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Transdermal delivery of naloxone: skin permeation, pharmacokinetic, irritancy and stability studies

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Abstract

The current investigation aims to evaluate ex vivo, in vivo performance, stability and irritancy potential of a transdermal formulation of naloxone (NLX) developed at our laboratory at different concentrations (10, 20 and 30 mg/g of gel) in a transdermal reservoir patch. Ex vivo permeation studies were performed by employing porcine and rat skins. In vivo performance was assessed in Sprague–Dawley rats by single and multiple application of the patch. Further stability of the formulation was established for 3 months at accelerated stability conditions as per ICH guidelines. Amongst the barriers used the rat skin was found to be more permeable than the porcine epidermis and the flux across each barrier increased with increasing thermodynamic activity of drug in the gel. Based on ex vivo data, the surface area (SA) of the patch was predicted to be 39.6 cm^2 in order to achieve therapeutic blood levels. Upon single dose administration, the steady-state levels were maintained from 4–48 h, which proves the clear advantage of transdermal delivery system over the current mode of administration, i.e., intravenous (i.v.) bolus which is effective upto a maximum of 1.5 h. Upon multiple dose administration, the sustained steady state for 12 h, even after patch removal proves the formation of drug depot in the skin. The formulations were found to be stable with respect to NLX assay and penetration enhancer efficacy upto 3 months under accelerated stability conditions. The alteration of penetration barrier function, as evidenced by increased trans epidermal water loss (TEWL) was not accompanied by any significant amount of skin irritation measured using laser doppler velocimetry (LDV). The developed transdermal delivery system of NLX is efficacious, stable and safe upon single and multiple dose applications each lasting for 48 h.

Keywords: Naloxone; Transdermal delivery; Stability; Laser doppler velocimetry (LDV); Trans epidermal water loss (TEWL); Pharmacokinetics

1. Introduction

Drug abuse involves non-therapeutic use of drugs resulting in physical, mental and social harm to an individual. Morphine is the most frequently abused drug (Gutstein and Akil, 2001). Tolerance develops from its

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chronic use and thus user has to increase dose to get euphoric effect. In US, around three million people are opioid dependent among which about 9,80,000 are long term users (Raisch et al., 2002). Naloxone (NLX), is a non-specific, competitive opioid antagonist and is used to reverse opioid induced CNS and respiratory depression. NLX shows a short biological half life (64 min) (Ngai et al., 1976), following its intravenous (i.v.) administration. Owing to extensive first pass metabolism leading to poor bioavailability of about 2%, peroral route is not effective for its delivery. NLX shows a very short duration of therapeutic effect thus requires frequent administration by i.v. or intramuscular (i.m.) routes which are invasive (Gourlay and Coulthard, 1983). Transdermal drug delivery (TDD), being non-invasive offers an improved approach to administration of drugs by maintaining a therapeutic concentration of drug in plasma over a prolonged duration extending to several days (Cleary, 1993). Development of a TDDS will thus circumvent the current delivery problems associated with NLX. Feasibility of transdermal delivery of NLX was demonstrated across excised rat skin by Jaiswal et al. (1999) and human skin by Aungst et al. (1986). Panchagnula et al. (2001) have also studied the effect of water, ethanol (EtOH), propylene glycol (PG) and their binary combination for selection of a suitable solvent system of NLX transdermal delivery. In continuation of that, we have developed and optimized a gel formulation of NLX containing 5% oleic acid as penetration enhancer (Panchagnula and Khandavilli, 2004; Khandavilli and Panchagnula, 2002), which can be effectively incorporated in a transdermal patch for its delivery across skin. In the present study, a TDDS for NLX was fabricated in which the gel formulation containing different concentrations of NLX was incorporated. Ex vivo studies were performed on either gel or using the prototype patch to evaluate permeation profile of NLX across excised rat skin and porcine epidermis. Further, in vivo studies using a prototype reservoir patch were performed for NLX at three concentrations (10, 20 and 30 mg/g). Pharmacokinetic evaluation of the patch at single dose (10 mg/g) was also assessed by its multiple application at different sites on skin. Further, the irritancy potential of gel formulation was assessed in vivo in Sprague–Dawley rats by histopathology, laser doppler velocimetry (LDV) and trans epidermal water loss (TEWL) measurements. The stability of the formulation with respect to drug content and penetration enhancer efficacy was established by accelerated stability testing for 3 months.

2. Materials and methods

2.1. Materials

NLX and Klucel-HF (hydroxypropylcellulose (HPC)) were gift samples from Mallinckrodt, USA and Signet Chemicals, India, respectively. ^{3}HNLX (specific activity 52 Ci/mmol) was procured from Amersham Pharmacia biotech, UK. EtOH was supplied by Merck, Germany, and PG, sodium azide and oleic acid by Sigma Chemicals, USA.

