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Comparison of skin permeability of drugs in mice and human cadaver skin

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In vitro percutaneous absorption of four antihypertensive drugs were carried out across the mice and human cavader skin in order to compare their skin permeability. An interesting trend was noticed in these experiments. Poorly water soluble drug prazosin hydrochloride showed 13 times enhanced flux in the mice skin whereas the steady-state flux of the water soluble drug propranolol hydrochloride was almost same in both human cadaver and mice skin. The permeation rate of prazosin hydrochloride and propranolol hydrochloride through the human cadaver skin fluctuated widely over time, but in mice skin, distinct trends were noticed. The study indicates that the overall permeation rate in mice skin is higher than that in the cadaver skin and the meeting of the target-flux in mice skin does not guarantee its good permeability in human skin.

With the renewed interest in the transdermal therapy, more and more researchers are trying to develop systems suitable for dermal delivery[1-4]. The first step of every transdermal screening is to conduct *in vitro* skin permeation experiments to find out, whether a drug has got the required skin-permeability. These experiments can lead to erroneous conclusions, if the models used for such studies fail to offer resistance, comparable to the human skin. Since the barrier function of the skin vary widely from species to species, it is customary to use of molecule of known penetration kinetics to assess the barrier function of skin[5]. This troublesome and lengthy assessment can be much simplified if the barrier properties of the skin belonging to different species could be studied and correlated[5].

In India, hairy mice are routinely used for transdermal screening because of their availability, viability and reproducibility. In west, researchers normally use human cadaver skin as substitute for viable epidermis to study the penetration kinetics of the drugs.

The aim of the present study is to investigate whether the resistance offered by human cadaver skin can be directly correlated to that of the mice skin with respect to four antihypertensive agents of different water solubility.

Materials and Methods

Drugs used in the study were Prazosin hydrochloride (Sun Pharmaceutical Industries, Gujarat) minoxidil (Dr. Reddy's Laboratories Ltd., Hyderabad), Atenolol (Parke-Davis Ltd., Hyderabad) and propranolol hydrochloride (Cipla Ltd., Mumbai). All the drugs were received as contributory sample. Chemicals used include methanol (Qualigens), Octanol (Rolex Lab.) and gentamicin sulphate (Nicholas Piramal India Ltd.). The cadaver abdominal skin was obtained from 23-35 years old males (Civil Hospital, Raichur). Swiss albino mice were purchased from National Institute of Nutrition, Hyderabad.

Solubility measurements

The solubility determinations were carried out by the method of Okumara *et al*[6]. An excess amount of the drug was taken and dissolved in measured amount of distilled water in a glass vial to get a saturated solution. The system was kept at rest for 24 hr at 37°C to assist the attainment of equilibrium. The supernatant was filtered and assayed spectrophotometrically (Hitachi U-2000, Japan) at the suitable wavelengths.

Partition coefficient determination

The octanol-water partition coefficient of the drugs were determined by the method described by Wells[7]. Ten ml of octanol was added to equal volume of aqueous solution of the drug of known concentration in a separating funnel. The system was kept for 24 hr at 37°C with intermittent shaking. Finally, the aqueous layer was separated, clarified by centrifugation and assayed.

Skin permeation procedure (mice skin)

The *in vitro* diffusion experiment of the drugs were carried out using 6-8 week oil Swiss albino mice (*Mus*

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musculus). Vertical type diffusion cells (Neutron Scientific, Calcutta) having a downstream volume of 20 ml were used. The excised hairy skin was mounted as such on the diffusion cell and receiver compartment was filled with 20 ml of normal saline containing 0.016% gentamicin sulphate to preserve the skin from deterioration. 5 ml of aqueous drug solution (1 mg/ml) was placed in the donor compartment and the temperature was maintained at 37°C. The sample from the receiver compartment were withdrawn at regular intervals and assayed.

Skin permeation procedure (cadaver skin)

Cadaver skin of 23-25 years old males were used for the permeation studies and the experiments were done according to the procedure of Roy *et al*[8]. Samples of skin were removed from the abdomen of cadavers within 48 hr after death. Epidermal layers were separated from the remaining skin by immersing each skin section in water at 60°C for 30 sec.

