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COMMUNICATION

Effect of Menthol on Permeability of an Optically Active and Racemic Propranolol Across Guinea Pig Skin

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ABSTRACT

The effect of menthol on the percutaneous penetration of S(−)-propranolol (SPL) and racemic form of propranolol (RSPL) was investigated in vitro using excised abdominal skin of guinea pig. In the presence of menthol, the permeability coefficient of SPL was high compared with that of RSPL. The enhancement factors for SPL and RSPL were 2.12 and 0.85, respectively. The lag times for SPL and RSPL were reduced considerably in the presence of menthol compared to those for control (without enhancer). The present findings suggest the enantio-selective permeation of SPL across the guinea pig skin in the presence of menthol.

INTRODUCTION

Propranolol (PL), the most widely prescribed beta-blocker, is administered as a racemate—a 50:50 mixture of two enantiomers, with S(−)-propranolol [S(−)-PL, or SPL] having 100-fold greater activity than that of the racemic form of PL [R(+)-PL, or RSPL] (1). The permeation rates of PL enantiomers have been studied across rat skin (2,3) and human skin (4). In the absence of permeation enhancers, systemic delivery of most drugs through the skin is restricted, mainly because of the barrier function of the stratum corneum. Permeation enhancers increase drug transport through the skin by altering the diffusion and/or partition coefficient. Recently,

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many attempts have been made to investigate the use of terpene enhancers such as menthol, limonene, and carvacrol to promote the transdermal transport of drugs (5–8). In the present study, the effect of menthol on percutaneous penetration of SPL and RSPL is investigated across the abdominal skin of the guinea pig.

EXPERIMENTAL

Materials

RSPL and SPL hydrochloride and l-menthol were purchased from Sigma Chemical Co. (St. Louis, MO). The racemic and optically active enantiomers of propranolol were prepared by precipitation of the base from aqueous solution with 1 N NaOH. Propylene glycol and poly(ethylene glycol) (PEG) 400 were obtained from Aldrich Chemicals (Milwaukee, WI). All other reagents were of analytical grade and were used without further purification.

In Vitro Permeation Studies

Guinea pig abdominal skin was used in this study. The abdominal skin was carefully excised and was gently teased of adhering fat and/or subcutaneous tissue. The excised skin was rinsed with distilled water and wiped carefully with tissue. The skin was then examined through a magnifying lens to ensure that it was free from any surface irregularities such as tiny holes or crevices. The thickness of the abdominal skin was 1.05 mm. The required surface area was cut by means of a punch. The epidermis was sandwiched between Valia-Chien "side-bi-side" diffusion cells (Crown Glass Co., Somerville, NJ) with the stratum corneum side facing the donor cell. The two half-cells were tightened by an adjustable clamp. The surface area of the exposed skin to the solution was 0.64 cm^2. The donor half-cell media contained 10 mg of SPL or RSPL free base with 20 mg of l-menthol. Control study was done without menthol as enhancer. The donor and receiver cells contained a volume of 3.2 ml, and the receiver cell had 30% v/v PEG 400 aqueous solution; the media and the donor cell contained drug, with or without menthol, in propylene glycol. The temperature was maintained at 37°C throughout the study period. The donor and receiver media were stirred at constant rpm during the experiment to ensure thorough mixing. Samples of 200 µl were collected at 0, 0.5, 1, 2, 3, 4, 6, 8, 10, and 12 hr. The withdrawn volume was replaced with a fresh 30% PEG 400 solution. The drug concentration was measured by HPLC. The results were expressed as mean ± SD of three successful experiments for each study.

Assay Method

The PL concentrations were measured by injecting appropriate sample volumes in a Waters (Milford, MA) HPLC with a UV detector (Waters 991PDA detector, 600E pump, and 715 UltraWisp sample processor). The drug concentration was measured by a modified USP 22 method. The assay was run at 290 nm on a 3 × 3 cm C18 reversed-phase column (Cartridge Pack, Perkin Elmer, Norwalk, CT) using external standard solutions of PL base. The mobile phase was composed of methanol 33% v/v, acetonitrile 33% v/v, sodium lauryl sulfate 0.2% w/v, and 0.15 M phosphoric acid aqueous solution 34% v/v; flow rate was 1 ml/min. Linear calibration curves over the concentration range of from 110 ng/ml to 40.5 µg/ml were generated. The standard deviation for replicate injections was not more than 2%.

