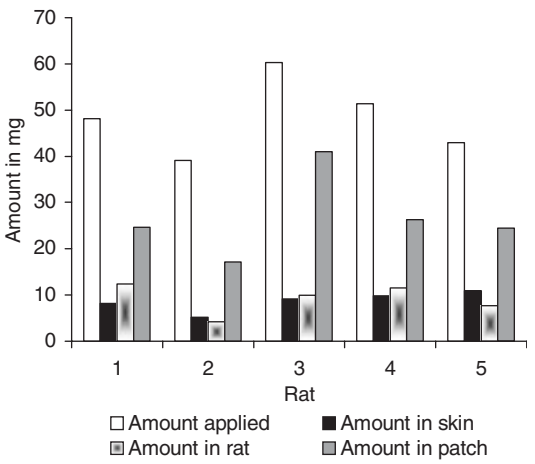

Figure 5. Distribution of IMH after 24 h application of transdermal patch in skin and body of rat, and amount remaining in patch applied to individual rat

Table 2. Pharmacokinetic parameters determined following i.v. and transdermal application of IMH, [mean (SD), $n = 6$]

Parameter	Administration route	
	i.v.	Transdermal
Dose (mg)	0.425	48.55 (8.16)
$AUC_{0-24\,h}$ ($\mu g.ml^{-1}.h$)	3.362 (1.65)	57.91 (10.97)
Amount absorbed (mg)	0.425	10.29 (3.29) in 24 h
α (h^{-1})	1.37 (0.480)	–
β (h^{-1})	0.012 (0.006)	–
k_{10} (h^{-1})	0.028 (0.004)	–
$t_{1/2}$ (h)	53.07 (17.950)	–
V_{ss} (l/kg)	13.40 (4.850)	–
Systemic bioavailability (in 24 h) (F, %)	100	21

the mass balance study therefore indicate that 55% of released IMH entered into the systemic circulation over the 24 h period that the patches were applied to the skin. The immediate absolute systemic bioavailability of IMH from the patches (i.e. over the initial 24 h) was therefore estimated to be 0.21. Although this may appear to be relatively low it should be remembered that it does not include the released drug present in the skin that formed a depot and from which IMH will continue to be absorbed. Also, it is probable that if the patches had not been removed, absorption of drug would have continued, resulting in increased bioavailability Table 2.

As can be seen from Figure 4, steady-state concentration levels were achieved after about 8 h and levels of IMH were maintained until the time of the last sample (24 h). Preliminary study results ($n = 2$) indicated that after 24 h, plasma concentrations of IMH (approximately 3 μg/ml) were maintained until 30 h (Figure 4); this highlights the ability of the delivery system to provide IMH input for an extended time period. The plasma concentrations of IMH achieved 20 h after the application of experimental patch are approximately 60–100 fold higher than those reported in rabbits following application of 160 mg of IMH in a 20 cm^2 transdermal patch [13]. In the present study higher plasma levels of IMH were achieved with the application of approximately 48 mg of drug, possibly due to the presence of penetration enhancers.

Histopathological studies were conducted to evaluate possible irritation caused by application of a transdermal patch containing penetration enhancers (menthol and oleic acid, 2.5% w/v each) with or without IMH. Total irritation scores (TIS) 24 h after the application of transdermal patch with or without IMH are given in Table 3. Microscopic findings and TIS after application of transdermal patches without IMH were similar to those found in the *in vitro* skin samples after treatment with the combination of penetration enhancers (Figure 6). After application of IMH transdermal patches, slight inflammatory responses in the form of cell infiltration were observed. It was noted that there was no significant change in microscopic findings between the *in vitro* skin samples after treatment with the combination of penetration enhancers and the skin over which a transdermal patch had been applied for 24 h.

Table 3. Irritation scores of rat skin before and after the application of patch with or without IMH

	Treatment	
	A	B
Epidermis liquefaction	1	0
Subepidermal edema	2	2
Dermis		
Collagen fibre swelling	1	1
Inflammatory cell infiltration	0–1	2
Total irritation score	4	5

Grade: 0, no change, 1, very slight; 2, slight; 3, moderate; 4, marked change. Treatment: A, transdermal patch without drug; B, transdermal patch of IMH.

Conclusions

The developed TDDS with an area of 12.5 cm^2 was found to attain plasma concentrations of 3.0 μg/ml in rats, which are approximately 15–35 times greater than the therapeutically effective concentration window (85 ng/ml to 250 ng/ml) in humans. Considering the fact that the human skin is less permeable than the rat skin, the results indicate the possibility of modifying the patch size ($\sim$50 cm^2) to achieve therapeutically effective concentrations in humans. Histopathological studies of rat skin after the application of transdermal patches for 24 h indicated that IMH caused little or no allergic response and no additional alteration in skin morphology even after occlusion for 24 h, providing evidence of the safety of the combination of penetration enhancers used in this transdermal system.

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