Synthesis, Stereoselective Enzymatic Hydrolysis, and Skin Permeation of Diastereomeric Propranolol Ester Prodrugs

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Abstract □ Four diastereomeric propranolol ester prodrugs (1*S*2*S*, 1*S*2*R*, 1*R*2*S*, 1*R*2*R*) were synthesized by treating pure *R*- and *S*-propranolol hydrochloride with pure enantiomers *R*- and *S*-phenylbutyryl chloride. A HPLC technique using α-1 acid glycoprotein (chiral AGP) column was developed to study the racemization of propranolol enantiomers during synthesis and hydrolysis studies. A reversed phase HPLC method was also developed to simultaneously analyze propranolol and the ester prodrug. Hydrolysis of these esters was studied in different rat tissue homogenates, i.e., liver, intestine, plasma, skin, brain, and pure plasma cholinesterases, i.e., butyryl cholinesterase (EC 3.1.1.8) and acetyl cholinesterase (EC 3.1.1.7). In vitro percutaneous permeation studies across full thickness shaved rat skin were performed using standard side-by-side diffusion cells at 37 °C. The disappearance of the diastereomeric ester prodrugs in rat tissue homogenates followed apparent first-order kinetics and was stereoselective. The ratio of brain to plasma hydrolytic rate constants are 27.8, 5.58, 6.07, and 2.97 for 1*S*2*S*, 1*R*2*R*, 1*R*2*S*, and 1*S*2*R* esters, respectively. Hydrolysis of all four diastereomeric ester prodrugs was faster by acetyl cholinesterase than butyryl cholinesterase and is stereoselective. The permeability coefficients [$K_p \times 10^3$ (cm h^{-1})] are 1.40 ± 0.30, 1.41 ± 0.27, 42.20 ± 1.24, 29.26 ± 3.41, 16.27 ± 3.12, 12.99 ± 2.84 for (*R*)-propranolol, (*S*)-propranolol, 1*S*2*S*, 1*R*2*S*, 1*S*2*R*, and 1*R*2*R* ester prodrugs, respectively. The results indicate that the 1*R*2*S* diastereomeric ester prodrug of propranolol shows greatest stability in liver and intestinal tissues while it exhibits fairly rapid conversion in plasma. The results also suggest the configuration on the second chiral carbon atom to be the determinant in the rate of hydrolysis of all the diastereomeric prodrugs in all biological media examined. The K_p of all four prodrugs markedly increased compared to that of the parent drug, with 1*S*2*S* showing a 30-fold increase in skin permeability, the highest among all four prodrugs.

Introduction

Propranolol (PL), an adrenergic β-blocker, is a widely accepted and clinically effective cardiovascular agent, indicated in the treatment of angina pectoris, hypertension, and cardiac arrhythmia. Very low and variable bioavailability of the drug follows oral administration, which may be attributed to extensive stereoselective hepatic first-pass metabolism.[1−4] Alternative noninvasive administration modes including rectal,[5] transdermal,[6] and nasal[7,8] routes have been explored to improve the systemic bioavailability of propranolol. These routes can allow the drug to reach the systemic circulation, bypassing the liver and escaping first-pass metabolism. Since one of the major metabolites is the *O*-glucuronide of PL,[9] O-acylation of PL may be a potentially useful way to reduce presystemic metabolism, as has already been demonstrated.[10,11]

The transdermal route of administration also allows systemic drug absorption bypassing hepatic first-pass effects, thus achieving higher bioavailability of the drug.[6,12] However, relative impermeability of lipoidal stratum corneum, the outermost keratinized layer of skin, offers considerable resistance to the permeation of PL through the skin. The prodrug approach represents an alternative and promising method of enhancing the skin permeability of drugs by increasing their lipophilicity. Permeability characteristics of individual enantiomers of chiral species in comparison to their racemates have been studied in some detail recently. Wearly et al.[13] reported that the maximum flux of a new antifungal compound from a saturated solution, through human cadaver skin, was 1 order of magnitude lower for the racemic compound than that of either of its enantiomers. Roy et al.[14] compared the permeability of pure enantiomers of keterolac acid, a potent analgesic, through human cadaver skin, and showed that there was no significant difference in the intrinsic permeability coefficients of the racemic compound and the enantiomers. Touitou et al.[15] proposed the Melting Temperature Membrane Transport concept, which showed the simple dependence of permeation flux ratio on the melting behavior of chiral compounds.