2.2. Analytical method

All skin/membrane permeation samples were analyzed by radiotracer method using liquid scintillation counting (Wallac 1409, Finland). It is specific to NLX and free from interference from skin proteins and plasma components (Sznitowska, 1991). ^{3}HNLX (radiochemical purity 97.8%) (Amersham Pharmacia Biotech, UK) was used as tracer molecule. A series of blank samples were always run to account for background activity. Further, a RP-HPLC method was developed and validated for the analysis of NLX in stability samples. The method was found to be specific, sensitive, rugged and reproducible in the sample matrix (Panchagnula et al., 2004).

2.3. Preparation of formulation

All experiments were performed using NLX gel, prepared at different concentrations (10, 20 and 30 mg/g of gel). Gels were prepared based on a previously optimized formulation (Panchagnula et al., 2001, 2004; Panchagnula and Khandavilli, 2004; Jaiswal et al., 1999; Khandavilli and Panchagnula, 2002), containing hydroxypropylcellulose, EtOH, PG and oleic acid. Polymer in the formulation was added after uniform mixing of all other components including radioactive tracer (2 μCi/g for ex vivo and 5 μCi/g for in vivo studies) and then subjected to further mixing for two more hours. Gels prepared were centrifuged to remove any entrapped air bubbles and stored at 2–8 °C overnight for complete swelling of polymer.

2.4. Preparation of skin

Protocols for all animal experiments were approved by Institutional Animal Ethics Committee or Biosafety committee, NIPER. The skin was prepared using a protocol reported earlier (Jain et al., 2002; Thomas and Panchagnula, 2003). Briefly, Sprague–Dawley rats (230–250 g) were obtained from central animal facility of NIPER and sacrificed by excess ether inhalation. Hair from dorsal side of skin was removed using a hair clipper (Aesculap, Germany) and whole skin was excised which is followed by removal of fat adhering to dermis with a scalpel. Any trace of fat adhering to skin was then finally removed by wiping it with cotton swabs soaked in isopropyl alcohol. Finally, skin was rinsed with phosphate buffer saline (pH 7.4) and stored at −20 °C (Densia, Germany) in aluminum foil till used (always used within a week) (Panchagnula et al., 1997).

The porcine ears were obtained from a local slaughterhouse. Hair on both the surfaces of ears were removed using a hair clipper (Aesculap, Germany). Shaved ears were immersed in water maintained at 60 °C for 2 min. Epidermis was peeled off gently from both the surfaces of ears. Finally, epidermis was rinsed with phosphate buffer (pH 7.4) and stored at −20 °C in an aluminum foil until used (always used within 6 h). Every time before permeation studies, skin was thawed at 37 °C for 30 min.

2.5. Ex vivo permeation studies

The skin permeation studies were performed ($n = 4$) using full thickness dorsal rat skin and porcine epidermis. Vertical Franz diffusion cells (jacketed) having a diffusional surface area of about 0.78 cm^2 with outer jacket maintained at 37 ± 0.2 °C were used to mimic physiological conditions. Phosphate buffer (pH 7.4) containing 0.005% sodium azide as preservative was used as receptor medium (5.3 mL). After equilibration of skin (placed dermis side in contact with receptor medium) with receptor phase, about 400 μL of gel was incorporated in the donor compartment and covered with a laboratory film (American National Can, USA) to prevent evaporation of volatile components (Panchagnula et al., 2001). At specific time intervals, samples (200 μL) were withdrawn from receptor compartment and replaced with fresh receptor medium. Samples were analyzed for cumulative amount of drug permeated by liquid scintillation counting.

2.6. In vivo evaluation of formulation

In vivo evaluation of different concentrations of NLX was carried out in Sprague–Dawley rats on dorsal surface. Transdermal patch was fabricated (area ∼ 2 cm^2) using an impermeable backing membrane (ScotchPak®, 3M, USA) and microporous polyethylene membrane that was sealed from three sides with the help of an impulse sealer (Sevana, India). About 500 μL of formulation was incorporated into the pouch using a positive pressure pipette (Brand, Germany) and fourth side was then finally sealed. Rats were anesthetized by urethane (0.65 g/kg) and patch was applied to the shaved skin and fixed using medical adhesives to provide occlusive conditions (Kim et al., 2001). Blood samples (250 μL) collected at specific time points till 48 h, were centrifuged (Heraeus, Germany) at 13,000 rpm for 10 min. Plasma (100 μL) was separated from samples and stored at −20 °C till analyzed. These were then mixed with 5 mL of biodegradable scintillation cocktail (Amersham, UK) and subjected to vortexing for 30 s. Patches were removed from rats after an interval of 48 h. Quantification of amount of NLX in plasma was done by measurement of radioactivity in samples.