RESULTS

The cumulative amount permeated versus time plot is shown in Fig. 1. In the absence of enhancer, SPL had

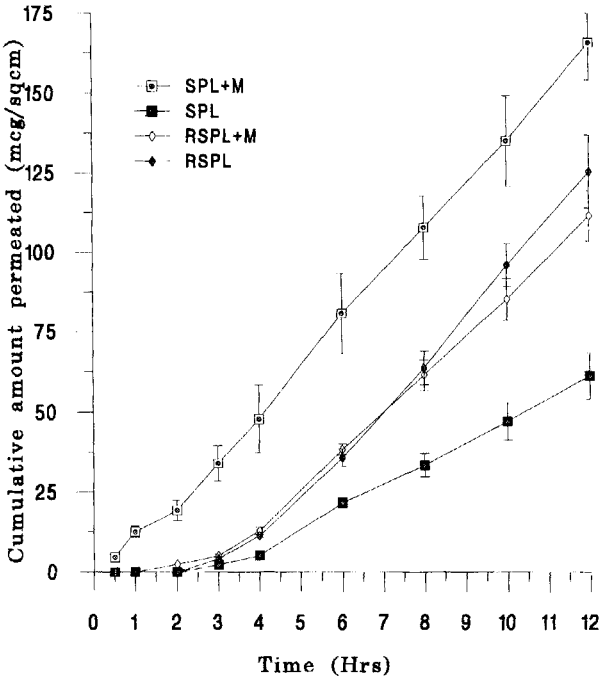

Figure 1. Permeability profiles of SPL and RSPL through the excised abdominal skin of guinea pigs with or without l-menthol (M). Each point represents the average of three experiments.

Table 1

Steady-State Flux, Lag Time, Permeability Coefficient, and Enhancement Factor Values for SPL and RSPL With or Without l-Menthol (M)

Drug	Flux ($\mu g\ cm^{-2}hr^{-1}$)	Lag Time (hr)	Permeability Coefficient ($cm^{-2}hr^{-1}$) $\times 10^{-5}$	Enhancement Factor
SPL + M	14.60	0.55	146	2.12
SPL	6.90	3.30	69	
RSPL + M	12.22	2.75	122	0.85
RSPL	14.44	3.15	144	

lower permeability than RSPL. However, SPL showed greater permeation across the guinea pig abdominal skin in the presence of menthol compared with that of control (without enhancer) at all time points studied. For RSPL, the amounts permeated were comparable at all time points in the presence and absence of menthol. The steady-state flux, lag time (T_{lag}), permeability coefficient (K_p), and enhancement factor (EF; steady-state flux with menthol/steady-state flux without menthol) for SPL and RSPL are summarized in Table 1. In the presence of menthol, there was a 2.1-fold increase in the steady-state flux of SPL compared with that of control (without enhancer). For RSPL, however, the flux values were comparable with or without menthol. The presence of menthol decreased the time to reach the steady-state flux (or T_{lag}) for RSPL as well as SPL, however the decrease in lag time with SPL was more pronounced compared to RSPL. The enhancement factors for SPL and RSPL were 2.12 and 0.85, respectively.

DISCUSSION

Enantioselectivity in pharmacology following administration of a racemic drug has been well recognized since the early part of this century. These differences were expected because of the diastereomeric relationship between chiral molecules and chiral receptors, which include enzymes and plasma proteins (9,10). It is reported that the bioavailability of SPL was less when administered as a single enantiomer compared to its bioavailability when administered as the racemic mixture (11). In the absence of menthol, the reduced permeability of SPL compared to RSPL is in agreement with the findings of Lindner et al. (11), suggesting that the presence of RSPL had a beneficial effect on the availability of SPL. However, in the presence of menthol, SPL exhibited higher transport across the skin compared to RSPL. The EF for SPL was relatively high (2.12) compared to that of RSPL (0.85), suggesting that there is an enantiomeric difference in their permeation through guinea pig skin in the presence of menthol. These differences may be attributed to differences in melting temperatures (MT) between enantiomers and racemate of PL. SPL has an MT of 72 $\pm$ 0.5°C, whereas RSPL has an MT of 92.6 $\pm$ 0.4°C. According to Touitou et al. (7), the difference between the melting points of the enantiomers and racemate is 21°C. Melting temperature–membrane transport concept (Touitou et al.) (7) predicts significant differences in skin transport rates for enantiomers and racemate having large differences in melting temperatures.

CONCLUSION

The preliminary results of our investigations suggest the potential use of chiral enhancers such as menthol in the development of SPL, a pharmacologically more active enantiomer for therapeutic applications.

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