Propranolol has two optical isomers, dextro-PL ((*R*)-PL) and levo-PL ((*S*)-PL). The drug shows stereoselective activity, i.e., the S-isomer is 100 times more potent than the R-isomer as a β blocker.[16] A number of studies have demonstrated stereoselective disposition of the drug in various species including man[17] and dog.[18] In addition, Stoschitzky et al.,[19] reported that half the dose of optically pure (*S*)-propranolol generates an equally effective β-adrenergic receptor agonist activity relative to the racemic mixture and that (*R*)-PL inhibits thyroid hormone activity.

Esterases are ubiquitous in nature and have broad substrate specificity. Stereoselective hydrolysis of ester prodrugs including PL prodrugs is largely dependent on the type of species and the tissue of origin.[20−25] Variability in the esterase content may have a significant effect on the rate of regeneration, tissue uptake, and disposition of the prodrug. Thus, consideration of stereoselectivity during prodrug design is very important. The chirality of a compound is critical to receptor-binding interactions: therefore, differences in biological activity are common. Chirality can also effect the way in which molecules fit together in a crystal structure. Diastereomers may differ in crystal structure and may cause differences in melting point and thereby differences in solubilities.[13] Therefore, diastereomeric prodrugs having at least two chiral centers may

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significantly alter the permeation of the enantiomeric parent drug.

This research article describes the influence of a chiral promoiety on the stereoselective hydrolysis and permeation of *R* and *S* propranolol prodrugs. Specifically the stereoselective hydrolysis and skin permeation of four diastereomeric PL ester prodrugs have been studied. 2-Phenylbutyryl chloride was chosen as a model chiral promoiety for the syntheses of four diastereomeric ester prodrugs of PL, as it offers both chirality and the aromatic group, which would aid in increasing the lipophilicity considerably.

Materials and Methods

Materials—The pure (*R*)- and (*S*)-propranolol hydrochloride, (*R*)- and (*S*)-2-phenylbutyric acids and pure esterases, acetyl cholinesterase (EC 3.1.1.7) and butyryl cholinesterases (EC 3.1.1.8), were obtained from Sigma Chemical Co. (St. Louis, MO). All other reagents were of analytical grade and used as received (Fisher Scientific).

Animals—Male Sprague Dawley rats (250−275 g) were obtained from Harlan Sprague Dawley, Indianapolis, IN. Animals were fasted overnight prior to conducting any experiment. Water was allowed ad libitum.

Synthesis of Acid Chloride—The acid chloride was synthesized by slightly modifying a previously published report.[26] Briefly, pure (*R*) or (*S*)-2-phenylbutyric acid (200 mg) was mixed with thionyl chloride (0.24 mL) (freshly redistilled) at 0 °C and then kept at 70 °C for 30 min. Dry benzene was added, and the mixture was evaporated to dryness. Another portion of dry benzene was then added, and the mixture was reevaporated to remove the last traces of thionyl chloride. The residue was dissolved in 2.4 mL of dry benzene and stored in the refrigerator in a glass-stoppered flask. The new acid chloride bands in the IR spectra appeared at about 1810 cm^{-1} with the concomitant disappearance of hydroxyl bands. The purity of the acid chloride was determined by TLC, HPLC, and ^1H NMR. No significant ($p > 0.05$) difference in the optical activity between the acid {$[\alpha]^{20}_D = -94/+93.8$ ($c = 0.9$, $C_6H_5CH_3$)} and acid chloride {$([\alpha]^{20}_D = -94.8/+94$ ($c = 0.9$, C_6H_5-CH_3)} was noticed for a particular enantiomer.