2.7. Skin affinity studies

Affinity of NLX to skin was estimated after 48 h of sampling. Skin samples were removed and wiped with 5% isopropyl alcohol, followed by normal saline to remove any adhered drug and stored in desiccator for drying. Accurately, weighed skin samples were solubilized using tissue solubilizer (custom made) at 37 °C in a shaker water bath (Julabo, Germany) for 24 h. Further, the solubilized samples were mixed with 6 mL of scintillation cocktail and amount of drug bound was measured.

2.8. Multiple dose pharmacokinetic study

To ensure clinical effectiveness, concentration of a drug has to be maintained above minimum effective level throughout the course of therapy. However, accumulation of drug may occur if drug intake exceeds

elimination and hence, multiple dose pharmacokinetic studies have to be performed to assess its behavior (Ritschel and Kearns, 1999). The transdermal patches containing 10 mg/g of NLX were applied to multiple sites of shaved skin of Sprague–Dawley rats for a period of 6 days. After 48 h, patch was removed and replaced with a fresh patch applied to a different site on skin. Blood samples (250 μL) were collected at specific time points in heparinized (10IU) microcentrifuge tubes and immediately centrifuged at 13,000 rpm for 10 min. Plasma (100 μL) was separated and stored at −20 °C till analysis.

2.9. Stability evaluation

Stability studies were performed for 3 months using NLX gel prepared at three different concentrations (10, 20 and 30 mg/g). All the stability samples (packed in the backing membrane (ScotchPak®, 3M, USA)) were prepared in triplicates and were kept at two stability testing conditions, viz. 4–8 °C serving as control and 25 °C/60% RH (Stability Chamber, WTC, Binder, Germany)) serving as test condition as per ICH Guideline Q1A. Stability samples were evaluated for drug assay and ex vivo permeation through porcine epidermis at each sampling point (1, 2 and 3 months).

2.10. Skin irritancy studies

Skin irritancy potential of the formulation upon in vivo application of the TDDS containing gel formulation, with and without drug was carried out by preliminary histopathological evaluation, LDV and TEWL. After 48 h of application, patch was removed and skin was excised and stored in 10% formalin solution in phosphate buffer saline (pH 7.4) followed by dehydration with alcohol. It was then treated with antimedia and embedded in paraffin for fixing. Sections of ∼5 μm thickness were cut from each skin piece and stained with hematoxylin and eosin (Wu et al., 2001). These samples were then observed under light microscope (Leica, Germany) and compared with control sample for epidermal liquefaction and edema of collagen fiber in dermis as well as hypodermis.

To another group the formulation without the radiotracer was applied for 48 h under occlusive conditions and LDV, TEWL measurements were carried out.

2.10.1. LDV measurement

LDV was measured on the application sites on the dorsal surface of rats using a Multichannel Laser Doppler System (Perimed, Sweden). The measuring fiber optic probe contains a diode laser with a fixed wavelength. Instrument was allowed to 'warm up' for 15 min after being turned on, giving the time for the laser light to stabilize and was never switched off between intermittent measurements. Probe was held gently on the skin to avoid vascular compression (Tanojo et al., 1998). To allow the output signal to stabilize readings were performed after 5 min. LDV measurements on nine rats were taken before starting the study that served as control. There after, on six animals formulation was applied and three animals were kept as control (without treatment). After 48 h, LDV measurements were taken and the values obtained were compared with that obtained at 0 h.

2.10.2. TEWL measurement

TEWL measurement was performed using Tewameter (Courage and Khazaka, Germany). Measurements were taken for 5 min by keeping the probe over shaved skin of anaesthetized rats. TEWL measurements on nine rats were taken before starting the study that served as control. Again, after 48 h, TEWL measurements were taken and the values obtained were compared with that obtained at 0 h.

2.11. Data analysis

Flux of drug permeated in case of in vitro as well as ex vivo studies was calculated from slope of the steady-state portion of permeation profile by linear regression analysis (Ritschel and Hussain, 1988). Lag time was calculated from back extrapolation of steady-state portion of the graph. Normalized diffusion coefficient (D/h^2) and permeability coefficient (K_p) was also calculated for the in vitro as well as ex vivo studies using Eqs. (1) and (2), respectively,

$$\frac{D}{h^2} = \frac{1}{6 \times T_{Lag}} \tag{1}$$

$$K_p = \frac{J_{SS}}{C_D} \tag{2}$$

where T_{Lag} is the lag time, J_{SS} the flux at steady state, C_D is concentration in donor compartment, D the

diffusion coefficient and *h* is the diffusion pathlength. Pharmacokinetic parameters after in vivo single dose administration were calculated using different model dependent as well as independent method of analysis. Area under the curve ($AUC_{0-\infty}$) was calculated using linear trapezoidal rule (Rowland and Tozer, 1995). Compartment model independent analysis was applied to estimate pharmacokinetic parameters of NLX after its in vivo administration. Data was subjected to statistical analysis employing one way analysis of variance (ANOVA) followed by Tukey test using Sigmastat (Jandel scientific, USA).

