International Journal of Pharmaceutics, 90 (1993) R5–R8

IJP 10059

Rapid Communication

In vitro skin penetration of propranolol enantiomers

C.M. Heard [a], A.C. Watkinson [b], K.R. Brain [a] and J. Hadgraft [a]

[a] *Welsh School of Pharmacy, UWCC, Cardiff CF1 3XF (UK) and* [b] *An-eX, Redwood Building, Cardiff CF1 3XF, (UK)*

(Received 22 December 1992)
(Accepted 4 January 1993)

Key words: Percutaneous penetration; Enantioselective transfer; Propranolol; Enantiomer; In vitro penetration

Summary

Transfer rates of individual enantiomers of propranolol across human skin were determined in vitro. Percutaneous penetration of propranolol from propylene glycol was negligible at the concentration previously reported to show enantioselective transfer in rat skin. Transfer of (*R*)- and (*S*)-propranolol from aqueous solutions of both the racemate and the pure enantiomers showed no differences in the rates of penetration demonstrating that the rate of transfer of propranolol across human skin from these solutions was independent of the stereochemistry of the drug. In addition there was no evidence for racemisation during the transfer process.

The transdermal delivery of many drugs has received considerable attention in recent years. However, surprisingly little attention has been paid to the transfer characteristics of individual enantiomers of chiral species. This is an important issue in view of the fact that significant differences are frequently found between the activities of drug enantiomers (e.g., Stevenson and Williams, 1988). As a consequence, regulatory authorities are demanding more information concerning all aspects of the administration of chiral drugs (Bridges, 1991), including those delivered transdermally.

Miyazaki et al. (1992) recently reported marked differences in the transfer rates of the enantiomers of the β-blocking drug propranolol (as free base) across rat skin and claimed that the process responsible for enantioselectivity resided within the stratum corneum. This communication describes similar experiments on the individual *R* and *S* enantiomers and the racemate using full-thickness excised human skin.

The (±)-propranolol hydrochloride (determined enantiomeric ratio 1.21:1, *R*:*S*) was a gift from ICI Pharmaceuticals plc. Enantiomerically pure (*R*)- and (*S*)-propranolol hydrochlorides were obtained from Cambridge Research Biochemicals Ltd, Northwich, U.K. Free propranolol base was prepared by neutralisation of an aqueous solution of the hydrochloride salt with 2 M sodium hydroxide. Sodium perchlorate was obtained from Aldrich. All other materials were AnalaR grade. Human male abdominal skin was obtained post-mortem.

Correspondence to: C.M. Heard, Welsh School of Pharmacy, UWCC, Cardiff CF1 3XF, U.K.

After removal of subcutaneous fat by blunt dissection the skin membranes were mounted, stratum corneum uppermost, in all-glass Franz-type calibrated diffusion cells of nominal diffusional area 0.8 cm^2 and receptor volume 2.2 cm^3. The receptor chambers were filled with pre-thermostatted phosphate-buffered saline (PBS) and the cells immersed in constant temperature water baths at 37°C such that the skin temperature was 32 ± 1°C. The receptor phases were continuously agitated by magnetic followers impelled by submersible multiple stirrer plates. After equilibration for 2 h, 1 ml of the relevant solution was added to the donor compartment which was then capped. At appropriate time points, 200 μl was withdrawn from each receptor chamber and retained for analysis. An equal volume of pre-equilibrated PBS was added to maintain the correct volume. A total of five replicates were used for each test.

Initially 100 μg ml^{-1} propranolol base in propylene glycol was applied to mimic the conditions employed by Miyazaki et al. (1992) with rat skin. Because the observed transfer rate was very low under these conditions, 0.01 M (2958 μg ml^{-1}) solutions in PBS (Green, 1988) of either (*R*)-, (*S*)- or racemic propranolol hydrochloride were also examined.

All samples were analysed by HPLC under the following conditions: LDC analytical constaMetric 3500 pump, LDC analytical membrane degasser, LDC analytical spectroMonitor 5000 detector (289 nm), Promis II autosampler with 100 μl loop, Daicel OD-R reversed-phase chiral column (derivatised cellulose/octadecyl), mobile phase 0.5 N sodium perchlorate/acetonitrile (60:40), flow rate 1 ml min^{-1}. Enantiomeric resolution was complete and the *R*(d) form had a shorter retention time than the *S*(l) form. A typical chromatogram is shown in Fig. 1.

When 100 μg ml^{-1} propranolol base in propylene glycol was used as donor phase, the quantity of drug found in the receptor chamber at the end of the experiment (72 h) was insignificant, certainly insufficient to determine the enantiomeric ratio. Although this result was contrary to the findings of Miyazaki et al. it is consistent with observations that rat skin is often considerably more permeable than human skin (Kao et al., 1985; Scott et al., 1987).

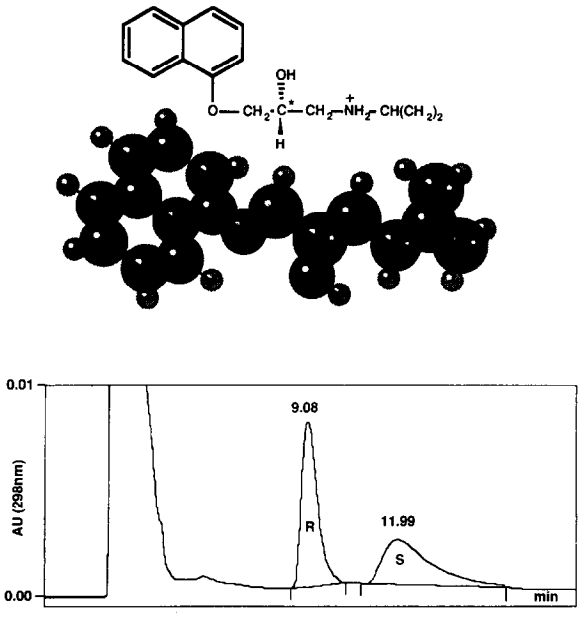

Fig. 1. Chemical structure of propranolol and typical HPLC chromatogram demonstrating complete enantiomeric resolution.