Synthesis of Propranolol Diastereomeric Ester Prodrugs—The synthetic method is a slight modification of the methods described previously.[27,28] Pure (*R*)- and (*S*)-propranolol HCl (100 mg, 0.34 mmol) was dissolved in chloroform and heated under reflux conditions for 5 h with pure (*S*)- or (*R*)-2-phenylbutyryl chloride (1.36 mmol). Excess acid chloride was removed under high vacuum, and the residue was repeatedly treated with toluene and evaporated to dryness to ensure removal of any remaining acid chloride. The diastereomeric esters were obtained as an oily residue. The ν_{max} at 1810 cm^{-1} in the IR spectrum due to acid chloride disappeared over 3 h and a strong ν_{max} at 1740 cm^{-1} due to O-acylation appeared. The structural identity and purity of the diastereomeric esters were confirmed by IR, ^1H NMR, mass spectrometry, TLC, and HPLC.

Solubility Determination—A normal equilibrium solubility determination was undertaken in 0.01 M acetate buffer (pH 4.0, 25 °C). To 1 mL of buffer, 2−3 mg of hydrochloride salts of ester prodrug or 300 mg of propranolol HCl was added. The samples were sonicated for 60 min at room temperature and subsequently shaken mechanically in a temperature-controlled water-bath at 25 °C for 16 h. The solution was then filtered through a 0.2 mm filter (Millipore) and, after dilution, the solubility of each diastereomer was determined by reversed phase HPLC. The solubility of each diastereomer was also determined in DMSO/acetate buffer mixture used in the skin permeation study according to the procedure outlined above.

Measurement of Partition Coefficient—Partition coefficients of propranolol and the prodrugs were determined in 1-octanol/pH 4.0 phosphate buffer ($\mu = 0.155$) since the prodrugs are most stable at pH 4.0. Aqueous buffer solution and 1-octanol were mutually presaturated at 25 °C before use. Propranolol or the prodrug concentration in pH 4.0 buffer was measured by a reversed phase HPLC method before and after shaking with equal volume of 1-octanol for 1 h. The partition coefficients were determined as the ratios between the concentrations measured in 1-octanol and pH 4.0 phosphate buffer.

Preparation of Liver and Intestinal Homogenates—Rats were sacrificed by decapitation and the upper two-thirds of the small intestine and liver were removed rapidly, washed with ice-cold 0.15 M KCl, blotted to dryness, and weighed. The intestine was cut into approximately 5 cm long sections, and the subsequent tissue manipulations were conducted at 4 °C. Fat and omentum from the small intestine were removed, and the lumen was washed with saline. The mucosal layer was collected by scraping with a glass slide. The mucosal cells and the liver were homogenized in five volumes of 1.15% KCl with a tissue homogenizer (Biospec) and then centrifuged at 10000*g* for 20 min at 4 °C. To adjust the protein content (2 mg/mL in liver sample and 1.5 mg/mL in intestine sample), the supernatant fraction was diluted with 1.15% KCl solution. Blood was collected in heparinized tubes, and plasma was separated by centrifugation (10000*g*) for 15 min at 4 °C. The protein content in the tissue homogenates was estimated by the method of Lowry et al.[29]

Preparation of Rat Brain Homogenate—Animals (male rats, Sprague−Dawley) were sacrificed by decapitation, and brains were removed and homogenized in ice-cold 1.15% KCl (w/v) solution [brain tissue/solution, 1:4] with a homogenizer (Tissue Tearor, Biospec). The homogenate was centrifuged at 9000*g* for 20 min. The supernatant fraction containing soluble and microsomal esterases was stored in aliquots at −80 °C until further use. Protein contents were determined by the method of Lowry et al. with bovine serum albumin as the standard.

Preparation of Skin Homogenate Fractions—All operations were carried out at 0−4 °C. After sacrificing the animal by decapitation, cutaneous strips were removed from the back and the abdomen. Fat and the surrounding muscular tissues as well as capillaries adhering to the dermis were removed. The skin was minced and mixed with 10 volumes of cold Tris-HCl buffer (pH 7.4) containing 0.15 M KCl and homogenized for 10 min. The whole homogenate was filtered with a funnel through buffer-soaked cotton and centrifuged at 10 000*g* for 20 min at 0−4 °C to remove mitochondria and nuclei. The postmitochondrial fraction was centrifuged at 100 000*g* for 1 h with a Beckman TL 100 ultracentrifuge, and the cytosolic supernatant was separated. The microsomes obtained from the pellet were resuspended in 5 mL of Tris buffer. Washed microsomes were isolated by recentrifugation at 100 000*g* for 1 h.

