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In-vitro permeation of some β-blockers across the hairless mouse skin

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Abstract—To support the development of a suitable transdermal dosage form for β-blockers, in-vitro, skin permeation studies of nine β-blockers were conducted at 37°C across the excised abdominal skin of hairless mouse mounted on the receptor compartment of a two-chambered Valia-Chien glass diffusion cell. The drugs varied in lipophilicity, whereas pK_a values were comparable. Permeability coefficients were calculated from the steady-state flux values. Agreement was found between the permeability coefficient and the drug lipophilicity, expressed as the octanol-buffer distribution coefficient.

At present, β-blockers are the most extensively used drugs for therapeutic management of various cardiovascular disorders and are recommended as the first-step approach for that purpose (Scriabine 1979). Controlled administration of a β-blocker via a transdermal delivery system could improve its systemic bioavailability and its therapeutic efficacy by avoiding first-pass effect, as well as decreasing the dosing frequency required for the treatment.

Schoenwald & Huang (1983) investigated the transcorneal permeabilities of several β-blockers and recently Le Brun et al (1989) reported the buccal transportation of four β-blockers. No systematic investigation on the transdermal permeation of β-blockers has been reported so far. In this paper, the permeation of nine β-blockers across the hairless mouse skin is reported.

Materials and methods

Drugs. All the drugs (except propranolol hydrochloride) used in this investigation were donated by the respective manufacturers indicated: bevantolol hydrochloride (Warner Lambert Company, Ann Arbor, MI, USA); levbunolol hydrochloride (Warner Lambert Company, Ann Arbor, MI, USA); metoprolol tartrate (Ciba-Geigy Company, Summit, NJ, USA); nadolol (E. R. Squibb and Sons, Princeton, NJ, USA); oxprenolol hydrochloride (Ciba-Geigy Company, Summit, NJ, USA); (±)-propranolol hydrochloride (Sigma Chemical Company, St Louis, MO, USA); penbutolol sulphate (Eli Lilly & Company, Indianapolis, IN, USA); sotalol hydrochloride (Bristol-Meyers Company, Evansville, IN, USA); timolol maleate (Merck Sharp & Dohme, Rahway, NJ, USA).

All other reagents, either HPLC grade or reagent grade, were used as obtained (Fisher Scientific Co., NJ, USA).

Animals. Breeding pairs of hairless mouse (Skh:Sr-1) were purchased from Skin Cancer Research Institute, Temple University, Philadelphia. All experimental animals (6–8 weeks old) used were obtained from the established in-house breeding colony.

Drug assay. An HPLC method was used for analysis of each drug in the receptor compartment (Schoenwald & Huang 1983). The HPLC system included a solvent delivery system (Model 6000A, Waters Associates, Milford, MA, USA), a UV-absorption detector (Model 440, Waters Associates, Milford, MA, USA), an injector (Model U6K, Waters Associates, Milford, MA, USA), stainless steel columns (μBondapak C18 and CN) and a recorder (Model 5211-1, Omniscribe, Houston Instruments, Austin, TX, USA). Quantitation was by the peak height ratio method.

Preparation of skin. Male hairless mice (6–8 weeks old) were killed by cervical dislocation of the spinal cord and a portion (about 3 × 3 cm) of the full-thickness abdominal skin was carefully excised. The dermal side of the skin was carefully cleared of any adhering subcutaneous tissues and blood vessels.

Skin permeation studies. The freshly excised full-thickness skin sample was mounted on the receptor compartment of the Valia-Chien glass diffusion cell (Chien & Valia 1984), with the stratum corneum side facing the donor compartment and the dermal side facing the receptor compartment. Donor solution composition was approximately 0·5% w/v (actual concentration was determined by sampling from the donor compartment at the beginning of each experiment) drug solution in pH 7·4 Sørensen's phosphate buffer and the same buffer solution without drug was used in the stirred receptor compartment. The temperature of the whole system was maintained at 37 ± 1°C by a circulating waterbath (Cole-Palmer Instrument Company, Model 1268–00).

Samples (200 μL) from the receptor compartment were withdrawn at predetermined time intervals and immediately replaced by an equal volume of fresh buffer solution (Chien & Valia 1984). Initial experiments confirmed the maintenance of sink conditions by this procedure. The drug content in the receptor compartment was then determined by HPLC. Each experiment was run in triplicate.