The epidermis was teased from the dermis using forceps. The separated epidermal layer was used as such for the skin permeation studies. The epidermal layers were cut into small circular patches and checked immediately for any leaks before application of the drug solution. Each membrane was carefully mounted on the diffusion cell and permeation experiments were performed as per mice skin.

Data analysis

The cumulative amount of the drugs ermeated per unit skin surface area was plotted against time and the slope of the linear portion of the plot was estimated as the steady-state flux (Jss)[9]. The permeability coefficient was calculated as,

$$Kp = \frac{Jss}{Cv}$$

where Cv is the total donor concentration of the solute.

In experiments, where steady-state could not be achieved the average permeation rate was calculated by dividing the cumulative amount permeated with the duration of the experiment.

Results and Discussion

Transdermal literature abounds in data obtained from permeation experiments carried out with various animal skins. Permeation studies carried out in such models have generated a plethora of information about the behaviour of drugs within physiologically active viable skin systems. However, data obtained from different skin models vary widely and any

Table 1 — Physicochemical parameters of the candidate drugs

Drugs	Molecular wt	Aqueous solubility at 37°C mg/ml	Octanol/ water partition coefficient
Prazosin hydrochloride	419.91	1.00	0.173
Minoxidil	209.25	8.79	0.815
Atenolol	266.34	31.2	0.374
Propranolol hydrochloride	295.84	104.2	0.041

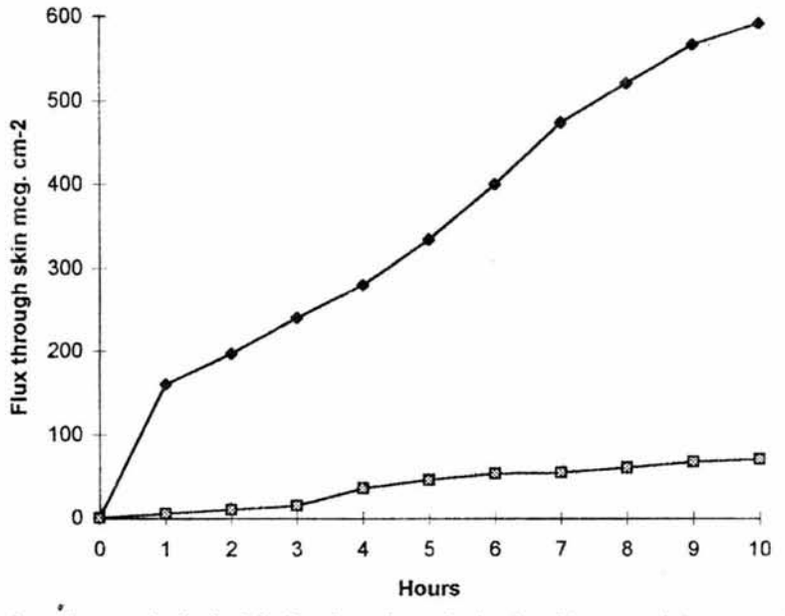

Fig.1 — Cumulative penetration of prazosin hydrochloride through excised mice skin (♦) and human cadaver skin ( ). In case of mice each data-point represents the mean of three experiments which in case of human cadaver is the average of two experiments.

actual evaluation is possible, only if these models could be graded according to the resistance, they offer towards the various penetrants.

In our study we have observed that all the candidate drugs have got higher permeation rate in the mice skin than in the human cadaver skin. Prazosin hydrochloride, a poorly water soluble drug had steady-state flux 13 times higher in the mice skin in comparison to its steady-state flux in the cadaver skin (Fig. 1, Table 2). Minoxidil and atenolol also show considerable higher permeation (3-4 times) in the mice skin (Figs 2&3). However, the freely water soluble drug propranolol hydrochloride has only slightly higher permeation rate in the mice skin than in the cadaver skin (Fig. 4). This contradicts the popular perception that water soluble drugs would show enhance permeation in the hairy skin, the main argument against the use of hairy species in transdermal screening. In a recent investigation also, researchers have observed that hairless guinea pig skin was much more permeable to retinoic acid than haired guineapig skin[10].