3. Results and discussion

3.1. Ex vivo skin permeation

With increasing concentration of NLX in gel the flux was observed to increase in case of both porcine epidermis and rat skin (Fig. 1). Further, it was also observed that lag time (T_{Lag}) showed no significant difference ($P > 0.5$) and remained independent of concentration among the different barriers used. Between barriers used the rat skin was found to be more permeable than the porcine epidermis. The flux of NLX from rat skin at all the concentrations tested was found to be 7–11 times more (Fig. 1B) and the lag time was found to be 1.7–2.2 times lesser (Fig. 1C) in case of rat skin in comparison to porcine epidermis. The constancy of flux and lag time ratio at all concentrations between barriers reflects essentially the influence of thermodynamic activity on the drug permeation. In other words, since only concentration of NLX has increased in all the cases, keeping rest of the formulation same, increase in thermodynamic activity of NLX was the contributing factor in increasing flux across different barriers. In literature, the porcine epidermis has been reported extensively to be similar to human skin with respect to structure and permeation of solutes (Andega et al., 2001; Marro et al., 2001; Fang et al., 1995), while less permeable than rat skin (Fang et al., 1995). Based on the ex vivo permeation of NLX across the porcine epidermis, the surface area (SA) of the patch predicted to be 39.6 cm^2 in order to achieve therapeutic blood levels (10 ng/mL) (Benet et al., 2001) using Eq. (3)

$$J_{Skin} \times SA = C_{SS} \times Cl_T \quad (3)$$

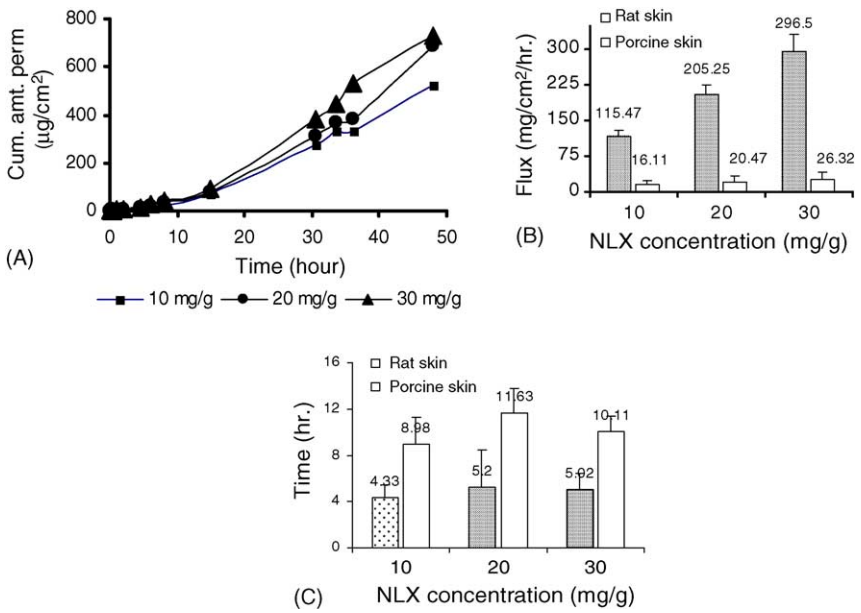

Fig. 1. (A) Permeation profile of NLX from gel formulations across porcine epidermis. (B) The comparison of flux values from porcine epidermis and rat skin at various concentrations of NLX in the gel formulations. (C) The comparison of lag time through porcine epidermis and rat skin at various concentrations of NLX in the gel formulations.

where J_{Skin} is the flux through porcine epidermis from ex vivo studies (26.32 μg/cm^2 h), C_{SS} the therapeutically effective plasma concentration of the drug at steady state (10 ng/mL) and Cl_T is the clearance total of drug (104 L/h in 70 kg adult) (Shibata et al., 2002). Since NLX is an antagonist whose clinically effective concentrations are dictated by the addiction status, higher concentrations may be achieved upto 12.6 ng/mL, by increasing the patch size upto 50 cm^2. However, the estimation of patch area must be cross-validated using the human skin data.

