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Pharmacokinetics of a losartan potassium released from a transdermal therapeutic system for the treatment of hypertension

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Monolithic transdermal therapeutic systems (TTS) were developed for sustained antihypertensive effect of losartan potassium using the polymers Eudragit E 100 and polyvinyl pyrrolidone VA 64. The developed formulations (polymeric films) were evaluated for physical characteristics, *ex vivo* (histopathology) and *in vivo* (pharmacokinetic studies). Pharmacokinetic parameters, such as C_{max}, t_{max}, and AUC were estimated. The transdermal formulation in the present study was found to enhance the relative bioavailability of losartan potassium by 2.2 times with reference to an oral delivery. The increased bioavailability might be due to elimination of hepatic first pass metabolism. Thus, the transdermal formulation F3E with polymeric composition of Eudragit E 100 and polyvinyl pyrrolidone VA 64 (5:3) was found to provide prolonged steady state concentrations of losartan potassium with minimal fluctuations and improved bioavailability.

1. Introduction

Losartan potassium (LP) is an angiostein II receptor (type AT1) antagonist which is successfully used alone or in combination with other against hypertension agents. The drug is generally given for a longer period of time. This causes daily dosing schedule and patient inconvenience. The low bioavailability (33% after oral dosing) is primarily due to incomplete absorption and partly due to pre-systemic metabolism (Thakur et al. 2009). Due to its low bioavailability after oral administration, and the inconveniences related to parenteral administration, the development of transdermal drug delivery device is reasonable.

Thus, it was planned to design a delivery system which provides the delivery of drug at a controlled rate across intact skin to achieve a therapeutic effective drug level for a longer period of time. Polymeric matrix systems are widely used to provide controlled delivery of drug substances because of their versatility, effectiveness, and low cost. Since the formulation was designed to deliver the drug by transdermal route, it will by-pass hepatic first pass metabolism and hence would provide better bioavailability compared to conventional dosage forms. Thus, with this background the main purposes of this study was: (i) to develop a stable, reproducible and patient compliance drug delivery system in the form of transdermal therapeutic system, (ii) to reduce the side effects by the optimization of the blood concentration time profile, (iii) to provide extended duration of activity, which allows greater patient compliance owing to elimination of multiple dosing schedules, (iv) to obviate low absorption, first pass effect, and formation of metabolites that cause side effects.

2. Investigations, results and discussion

The drug LP is easily soluble in methanol. The selected polymers Eudragit E100 and Copovidone (polyvinyl pyrrolidone VA-64) also exhibited good solubility in methanol. Eudragits are basically water insoluble and soluble in most organic solvents. Various drug formulations were developed i.e., F1E, F2E, F3E, F4E and F5E and evaluated for physical properties (Table 1), *ex vivo* and *in vivo* (Fig. 1, 2, 3).

All the films were found to be thin (< 0.3 mm) with uniform thickness and weight variation. The uniform thickness and weight of the films suggested the uniform distribution of drug and polymer over the surface selected for film-forming. The moisture uptake capacity was very low, which defends the material from microbial contamination and bulkiness of the patches (Arora and Mukherjee 2002). The folding endurance measures the breaking ability of the films during use. Sufficient folding endurance was found for the films of each batch, suggesting the use of the system for a period of 24 h or more without breaking or cracking (Table 1). The drug content in each of the film was found to be high (>90 %) suggesting a high retaining capacity of LP that will be available to permeate transdermally.

The cumulative amount of drug permeated through rat skin was calculated for each patch at various time intervals up to 48 h i.e., the desired time period for which transdermal patches were planned (Fig. 1). The formulations with permeation enhancer (F1E, F2E, F3E, F4E, and F5E) exhibited higher permeation than the formulations without. Among these five formulations, only two formulations F2E and F3E gave satisfactory permeation up to 48 h i.e., 65.96% and 70.77% respectively. The permeation enhancer to be included in the formulation was selected amongst the enhancers studied including orange oil (5% v/v), dimethyl formamide (5% v/v, DMF), methyl pyrrolidone (5% v/v, MP), rose oil (5% v/v) and eucalyptus oil (5% v/v). An optimum 10% MP was selected for the formulation of transdermal therapeutic system based on the increase in flux. A corresponding increase of permeability coefficient was also seen with MP. The effectiveness of these permeation enhancers was also ascertained by determination of the enhancement factor (E_f) of each permeation enhancer. Methyl pyrrolidone might increase