Fig. 2 shows the percentage of the dose of propranolol transferred from PBS solutions of the individual *R* and *S* enantiomers. No difference in transfer rate was apparent and in addition no racemisation was observed. These results were reinforced by the data in Fig. 3 from a PBS

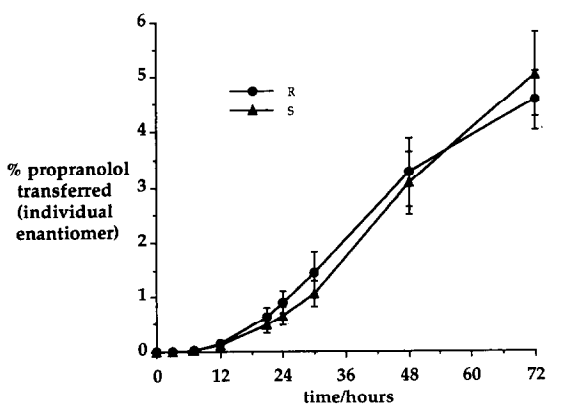

Fig. 2. Percentage propranolol transferred as pure enantiomer.

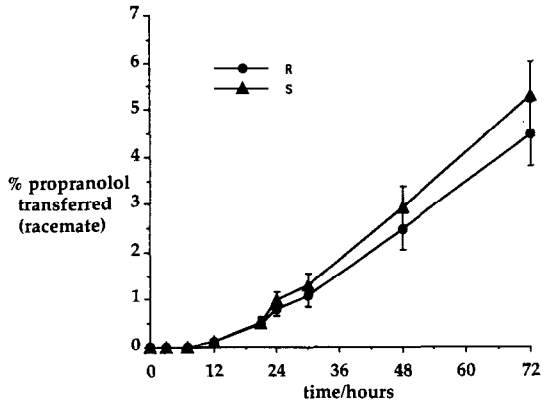

Fig. 3. Percentage propranolol transferred from racemate.

solution of (±)-propranolol (correcting for *R* excess). Table 1 gives the steady-state flux and permeability coefficient data. Using analysis of variance no statistically significant differences were found between the transfer characteristics of the enantiomers.

Enantioselectivity might be expected to be particularly sensitive to small changes in molecular environment such as pH and vehicle. The pK_a of propranolol is 9.2. On solution of propranolol free base in propylene glycol the degree of protonation would be minimal, although protonation may occur on contact with the acidic environment (~ pH 5.5) of the skin surface. On the other hand, propranolol will exist almost entirely in its protonated form (Fig. 1) when in a buffer solution of pH 7.4. The distance between the amine group in propranolol and the chiral centre is quite small and protonation of this group may interfere with a stereospecific interaction involved in percutaneous transport. However, it has been observed previously (Heard et al., 1992) that enantioselective ion-pair transfer of propranolol is possible using an artificial solid-supported liquid membrane containing a stereospecific carrier molecule.

Alternatively, human stratum corneum may not contain an enantioselective feature which is present in rat skin. This has significant implications where animal skin is used as a predictive model for human skin and the possibility of species differences in transdermal enantioselectivity requires further investigation.

References

Bridges, J.W., A regulatory view of the importance of chiral discrimination. In Stevenson, D. and Wilson, I.D. (Eds), *Recent Advances in Chiral Separations*, Plenum, London, 1991, pp. 1–3.

Green, P.G., Physicochemical aspects of enhanced skin permeation of cationic drugs, Ph.D. Thesis, University of Wales (1988).

Heard, C.M., Brain, K.R. and Hadgraft, J., Differential facilitated transfer of propranolol and bupranolol enantiomers across a liquid membrane. In *Proceedings of the 1st annual UKaps Conference* 1992, p. 28.

Kao, J., Patterson, F.K. and Hall, J., Skin penetration and metabolism of topically-applied chemicals in six mammalian species, including man : an in vitro study with benzo-*a*-pyrene and testosterone. *Toxicol. Appl. Pharmacol.*, 81 (1985) 502–510.

Miyazaki, K., Kaiho, F., Inagaki, A., Dohi, M., Hazemoto, N., Haga, M., Hara, H. and Kato, Y., Enantiomeric difference in percutaneous penetration of propranolol through rat excised skin. *Chem. Pharm. Bull.*, 40 (1992) 1075–1076.

TABLE 1

Steady-state flux and permeability coefficient values for transfer of propranolol enantiomers across human skin

Enantiomer type		Flux ($\mu g\ cm^{-2}\ h^{-1}$)	*p*	Permeability coefficient ($\times 10^{-3}$)(cm h^{-1})	*p*
Racemate	*R*	1.81 ± 0.27	0.82	1.12 ± 0.17	0.51
	S	1.73 ± 0.25		1.30 ± 0.19	
Individual	*R*	4.49 ± 0.67	0.21	1.14 ± 0.14	0.20
	S	3.37 ± 0.43		1.52 ± 0.23	

Scott, R.C., Dugard, P.H., Ramsey, J.D. and Rhodes, C., In vitro absorption of some *o*-phthalate diesters through human and rat skin. *Environ. Health. Perspect.*, 74 (1987) 223–227.

Stevenson, D. and Williams, G.A., The biological importance of chirality and methods available to determine enantiomers. In Stevenson, D. and Wilson, I.D. (Eds), *Chiral Separations*, Plenum, New York, 1988, pp. 1–9.