3.2. Single dose in vivo study using transdermal patch

The plasma time concentration curve upon single patch application, for 48 h at different concentrations of drug is shown in Fig. 2. The pharmacokinetic parameters determined were compared at all doses (Fig. 3). Area under the curve, reflecting the amount of drug absorbed to body, is a measure of dose proportionality (Rowland and Tozer, 1995). The AUC increased significantly ($P < 0.05$) with each increment of concentration from 10 to 30 mg/g (Fig. 3A). Similar to the increase in AUC of drug concentration in systemic circulation, the skin affinity also has increased with increasing drug concentration in formulation (Fig. 5). However, in ex vivo permeation studies using the rat skin no such trend in skin affinity was apparent. Upon single dose administration, an increase in steady-state plasma levels was observed with increasing concentration (Fig. 3B). Steady-state plasma levels observed in case of 10 and 20 mg/g of gel was not significantly different from each other ($P < 0.001$). However, steady-state plasma levels obtained in case of 30 mg/g of gel were found to be significantly higher than the former two concentrations. Further, T_{SS} was not found

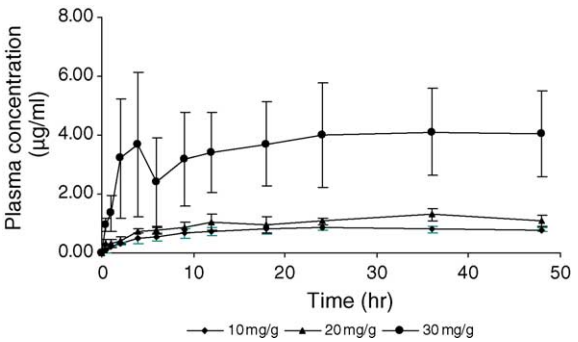

Fig. 2. Plasma concentration time profiles obtained after single dose transdermal administration of NLX at different concentrations to Sprague–Dawley rats for 48 h. Each time point represents mean ± S.D. ($n = 6$). Plasma concentrations obtained for the concentrations of 10 and 20 mg/g were not significantly different with each other, while that obtained for concentration of 30 mg/g of gel is significantly different ($P < 0.001$).

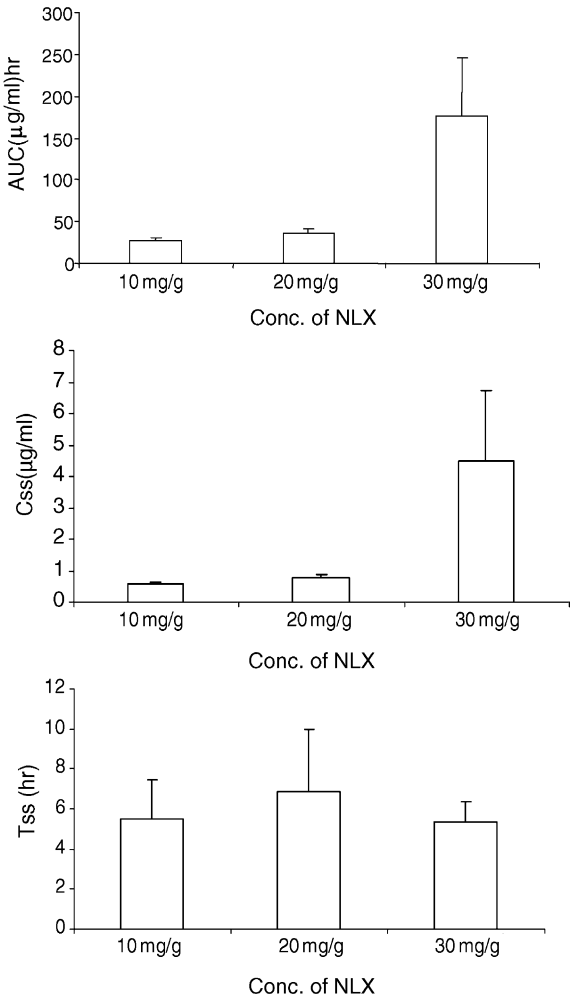

Fig. 3. Comparison of different pharmacokinetic parameters obtained after in vivo single dose administration of NLX at different concentrations. Each histogram represents mean ± S.D. ($n = 6$). Different pharmacokinetic parameters evaluated were (A) area under the curve (AUC); (B) concentration at steady state (C_{SS}) and (C) time to reach steady state (T_{SS}). In case of (A) and (B), histograms at 10 and 20 mg/g were not significantly different ($P < 0.001$). The values for T_{SS} in case of (C) were not significantly different ($P > 0.05$).