Table 1: Physicochemical properties of medicated transdermal films (with enhancer)

Medicated films	Physical parameters				
	Thickness (mm) n = 3, ± SD	Weight variation(mg) n = 3, ± SD	Folding endurance n = 3, ± SD	%Moisture uptake n = 3, ± SD	% Drug Content
F1E	0.237 ± 0.071	214.27 ± 1.83	41 ± 1.19	3.21 ± 0.924	99.94
F2E	0.232 ± 0.061	217.43 ± 1.71	35 ± 1.24	3.16 ± 0.853	99.94
F3E	0.228 ± 0.064	211.91 ± 1.97	37 ± 1.16	3.08 ± 1.119	99.99
F4E	0.229 ± 0.063	213.29 ± 1.59	38 ± 1.17	3.13 ± 0.972	99.93
F5E	0.233 ± 0.067	215.62 ± 1.93	40 ± 1.21	3.19 ± 1.014	99.94

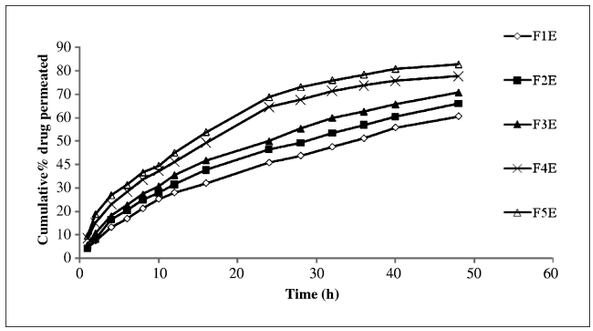

Fig. 1: *In vitro* permeation studies of transdermal therapeutic system

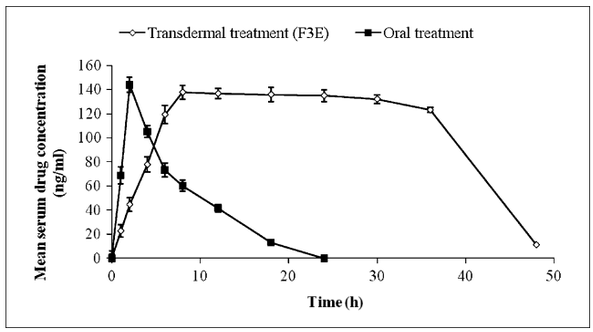

Fig. 2: Concentration of LP in rat serum after transdermal and oral treatment at different time intervals

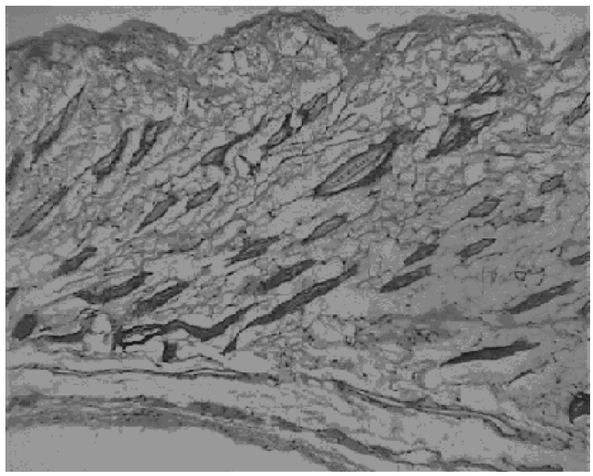

Fig. 3: Histopathology of rat skin (untreated –control) (under light microscope, 100x)

Table 2: Mean values of pharmacokinetic parameters for oral and transdermal treatment

Mean values of pharmacokinetic parameters	Oral formulation (Marketed)	Transdermal formulation (F3E)
T_{max} ± SD (h)	2 ± 0.21	8 ± 1.71
C_{max} ± SD (ng/ml)	143.87 ± 19.84	137.6 ± 6.53
$AUC_{0\text{-}t}$ ± SD (ng.h/ml)	1065 ± 132	2453 ± 211
F%	–	2.2

the epidermal permeability through a mechanism involving the perturbation of stratum corneum lipid bilayers and lacunae formation to enhance transdermal drug delivery (Jiang and Zhou 2003). From the above observations, it was seen that formulations F2E and F3E led to better permeation.