Hydrolysis Studies—*In Buffer*—Stock solution of diastereomeric ester prodrugs (0.05 M) was prepared in dimethyl sulfoxide. An aliquot (20 μL) was added to 10 mL of preincubated (37 °C) buffer solution (pH 7.4, $\mu = 0.155$) in a shaker water bath to initiate the hydrolysis reaction. At appropriate time intervals, samples (100 μL) were withdrawn and directly analyzed by HPLC.

In Plasma—Five microliters of stock solution of each diastereomeric prodrug was added to 2.5 mL of fresh plasma preincubated at 37 °C for 5 min to initiate the reaction. At appropriate time intervals 20 μL samples were withdrawn and immediately added to 100 μL of acetonitrile kept in an ice−water bath to precipitate the plasma proteins. The mixture was vortexed and centrifuged at 10 000*g* for 10 min at 4 °C. The supernatant was separated and stored at 4 °C until analysis by HPLC.

In Liver, Intestinal, and Brain Homogenates—Stock solutions of the liver, mucosal cells, and brain homogenate preparations were diluted to approximately 1 mg/mL protein content with ice-cold phosphate buffer (pH 7.4; $\mu = 0.155$). The rest of the procedure was similar to that described for hydrolysis in plasma.

In Skin Preparations—Protein concentrations of the cytosol and microsomal fractions were adjusted to 2 mg/mL with ice-cold pH 7.4 phosphate buffer ($\mu = 0.155$). The rest of the procedure was similar to that described for hydrolysis in plasma.

In Pure Esterase—Pure cholinesterases, i.e., buytyryl and acetyl, were diluted with phosphate buffer (100 mM, pH 7.4) to a suitable protein concentration (1 mg/mL) and were used to study the hydrolysis kinetics of diastereomeric propranolol esters. The rest of the procedure is similar to that described above.

Chromatography—A Beckman 100 A liquid chromatographic system equipped with a fluorescence spectrophotometer (Schoeffel FS 970) was used at excitation (λ_{ex}) and emission (λ_{em}) wavelengths of 290 and 340 nm, respectively. Propranolol and diastereomeric ester prodrug concentrations were determined with a C_{18} Novapak column (150 mm × 3.9 mm i.d., 5 mm, Waters). A chiral AGP column (100 mm × 4 mm, 5 mm, Alltech) was used to separate enantiomers of propranolol. The eluent systems were composed of acetonitrile: 2% v/v acetic acid (70:30), pH 4.0 adjusted with

ammonia (C_{18} column), and 20 mM ammonium acetate buffer (pH 4.1)/acetonitrile (98:2) (AGP column). A constant flow rate of 1.5 mL/min (C_{18} column), and 1.0 mL/min (AGP column) was maintained. The chromatographic separations were carried out at ambient temperature. Samples of 20 μL were injected onto the chromatograph. The retention times were 11.5, 13, 14.5, and 17 min for 1*S*2*R*, 1*S*2*S*, 1*R*2*R*, and 1*R*2*S*, respectively.

In Vitro Permeation Study—In vitro percutaneous permeation study was performed with a side-by-side diffusion cell apparatus. The rats were sacrificed by decapitation, and the hair of the abdominal region was removed immediately using an animal clipper (Oster A2) and a razor. A rectangular section of dorsal skin was excised from the animal with surgical scissors. Adhering fat and visceral debris were removed from the undersurface with tweezers. The excised skin was immediately mounted between the half-cells, with the dermis side in contact with the receptor fluid (0.01 M acetate buffer, pH 4). The area of skin available for diffusion was 0.636 cm^2. The prodrugs (7.5 mg) 1*S*2*S*, 1*S*2*R*, and 1*R*2*R* were solubilized in 7% v/v DMSO, and the volume was subsequently adjusted to 3 mL with 0.01 M pH 4 acetate buffer and added to the donor half-cell. The 1*R*2*S* prodrug (7.5 mg) was solubilized in 20% DMSO since this prodrug was the least soluble one. The saturation solubility of the prodrugs, determined in the buffer−DMSO system, was used for calculating the permeability. The solubility of the prodrugs in these systems ranged from 1 mM (1*R*2*S*) to 1.6 mM (1*S*2*S*). Enantiomer transport of propranolol hydrochloride from actate buffer (pH 4.0) was studied using 230 mM donor concentration. The half-cells were maintained at 37 °C connected to a circulating water bath (Isotemp, 1016P, Fischer Scientific). A 200 μL sample was withdrawn periodically for 24 h and replaced with equal volume of buffer. The fractions were stored at 4 °C until analyzed by HPLC for regenerated propranolol and the intact prodrugs. Cumulative amount of propranolol and the prodrugs transported (sum of intact prodrug and regenerated propranolol) was plotted against time, and the flux was obtained from the apparent steady-state linear portion of the graph.