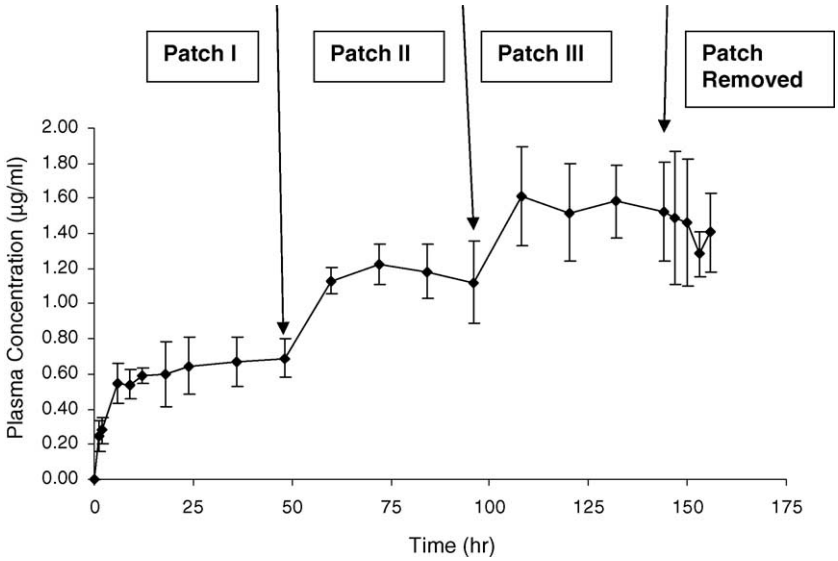

Fig. 4. Plasma concentration time profile of NLX after multiple (three) applications of prototype patch in Sprague–Dawley rats. Each time point represents mean ± S.D. ($n = 6$). The patches were applied to skin for a period of 48 h, after which it is removed and replaced with a fresh patch at new site. Study was conducted for a period of 6 days in which a total of three patches were applied.

to be significantly different ($P > 0.5$) amongst all the concentrations (Fig. 3C). These results showed that pharmacokinetics of NLX after single dose in vivo administration by transdermal patch is independent of concentration for 10, 20 mg/g of gel, whereas at a higher concentration of 30 mg/g when there is saturation of the skin by NLX, a deviation from this trend was observed. The steady-state levels were maintained from 4–48 h in all the cases (Fig. 2). This proves the clear advantage of transdermal delivery system over the current mode of administration, i.e., i.v. bolus which is effective upto a maximum of 1.5 h (Hanes, 1999), because of short $T_{1/2}$ of 64 min (Ngai et al., 1976). Further, for the treatment of intoxication with other opioid agonists of such as methadone with higher $T_{1/2}$ (23 h), in which cases it is currently given as an infusion, transdermal delivery could be indispensable.

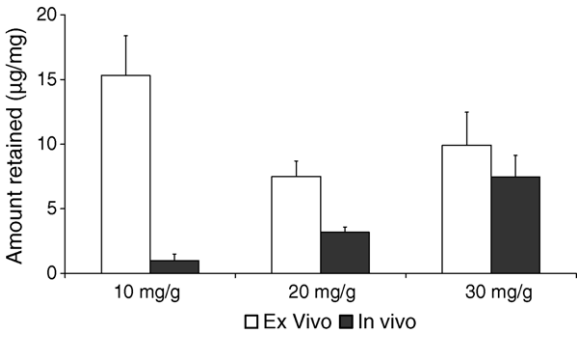

Fig. 5. Skin affinity values of NLX when applied from gel formulation both ex vivo and in vivo at different concentrations. Each histogram represents mean ± S.D. ($n = 4$).

3.3. Multiple dose study

In order to simulate the clinical situation, where in more than one patch will be applied consecutively for a prolonged period of time, a multiple dose pharmacokinetic study was conducted. In this case, the fresh patch was applied at a new site on the dorsal skin immediately after the removal of previous patch, every 48 h. Therefore, whenever a new patch is applied, increase in plasma concentration resulting from new patch and the decreasing drug from previous patch reservoir overlaps to show constant blood levels (Berner and John, 1994). Different pharmacokinetic parameters were derived from the plasma concentration time curve (Fig. 4), which are enlisted in Table 1. After application of first patch, steady state was achieved within 8 h and maintained till its removal, i.e., 48 h. AUC also increased proportionately showing the accumulation of drug with subsequent applications as shown by increasing ratio of

Table 1
Pharmacokinetic parameters obtained after multiple application of transdermal patch of NLX at a dose study of 10 mg/g

Pharmacokinetic parameters	Values
First patch	
C_{SS}	0.609 ± 0.061
T_{SS}	8.5 ± 1.22
$AUC_{(0–48\,h)}$	28.74 ± 3.21
Second patch	
C_{SS}	1.165 ± 0.121
$AUC_{(0–48\,h)}$	43.54 ± 4.29
Third patch	
C_{SS}	1.560 ± 0.216
$AUC_{(0–48\,h)}$	57.681 ± 7.62
Ratio	
AUC (first to second patch)	0.660
AUC (second to third patch)	0.755

C_{SS}, concentration at steady state; T_{SS}, time to reach steady state; AUC, area under the curve from 0 to 48 h, $AUC_{(0–48\,h)}$ and C_{SS} values for all the three patches are found to be significantly different at $P < 0.001$. Each value represents mean ± S.D. ($n = 6$).

