ENANTIOMERIC DIFFERENCE IN PERCUTANEOUS PENETRATION OF PROPRANOLOL THROUGH RAT EXCISED SKIN

Keisuke MIYAZAKI, Fusao KAIHO,* Aiko INAGAKI, Masahiko DOHI, Norio HAZEMOTO, Makoto HAGA, Hiroshi HARA and Yuriko KATO

Faculty of Pharmaceutical Sciences, Science University of Tokyo, Ichigaya, Funagawara-machi, Shinjuku-ku, Tokyo 162, Japan

The percutaneous penetration of *R*-(+)- and *S*-(-)-propranolol (PL) through rat excised skin was investigated *in vitro*. The flux of *S*-(-)-PL after application to normal skin was high compared with that of *R*-(+)-PL. On the other hand, in damaged rat skin, the flux of *R*-(+)-PL was almost equivalent to that of *S*-(-)-PL. It is suggested that there is an enantiomeric difference between *S*-(-)- and *R*-(+)-PL in terms of penetration through rat stratum corneum.

KEYWORDS percutaneous penetration; enantiomeric difference; *R*-(+)-propranolol; *S*-(-)-propranolol; normal skin; damaged skin; rat; flux; *in vitro*

Recently, many attempts have been made to investigate the percutaneous penetration of drugs through the skin *in vitro*. Nevertheless, little or no attention has been paid to the effect of enantiomeric difference of drugs on the permeation through the skin. In this paper, we wish to report the influence of enantiomeric difference on the permeation of *R*-(+)- and *S*-(-)-propranolol (PL) through the skin of the rat.

MATERIALS AND METHODS

Materials *R*-(+)- and *S*-(-)-PL hydrochloride and labetalol hydrochloride were purchased from Sigma Chemical Co., Ltd. All the solvents used in this experiment were of reagent grade from Kanto Chemical Co., Ltd.

Preparation of Test Solution The test solution used for this study was prepared as follows. Two enantiomers of free PL derived from the corresponding optically pure PL hydrochlorides by the method of Ogiso *et al.*[1] were dissolved in pure propylene glycol so as to give 0.01 w/v%.

***In Vitro* Study** Franz-type diffusion cells, designed with a volume of 27.5 ml in receptor phase and diffusion area of 5.37 cm^2, were employed. The normal and damaged abdominal skin of male Wistar rats weighing 230-250 g were used as diffusion membranes. Hair was removed from rats using an animal clipper and a razor. To obtain damaged skin, the hair of the abdominal region was cut and removed, and then the stratum corneum was removed by 20 successive strippings using cellophane adhesive tape according to the method of Washitake *et al.*[2] The excised normal or damaged skin was mounted on the diffusion cell, and the receptor phase was filled with saline. 1 ml of test solution was applied to the skin surface on the donor side. The cell was maintained at 37°C. The receptor phase was stirred constantly at 1000 rpm with a magnetic bar. An aliquot (250 μl) of receptor fluid was withdrawn periodically and replaced with the same volume of fresh saline.The concentration of PL in the receptor fluid was determined by high-performance liquid chromatography (HPLC).

Analytical Method The analysis of PL in the receptor fluid was carried out according to the method of Drummer *et al.* with some modifications.[3] 100 μl of internal standard (labetalol hydrochloride, 1 mg/ml) aqueous solution was added to 250 μl of sample in a centrifuge tube. After being shaken for 1 min vigorously, 30 μl of this solution was injected into HPLC. The chromatographic system consisted of a pump (5GK-30, Nippon Seimitu Co., Ltd.), an injector (Rheodyne-7125, Cotai Co., Ltd.), and a fluorescence detector (S-3350, Soma Kougaku Co., Ltd.) set at respective excitation and emission wavelengths of 295 and 360 nm. The conditions for analysis were as follows : mobile phase, 10 mM monobasic potassium phosphate buffer (pH 3.4) - methanol (1:1, v/v) ; column, SSC-ODS-262 (6.0 mm i.d. x 100 mm, Senshu Scientific Co., Ltd.) ; flow rate, 1.5 ml/min ; and column temperature, room temperature. The signal from a detector was fed into an integrator (Chromatocorder 12, System Instrument Co., Ltd.). *R*-(+)- and *S*-(-)-PL used in this study were checked to be

homo-chiral by chiral stationary-phase liquid chromatography.[4] Optical purity of PLs in the receptor fluid was intact with no isomerization through the skin.