Previously, drug permeation was thought to take

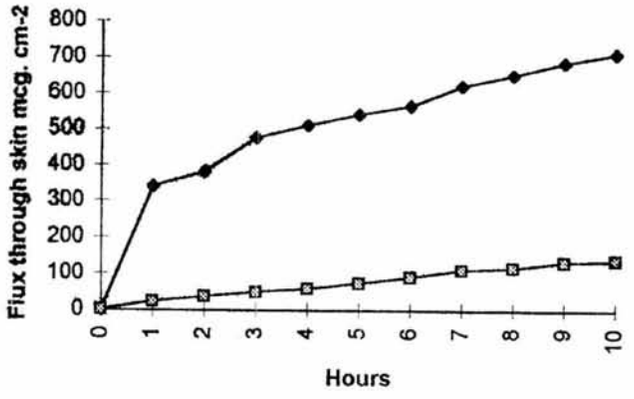

Fig.2—Cumulative penetration profile of minoxidil through the excised mice skin (♦) and human cadaver skin ( ). In case of mice each data-point represents the mean of three experiments which in case of human cadaver is the average of two experiments.

Table 2—Steady-state fluxes (Jss) and permeability coefficient (k_p) of candidate drugs through mice and human cadaver skin

Drugs	Skin type	Steady state flux $\mu g/cm^{-2} hr^{-1}$	Permeability co-eff $cm\ hr^{-1}$
Prazosin hydrochloride	Mice	93.66	9.3×10^{-2}
Prazosin hydrochloride	Human cadaver	7*	7.0×10^{-3}
Minoxidil	Mice	38.29	3.8×10^{-2}
Minoxidil	Human cadaver	12.27	1.2×10^{-2}
Atenolol	Mice	278.78	2.8×10^{-1}
Atenolol	Human cadaver	62.16	6.2×10^{-2}
Propranolol hydrochloride	Mice	75.06	7.5×10^{-2}
Propranolol hydrochloride	Human cadaver	71.24*	7.1×10^{-2}

*Represents average permeation rate.
Values are mean of three experiments in case of mice and average of two experiments in case of human cadaver skin.

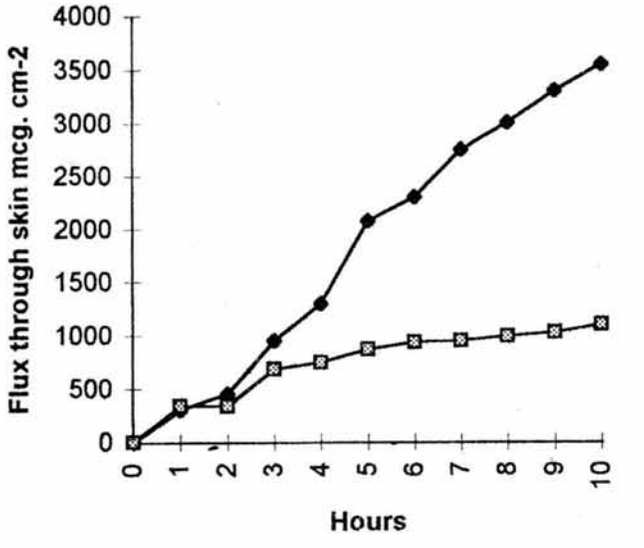

Fig.3—Cumulative penetration profile of atenolol through the excised mice skin (♦) and human cadaver skin ( ). In case of mice each data-point represents the mean of three experiments which in case of human cadaver is the average of two experiments.