AUC (first to second patch and second to third patch). The accumulation of drug was also evidenced by sustained plasma drug levels upto 12 h after the removal of third patch. This sustained steady state even after patch removal for the drug with very short half life (64 min) is due to the formation of drug depot in the skin. This drug depot formation is also supported by high skin affinity values at all the doses observed after the single dose study. The molecule being lipophilic it was reported to have propensity to form depot inside skin (Jaiswal et al., 1999). It was observed from Fig. 5 that the amount of drug bound to skin has increased from 10 to 30 mg/g of gel concentration. Under the conditions of occlusion and the use of penetrants, initially there may be a relatively high level of NLX in upper strata of stratum corneum (SC). This drug may slowly diffuse to lower layers of SC, epidermis and dermis from where it is accessible to the micro blood capillaries. Thus the drug, which might have formed reservoir in the stratum corneum, maintained the blood levels even after its removal.

3.4. Stability evaluation

The formulations were subjected to accelerated stability testing, as per ICH guidelines for semi solid preparations, at 25 °C/60% RH keeping refrigerated conditions (4–8 °C) as control. The formulations were found to be stable with respect to NLX assay when analyzed by HPLC (in the range 75–125%) at all concentrations (10, 20 and 30 mg/g) of NLX in gel (Fig. 6). Further, in order to assess the integrity of penetration enhancer system, oleic acid together with vehicle (EtOH:PG 2:1) in this case, the transdermal permeation of the formulations was conducted at all concentrations through the porcine epidermis. For this purpose, ^{3}HNLX was spiked into the cold stability formulations just before the permeation study by

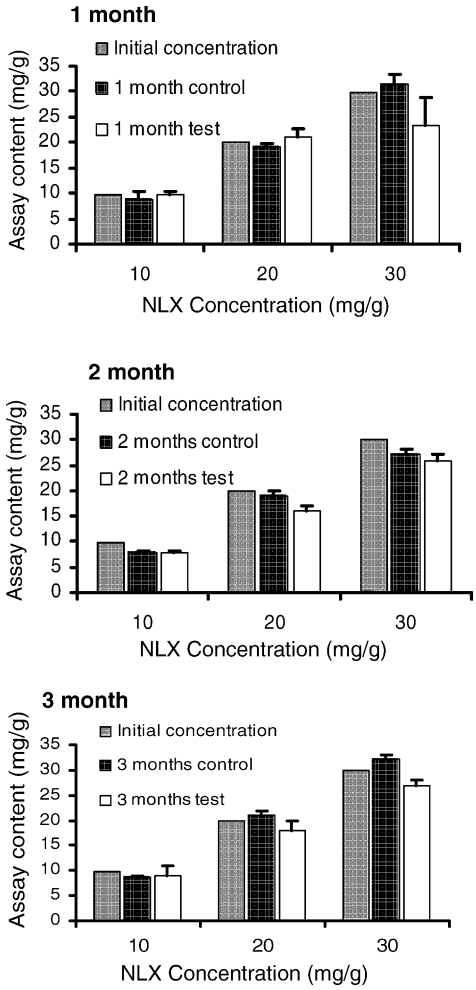

Fig. 6. The assay of NLX from stability samples. All stability samples (at three concentration levels) were prepared in triplicate and were kept at two stability testing conditions, viz. 4–8 °C serving as control and 25 °C/60% RH (Stability Chamber, WTC, Binder, Germany) serving as test condition.

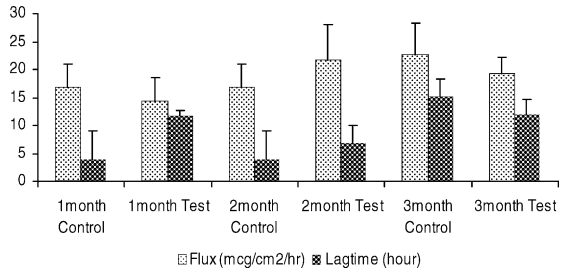

Fig. 7. The permeation parameters of NLX through porcine skin from stability samples (10 mg/g). All the stability samples were prepared in triplicate.

thorough mixing in order to rule out the probability of tritium exchange during stability period. Flux and lag time for NLX permeation did not alter significantly in the 3-month long study at all the stability conditions, showing the integrity of penetration enhancer system (Fig. 7).

3.5. Skin irritancy studies

Morphological changes in different layers of rat skin after application of the prototype reservoir patch containing NLX at a concentration of 10 mg/g were evaluated as shown in Fig. 8. In this histopathological evaluation, epidermal liquefaction, edema of collagen fibers was considered as the key criteria to distinguish the effect of drug (Narishetty and Panchagnula, 2004). It was observed that there was no apparent change in skin morphology after the application of control patch containing vehicle (EtOH:PG 2:1). However, when compared with the skin treated with patch containing NLX, slight swelling of the sub epidermal layer was observed.