A pharmacokinetic study was carried out on Wistar rats to judge the efficacy of the developed formulation against the oral dosage form. The data so obtained were subjected to pharmacokinetic analysis. The mean T_{max} of LP was 2 h for oral treatment and 8 h for transdermal treatment. A considerable difference in T_{max} value was experiential between transdermal and oral treatment ($p < 0.05$). An increase in T_{max} value by transdermal treatment indicated the controlled release performance of the formulation. The C_{max} values for both (Table 2) were almost similar, however in the case of oral treatment fluctuations in the plasma concentration were seen whereas after transdermal treatment a steady state plasma concentration level was maintained over more than 40 h (Fig. 2). There was a considerable difference between the $AUC_{0\text{-}t}$ values for transdermal and oral treatment ($p < 0.05$) (Table 2) which clearly reflects the high first pass metabolism of LP after oral administration. Thus, the transdermal formulation in the present study was found to enhance the relative bioavailability of LP by 2.2 times with reference to an oral delivery. Furthermore, the transdermal formulation F3E was found to provide a prolonged steady state plasma concentration of LP with minimal fluctuations and improved bioavailability for more than 40 h.

For histopathological examination the treated skin was collected in formalin and after staining a light power photomicrograph was taken and compared to a photomicrograph of control skin to detect any kind of disruption or changes in the skin treated with the formulation. The photomicrograph of untreated rat skin (control) shows well defined epidermal and dermal layers with layer of corneocytes and skin appendages (sweat glands, hair follicles) (Fig. 3). When the skin was treated with transdermal patch definite changes were observed in the skin morphology. The disruption and extraction of lipid bilayers was clearly evident as distinct voids and empty spaces visible in the epidermal region (Fig. 4). The extraction of subcutaneous lipids might cause dehydration of subcutaneous tissue with significant loss of moisture. As visible in the photomicrograph no significant alteration was seen in terms of skin cells with the treatment of

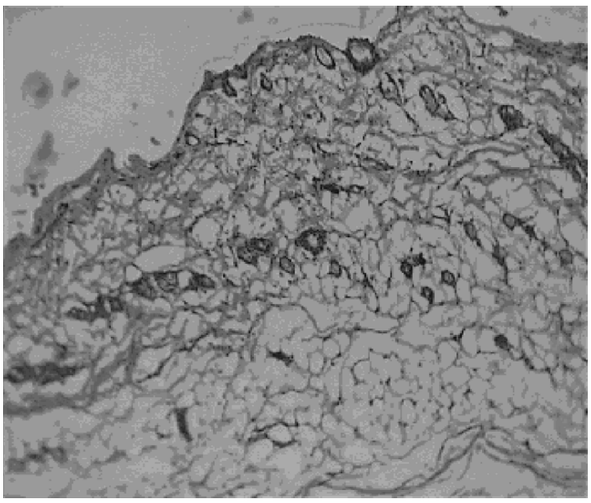

Fig. 4: Histopathology of rat skin after applying transdermal patch F3E (treated skin) (under light microscope-100 x)

Table 3: Formulae of medicated transdermal patches for one ring of casting assembly

Ingredients	Formulation code				
	F1E	F2E	F3E	F4E	F5E
Eudragit E 100 (mg)	700	600	500	450	400
Polyvinyl pyrrolidone VA 64 (mg)	100	200	300	350	400
Dibutylphthalate (ml)	0.1152	0.1152	0.1152	0.1152	0.1152
Glycerol (ml)	0.02	0.02	0.02	0.02	0.02
MP (%)	10	10	10	10	–
Methanol (ml)	8	8	8	8	8

the transdermal patch to the skin after 48 h. The stratum corneum remained intact. Thus, the transdermal formulation F3E seems to be safe in topical delivery.

Accordingly, the system can be used for sustained antihypertensive effect that would obviate the problems of low bioavailability and inconveniences related to parenteral administration and oral administration. It can be further evaluated for clinical trials on human volunteers.

3. Experimental

3.1. Materials

The materials used include losartan potassium (Ranbaxy Laboratory Ltd. India), ethanol AR (Merck, India Ltd.), propanol AR (Merck, India Ltd), isopropyl alcohol (Merck, India Ltd), Eudragit E-100 (Dr.Reddy's Laboratories, Hyderabad, India), Copovidone (PVP VA 64) (Jubilant Organosys ltd., Noida, India), acetone (S.D Fine Chemicals, India), dichloromethane (Merck, India Ltd), n-octanol (Merck India Ltd. India), methanol (S.D Fine Chemicals, India), polyethylene glycol – 400 (Central Drug House, New Delhi, India), methyl pyrrolidone (S.D Fine Chemicals, India), dibutyl phthalate (Merck Limited, Mumbai, India), ammonia solution (Thomas Baker (Chemicals) Ltd, Mumbai, India), other chemicals were of analytical reagent grade.