The permeability coefficients of propranolol and its prodrugs were calculated[30] by

$$K_p = J_s/C_s$$

where K_p is the permeability coefficient, J_s is the steady-state flux, and C_s is the saturation solubility of the prodrugs in the buffer−DMSO system or PL in acetate buffer.

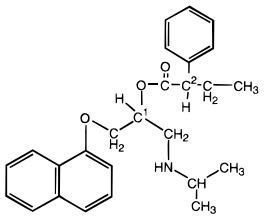
Statistical Analysis—Results of hydrolysis and permeation experiments were expressed as the mean ± SD. The student's *t* test was applied, where necessary, to evaluate significance of difference.

Results and Discussion

A major problem associated with the preparation of *O*-acetyl derivatives of propranolol and similar O,N-bifunctional compounds is the competing *N*-acetyl reactions. Indeed, the previously reported hemisuccinate derivative of propranolol has been shown to have the N- rather than the *O*-acetyl structure.[31] Irwin and Belaid[28] showed that O-acetylation is possible only by allowing the reaction of propranolol hydrochloride to take place with an appreciable, i.e., 4−5-fold, excess of the acid chloride without a catalyst. Under either synthetic or hydrolysis reaction conditions racemization at any one/two of the chiral centers of the prodrug can occur. It has been reported that, oxazepam undergoes racemization in aqueous medium.[32] The factors and the implications of racemization of enantiomers were discussed in detail elsewhere.[33] Such possible racemization was checked by reversed phase HPLC. Racemization of propranolol after prodrug hydrolysis is also possible, and this was also cross-checked under chiral column HPLC conditions. Interestingly, neither O to N transfer nor racemization were observed during synthesis or hydrolysis of the prodrugs.

Physicochemical Properties—Physicochemical parameters, such as aqueous solubility, have been shown to influence membrane flux, therapeutic activity, and pharmacokinetic profiles of medicinal agents. Therefore the physicochemical properties of propranolol and the prodrugs are important, especially in determining loading and release properties from a transdermal therapeutic device. The ester derivatives of propranolol used in this study undergo chemical hydrolysis, particularly under alkaline conditions as described later. Therefore the compounds are relatively unstable for normal equilibrium aqueous solubility determination around neutral pH range. To overcome this problem, the solubility of the prodrugs was determined at pH 4 (acetate buffer; $\mu = 0.155$) in which the prodrugs are found to be most stable. Analysis by HPLC showed insignificant degradation (<0.05%) of the esters during the solubility determination. The physicochemical properties such as solubility and partition coefficients (PC) of the four diastereomeric prodrugs are shown in Table 1. Diastereomers by definition exhibit different physicochemical properties. However, as shown in Table 1, only 1*R*2*S* showed significantly ($p < 0.05$) different aqueous solubility compared to the other diastereomers. The aqueous solubilities of the PL diastereomeric esters decreased and the partition coefficients increased compared to those of PL. Lipophilicity is very important for transdermal permeation because the stratum corneum, the major barrier to drug permeation, is lipid in nature and generally favors the permeation of lipophilic drugs. It has also been reported recently that an effective dermal prodrug should possess not only high lipophilicity but also adequate aqueous solubility.[34]

Table 1—Solubility and Partition Coefficients (PC) of Propranolol and Its Diastereomeric Ester Prodrugs



compound	solubility,[a] mg/mL	PC[b]
propranolol HCl	116.521[38]	2.50
1*S*2*S*	0.303 ± 0.031	11.85
1*R*2*R*	0.231 ± 0.005	17.90
1*S*2*R*	0.301 ± 0.021	11.00
1*R*2*S*	0.040 ± 0.006 *	20.70

[a] Determined in pH 4.0 acetate buffer at 25 °C. [b] Done in octanol–pH 4.0 phosphate buffer ($\mu = 0.155$) at 25 °C; *$p < 0.05$ compared to the other prodrugs.