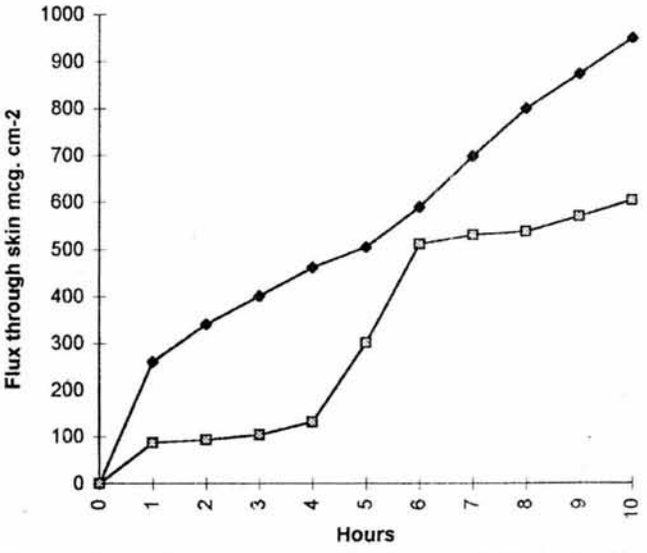

Fig. 4—Cumulative penetration profile of propranolol hydrochloride through the excised mice skin (♦) and human cadaver skin ( ). In case of mice each data-point represents the mean of three experiments which in case of human cadaver is the average of two experiments.

place through two parallel pathways only (lipid and pore pathway)[11]. It was suggested that very hydrophilic compounds diffuse through the aqueous pore pathways as well as through the lipid bilayers. However, a recent study about the mechanism of drug permeation through the human skin revealed that lateral bilayer diffusion is the primary transport mechanism of solute permeation through the human stratum corneum[12]. In the lateral diffusion, the drugs of low molecular weight travel through the narrow lipid slits that laterally separate the keratinocytes. Hence, lateral diffusion shows a strong size dependence for small solutes (<300 Da). Therefore, the good permeability of the atenolol, minoxidil and propranolol hydrochloride in the cadaver skin could be attributed to their ease of movement through the lipid bilayers because of their small size. Similarly, the poor permeability of prazosin hydrochloride in human cadaver skin could be related with their comparatively higher molecular weight.

It can be concluded from the study, that overall permeation rate of the drugs through the mice skin is higher than that through the cadaver skin and in spite of the influence of various physicochemical parameters (water solubility, partition co-efficient etc) on the drug permeation, a 3 to 4 times flux enhancement in the mice skin is not unexpected. This study also confirms the hypothesis of Zesch[13] that data from animals are not transferable to man and also nullifies the perception that hairy skin favours the permeation of water soluble drugs.

Finally, the high permeability co-efficient of the propranolol hydrochloride through the human cadaver skin indicates it has a strong possibility to be successfully developed with a transdermal dosage form.

References

1 Godwin D A, Michniak B B & Creek K E, *J Pharm Sci*, 86 (1997) 1001.

2 Kubota K, Yamada T, Kikuchi K, Koyama E & Ishizaki T, *Eur J Clin Pharmacol*, 44 (1993) 493.

3 Krishna R & Pandit J K, *J Pharm Pharmacol*, 48 (1996) 367.

4 Kakkar A P & Gupta A, *Indian Drugs*, 29 (1992) 308.

5 Gummer C L., in *Transdermal drug delivery*, edited by Jonathan Hadgraft & Richard H Guy (Marcel Dekkar Inc, New York) 1989, 193.

6 Okumara M, Sugibayashi K, Ogawa K & Morimoto Y, *Chem Pharm Bull*, 37 (1989) 1404.

7 Wells J I, in *Pharmaceutical preformulation*, edited by M H Rubinstein (Ellis Horwood Ltd), New York, 1988, 46.

8 Roy S D, Gutierrez M, Flynn G L & Cleary G W, *J Pharm Sci*, 85 (1996) 491.

9 Elias P M, Williams M L, Maloney M E, Bonifas J A, Brown B E, Grayson S & Epstein E H, *J Clin Invest*, 74 (1984) 1414.

10 Hisorie G & Bucks D, *J Pharm Sci*, 86 (1997) 398.

11 Morimoto Y, Hatanaka T, Sugibayashi K & Omiya H, *J Pharm Pharmacol*, 44 (1992) 634.

12 Johnson M E, Blankschtein D & Langer R, *J Pharm Sci*, 86 (1997) 1162.

13 Zesch A, in *Dermal and transdermal absorption*, edited by Brandau R and Lippold B C (Wissenchaftliche Verlagsgesellschaft stuttgart) 1982, 116.