3.2. Development of the transdermal therapeutic system

Accurately weight LP (168.12 mg) was dissolved in methanol. The different proportions of polymers (Eudragit E 100 and polyvinyl pyrrolidone VA 64), plasticizers (dibutyl phthalate (DBP) and glycerol) and permeation enhancer (methyl pyrrolidone – 10% w/w of total weight of polymers) were then dissolved in methanol using a magnetic stirrer (Table 3). The resulting solution was poured carefully into a film casting assembly fabricated for this purpose. It consisted of two stainless steel plates of the same external diameter. The upper plate was made hollow such that the internal diameter was 7.90 cm. Aluminum foil was placed in between the plates and screwed tightly to prevent solvent leakage. An inverted funnel was placed over the casting assembly to control the evaporation rate of solvent. This minimized the chances of cracking or wrinkling the films. The open end of the funnel was plugged with cotton to allow uniform evaporation of the solvents. The solvent was allowed to evaporate undisturbed. The films got dried up in approximately 24 h.

3.3. Evaluation of physical properties of polymeric films

The thickness of the films was assessed at three different points using a screw gauge and the average thickness was determined. The number of times the film could be folded at the same place without breaking /cracking gave the value of folding endurance. This was determined by repeatedly folding the film at the same place until it broke. Three films from each batch, were weighed individually as a whole and the average weight was calculated. The accurately weighed films kept in a desiccator at 40 °C for 24 h were taken out and exposed to two different relative humidities of 75% (saturated solution of sodium chloride) and 93% (saturated solution of ammonium hydrogen phosphate) in two different desiccators at room temperature until a constant weight for the films were obtained. The percentage of moisture uptake was calculated as the difference between final and initial weights with respect to initial weight.

$$\text{Percent moisture uptake} = \frac{\text{final weight} - \text{initial weight}}{\text{initial weght}} \times 100 \quad (1)$$

A film of size 9.616 cm^2 was dissolved in 100 ml of methanol. This was shaken in a mechanical shaker for 30 minutes to obtain a homogeneous solution and filtered. The drug content was determined by U.V. spectrophotometry at λ_{max} 234.2 nm after suitable dilution and calculated using the following formula:

$$\text{Drug content(mg)} = \frac{\text{concentration} \times \text{dilution factor} \times \text{volume}}{1000} \quad (2)$$

3.4. Skin permeation studies

The *in vitro* skin permeation studies were performed in a vertical diffusion cell fabricated by a local glass manufactures similar to the Keshary Chein diffusion cell [jacketed] (Dahlöf et al. 2002). The temperature of the cell was maintained at 37 ± 0.5 °C by circulating water through the water jacket of the cell. The vertical double walled diffusion cells consisted of two half-cells (chambers), a donor chamber and a receiver chamber (33 ml) with an area of diffusion between two half-cells of 9.616 cm^2.

The albino rats were sacrificed in chloroform environment for obtaining the skin from the abdominal region. The excised skins were stored in the deep freezer (−20 °C) until use. On the day of experiment, the skin was brought to room temperature and then treated with 0.32 M ammonia solution for 1 h and hair and fat were removed manually. The skin was washed with water and examined for cuts or holes.

After pre-treatment, skin was mounted between the two half cells of the apparatus and the extra skin was cut and trimmed to prevent lateral diffusion. The stratum corneum side of the skin was facing the donor compartment whereas the dermis faced the receiver compartment and the apparatus was assembled with springs. Both the compartment was filled with isotonic phosphate buffer (IPB) of pH 7.4. The receiver fluid was stirred with a magnetic bead at a speed of 600 rpm and the assembled apparatus was placed on a magnetic stirrer. Hot water was circulated through the jackets of the cells in order to maintain 37 ± 0.5 °C temperature. The receiver fluid was replaced every 15 min to stabilize the skin. It was found that the sample at 4 h and beyond showed negligible UV absorption indicating the complete stabilization of the skin. The transdermal patch was placed above the skin and the release surface faced the stratum corneum. The samples were withdrawn via the sampling port at specified intervals up to 48 h, filtered and analyzed for drug content spectrophotometrically (Ansari et al. 2004). Cumulative amount of drug permeated was plotted against time for each formulation.