Data analysis. From the concentration profiles of β-blocker in the receptor solution, steady-state skin permeation flux, J (μg cm^{-2} h^{-1}), was calculated using a modified Fick's law equation:

$$J = \frac{V\,(dC/dt)}{A}$$

where, dC/dt is the steady-state slope of the concentration vs time plot (μg cm^{-3} h^{-2}), V is the volume of the receptor compartment (cm^3), and A is the diffusional area of the skin (cm^2). The permeability coefficient P_T (cm h^{-1}) was then determined by dividing the steady-state flux by the donor concentration C_d (mg mL^{-1}) using the following relationship:

$$P_T = \frac{J}{C_d}$$

The octanol/pH 7·4 buffer distribution coefficients, DC, of β-blockers were calculated from the reported partition coefficient, PC (Schoenwald & Huang 1983), using the relationship:

$$DC = \frac{PC}{1 + \text{antilog}\,(pK_a - pH)}$$

Results and discussion

The skin permeability coefficients of the β-blockers calculated by the method described above are listed in Table 1 along with their

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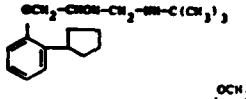
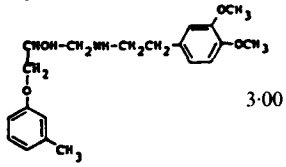
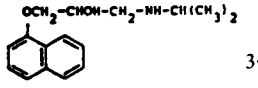
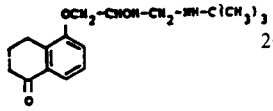
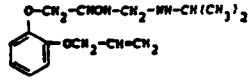
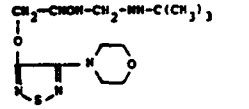
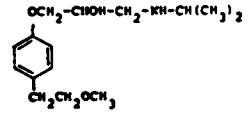
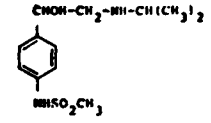
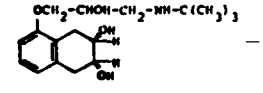
Table 1. Permeability coefficients and physical constants of β-blockers.

Drug	Structure	log PC	log DC	log P_T
Very lipophilic				
Penbutolol		4·15	2·3	−1·59
Bevantolol		3·00	2·0	−1·88
Propranolol		3·21	1·4	−2·13
Lipophilic				
Levobunolol		2·40	0·48	−2·81
Oxprenolol		2·37	0·45	−2·28
Timolol		1·91	0·079	−2·81
Metoprolol		1·88	0·041	−2·56
Hydrophilic				
Nadolol		0·93	−1·07	−2·91
Sotalol		−0·62	−1·44	−3·43

P_T represents the permeability coefficient across the intact excised hairless mouse skin in cm h^{-1}; DC represents distribution coefficient; PC represents partition coefficient.

structures and octanol/pH 7·4 buffer distribution coefficients. Agreement was found between the permeability coefficient and the drug lipophilicity expressed as the octanol-buffer distribution coefficient (Fig. 1). The distribution coefficients of the compounds studied varied over a fourfold log range. This is a consequence of the differences in aromatic substitution which enabled them to be grouped as very lipophilic, lipophilic and hydrophilic compounds. The very lipophilic compounds contain hydrophobic substituents. The cyclopentyl group on the benzene ring of penbutolol, dimethoxybenzyl substituent on bevantolol and an additional benzene ring on propranolol give rise to high distribution coefficients for these compounds. Skin permeation coefficients of these compounds were also found to be high. Direct correlation between permeability coefficients and distribution coefficients was observed. Hydrophilic compounds, in addition to the amino group, also contain relatively polar substituents on the aromatic ring. Therefore, hydrogen bonding interactions with water are greater for nadolol which contains a dihydroxy function and for sotalol, which contains a sulphonamido group. As a consequence, skin permeation coefficients of these compounds were found to be very low. The remaining compounds (levobunolol, oxprenolol, timolol, and metoprolol) contain substituents of an intermediate nature in terms of polarity and their permeability coefficients also lie in the intermediate range.

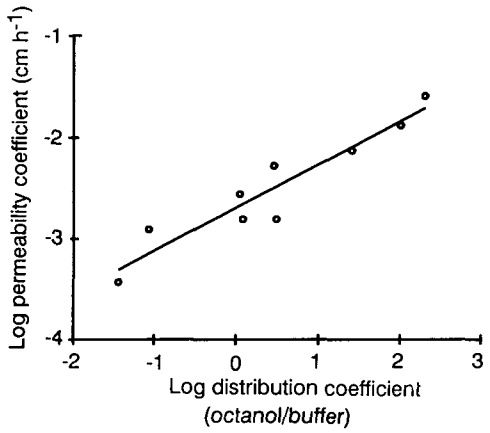


FIG. 1. Log-log plot of permeability coefficient and distribution coefficient (pH 7·4).

For buccal transport (Le Brun et al 1989), a linear correlation was found between permeability coefficient and distribution coefficient, although no optimum or plateau value of distribution coefficient was found. For corneal permeation (Schoenwald & Huang 1983), permeability coefficients increased with the corresponding increase in distribution coefficients to a plateau at a log distribution coefficient of 2·5. Like buccal transport, no optimum value of distribution coefficient for transdermal permeation has yet been found, although a plateau for drugs with a higher lipophilicity is expected. Apart from lipophilicity other factors such as chemical structure and skin protein binding may play a role in permeability behaviour, as pointed out by Michaels et al (1975). Conducting studies on skin/buffer partitioning may explain the phenomena better. Nevertheless, these preliminary data will be useful in screening β-blockers for transdermal delivery.

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