In routine transdermal studies, penetration enhancers for enhancing passive permeation of drug molecule are commonly used. However, regulatory authorities have also given importance to the irritation potential of penetration enhancers. Hence, many non-invasive techniques for assessment of skin irritation potential in vivo are routinely employed for example TEWL and LDV measurement (Kandimalla et al., 1999; Tanojo et al., 1999). In order to assess the efficacy of the patch, TEWL was measured before and after the application of formulation under occlusion. The occluded area using a teflon ring was used as control.

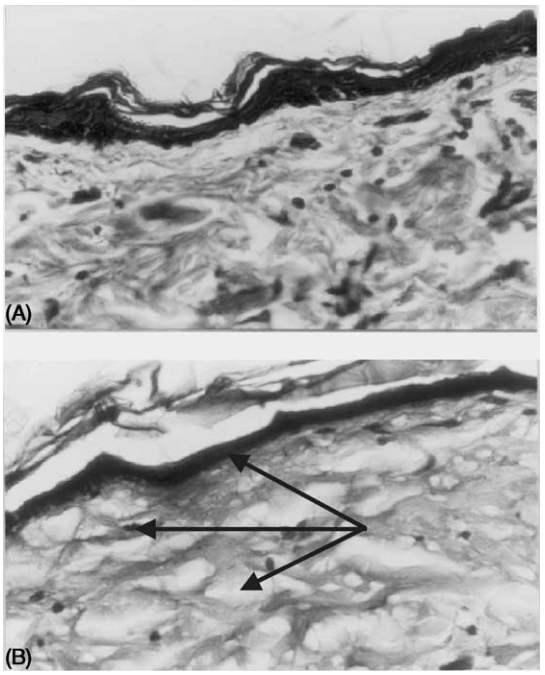

Fig. 8. Light microscopic photographs of rat skin after 48 h of application of patch containing gels without (A) and with (B) NLX. Evaluation was carried out after multiple dose study, in which patch was applied to dorsal side of Sprague–Dawley rats. Micrographs were obtained at a magnification of 25×. The arrows represent sites under the skin surface, in which the evaluation was carried out.

The TEWL enhancement ratio calculated with respect to control, has increased significantly ($P \leq 0.001$) from 0.39 ± 0.04 to 3.83 ± 1.24 (Fig. 9A). This reflects the efficacy of the penetration enhancer system. However, this alteration of penetration barrier function was not accompanied by any significant amount of skin irritation measured using LDV. LDV is a rapid and sensitive technique, which allows continuous and reliable assessment of skin irritation potential of penetration enhancer in terms of altered blood flow. Skin irritation results in increased cutaneous blood flow, which also results in high LDV value when compared to basal LDV value that is LDV value of normal skin. The irritation index calculated in terms of ratio of blood flow rate with respect to occlusion control before (0.81 ± 0.07) and after (0.94 ± 0.34) the formulation application did not change significantly ($P > 0.05$) (Fig. 9B).

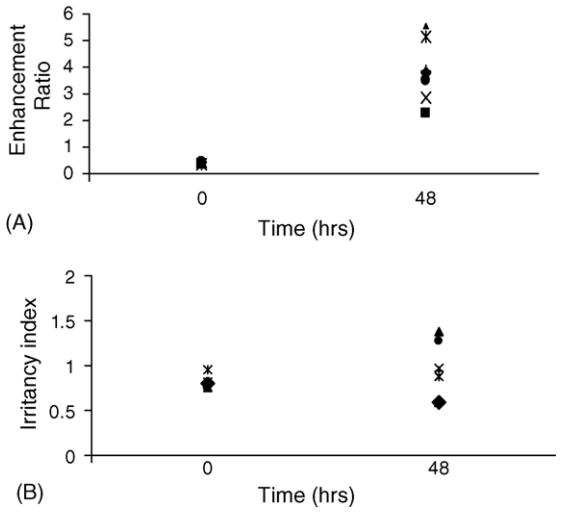

Fig. 9. (A) The trans epidermal water loss (TEWL) enhancement ratio before and after treatment of rats with gel formulation. The ratio is calculated with respect to occlusion control. Enhancement ratio: TEWL test/TEWL control. (B) The cutaneous irritancy index measured as the enhancement of cutaneous blood flow with laser doppler velocimetry (LDV) before and after treatment of rats with gel formulation. The ratio is calculated with respect to occlusion control. Irritancy index: LDV test/LDV control.

4. Conclusions

The transdermal gel formulation and the prototype patch were shown to be efficacious, safe, stable and non-irritant to skin. The flux value obtained ex vivo can be translated into a patch of 40 cm^2 area in order to provide therapeutically effective drug levels in vivo. The establishment of steady-state levels in vivo for 48 h shows the clear advantage of transdermal patch over current modes of administration. The formation of drug depot in skin could have an important bearing on the multiple applications and the interval between subsequent applications. The formulation was shown to be stable for at least 3 months with respect to drug content and penetration enhancer efficacy. The formulation was shown to be non-irritant and safe upon multiple applications.

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