3.5. Pharmacokinetic studies

In vivo pharmacokinetics studies were conducted in order to assess the efficacy of optimized formulations. The effectiveness was carried in terms of measurement of drug concentration in serum of rats. Approval to carry out *in vivo* studies was obtained from the institutional animal ethics committee, Jamia Hamdard, New Delhi (India) and their guidelines were followed throughout the studies.

Twelve Wistar rats of either sex weighing between 150–180 g were procured and fed with standard feed and drinking water and monitored on a regular

basis. The experimental rats were then divided into two groups (group I and II) each comprising six animals. Before starting the experiment, rats were fasted overnight with free excess to water to avoid any food effect. Group I was subjected to TTS treatment with formulation F3E. The selection of the formulation F3E was based on its physicochemical properties. The formulations were applied to shaved rat skin with the entire release surface in intimate contact with the stratum corneum. The micro porous adhesive tape was rolled over to keep the patch secured at the site of application. Group II received marketed LP tablets in suspension form equivalent to normal daily dose (50 mg) according to the weight of rat by calculation in respect to human dose.

The rats were anesthetized using ether, and blood samples (500 µl) were collected at different time intervals (0, 1, 2, 4, 6, 8, 12, 18, 24, 30, 36, 48 h) from the tail vein. The collected sample of blood was placed into a test tube and vortex-mixed for 10 s and kept at room temperature for 10 min. After adding chloroform (2 ml), the tube was vortex-mixed for 30 s, then centrifuged at 1500 rpm for 10 min. The aqueous layer was completely removed and transferred to a clean test tube. An aliquot of 50 µl phosphate precipitation solutions was added to the test tube and then mixed. After the addition of chloroform (2 ml) the tubes were vortex mixed for 30 s and centrifuged for 3 min at 1500 rpm. The chloroform layer was completely removed and transferred to a clean test tube and evaporated to dryness at 50 °C in a water bath under nitrogen. The residue was reconstituted in 200 µl of 0.05 M sodium hydroxide and 20 µl of the solution was injected into the HPLC system. The drug concentrations in the collected samples were analyzed using a reported HPLC method with slight modifications (Ozkan 2004) and the graph was plotted between drug plasma concentration and time. The unknown serum-sample concentrations were calculated from the calibration curve plotted between peak area ratios of LP to IS against corresponding LP concentrations.

The various pharmacokinetic parameters such as maximum serum concentration (C_{max}), time to reach maximum serum concentration (T_{max}), and area under the serum concentration–time curve (AUC) were calculated. The values of C_{max} and T_{max} were interpreted directly from the arithmetic plot of time versus serum concentration of LP. The trapezoidal method of AUC determination was followed (Shakeel et al. 2008). The relative bioavailability of LP after the transdermal versus oral administration was calculated as follows:

$$F(\%) = \frac{(AUC)_{sample} \times (Dose)_{oral} \times 100}{(AUC)_{oral} \times (Dose)_{sample}} \quad (3)$$

The pharmacokinetic data between transdermal and oral formulations were compared for statistical significance by one-way ANOVA followed by Tukey–Kramer multiple comparisons test using GraphPad Instat software (GraphPad Software Inc., CA).

3.6. Histopathological studies

For histopathological examination the transdermal patch (F3E) was applied on the abdominal skin of Wistar rats. After 48 h, rats were sacrificed and the skin samples from untreated and treated area were collected. Each specimen was stored in 10% formalin solution in isotonic phosphate buffer of pH 7.4. The specimen was vertically cut into sections. Each section was dehydrated using ethanol, embedded in paraffin for fixing and stained with hematoxylin and eosin. These samples were then observed under light microscope (Motic, Japan) and compared with control sample. From each skin sample, three different sites were scanned (Fang et al. 2003; Aqil et al. 2004).

3.7. HPLC method of analysis

The HPLC method of Ozkan et al. (2004) was modified according the need in the present research work. A HPLC system of Shimadzu class VP series was used. Separation was achieved using a RP C-18 column (25 × 4.6 mm internal diameter). A shimadzu SPD-10 AVP UV detector was used to detect LP at 234.0 nm. The mobile phase used was a mixture of 0.02 M KH_2PO_4 (pH 3.2): acetonitrile (55:45). The pH was adjusted to 3.2 with phosphoric acid. The flow rate was set to 0.9 ml/min. The retention time was found to be 6.325 min which is within the reported value.

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