RESULTS AND DISCUSSION

Fig.1-A shows the permeation profiles of *R*-(+)- and *S*-(-)-PL from test solution through the normal abdominal skin of rats. The flux of *S*-(-)-PL after application to the normal skin was high compared with that of *R*-(+)-PL, and further, the amount of the former in the receptor fluid was observed to be about 4 times higher at 12 h than that of the latter. On the other hand, as shown in Fig.1-B, the flux of *R*-(+)-PL from test solution through damaged skin of rats was almost equivalent to that of *S*-(-)-PL.

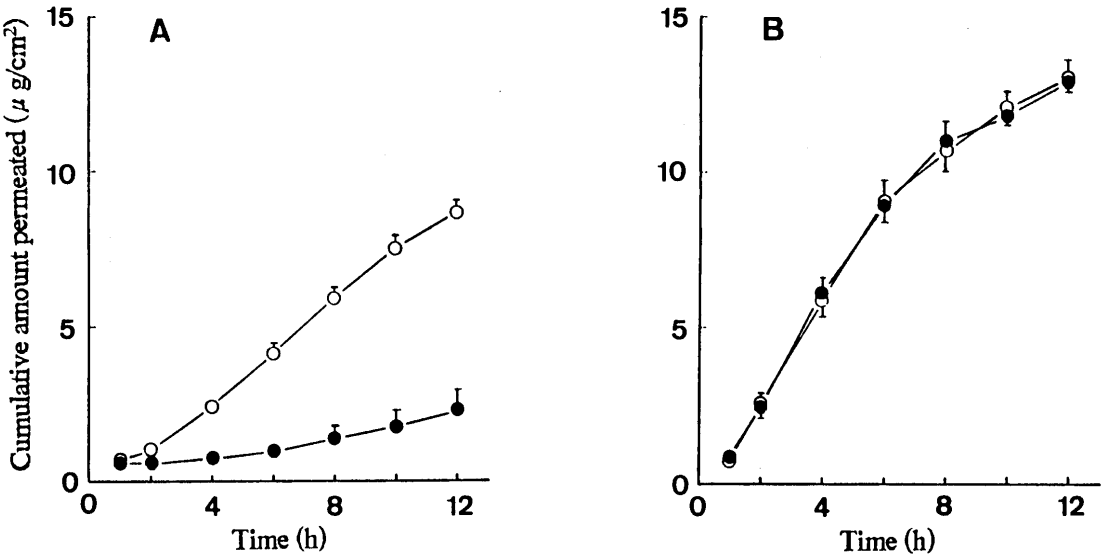

Fig.1. Permeation Profiles of *R*-(+)- and *S*-(-)-Propranolol (PL) through the Excised Skin of Rats
A, normal skin; B, damaged skin; ●, *R*-(+)-PL; ○, *S*-(-)-PL
Each point represents the average of four experiments, and bars represent the standard error.

These results suggest that there is an enatiomeric difference between *S*-(-)- and *R*-(+)-PL in terms of penetration through rat stratum corneum. However, the factors which give rise to the permeability gaps between *S*-(-)- and *R*-(+)-PL through rat skin are still unclear. Experiments to elucidate these factors are now in progress.

REFERENCES

1) T. Ogiso, Y. Ito, M. Iwaki and A. Shintani, *J. Pharmacobio-Dyn.*, **11**, 349 (1988).
2) M. Washitake, T. Yajima, T. Anmo, T. Arita and R. Hori, *Chem. Pharm. Bull.*, **21**, 2444 (1973).
3) O. H. Drummer, J. Mcneil, E. Pritchard and W. J. Louis, *J. Pharm. Sci.*, **70**, 1030 (1981).
4) H. Takahashi, S. Kanno, H. Ogata, K. Kashiwada, M. Ohira and K. Someya, *J. Pharm. Sci.*, **77**, 933 (1988).

(Received January 27, 1992)