Hydrolysis in Buffer and Tissue Preparations—Hydrolysis of all four diastereomeric prodrugs was investigated in aqueous solution at 37 °C over a wide pH range. At constant pH and temperature, the disappearance of the prodrugs displayed apparent first-order kinetics for several half-lives (data not shown). The prodrugs were completely hydrolyzed to propranolol in buffer (pH 1.2−8.0) although the propranolol prodrugs can undergo both hydrolysis and intramolecular aminolysis in alkaline solution.[35] The hydrolysis rate of prodrug increased with increasing pH. The shape of the pH−rate profile indicated proton- and hydroxyl-catalyzed degradation of the protonated prodrug together with a hydroxyl-catalyzed degradation of the unprotonated form. The V-shaped pH−rate profile (slope close to unity [0.78]) (Figure 1) indicated that water-catalyzed spontaneous degradation does not significantly contribute to the overall reaction. The maximum stability of the prodrugs was observed at around pH 4.0. No significant ($p > 0.05$) differences were observed among the hydrolytic rate constants of four diastereomeric PL ester

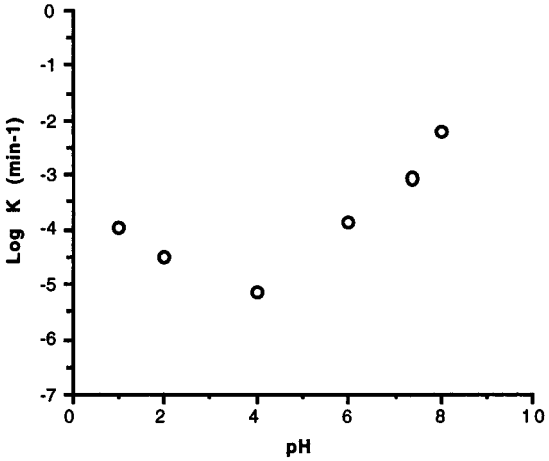


Figure 1—Typical pH–rate profile for the hydrolysis of diastereomeric ester prodrug (1*S*2*S*) in aqueous solution at 37 °C.

prodrugs in the phosphate buffer solution at a given pH, suggesting that H^+, OH^-, or spontaneous hydrolytic rate constants for an ester may not depend on the stereochemistry of the attached R group. The features of chemical kinetics are in agreement with those findings earlier reported for other propranolol esters.[20,35]

The first-order degradation of the diastereomeric ester prodrugs in plasma and liver homogenates is shown in Figure 2, and apparent first-order rate constants were determined from the slopes of the linear plots. Absence of any detectable peaks other than the propranolol and prodrug indicated the possibility of ester hydrolysis without rearrangement. Similar findings have been reported for other propranolol esters in rabbit serum.[36] The rate constants for the hydrolysis of four diastereomeric PL ester prodrugs with various tissue preparations are summarized in Table 2. Hydrolysis was accelerated by the addition of plasma or tissue homogenate, and stereoselectivity was observed. The order of hydrolytic rate constant of diastereomeric esters was found to be (1*S*2*S* < 1*R*2*S* < 1*R*2*R* < 1*S*2*R*) in plasma, and brain tissue homogenates. However, the order was changed in liver homogenates (1*R*2*S* < 1*S*2*S* < 1*S*2*R* < 1*R*2*R*) and intestinal homogenate (1*S*2*S* < 1*R*2*S* < 1*R*2*R* < 1*S*2*R*). The data clearly suggests that the configuration on the second chiral carbon atom (see prodrug structure in Table 1) to be the determinant in the rate of hydrolysis of all the diastereomeric prodrugs in all biological media examined (slowest 1X2S and fastest 1Y2R). The rate of hydrolysis of each diastereomer shows tissue dependency, with highest rate constants observed in intestinal homogenate and the lowest being in the plasma.

As judged from the rate constants shown in Table 2, the diastereomeric PL esters may be hydrolyzed mainly by esterases in the intestine and liver. These esterases consist of several isozymes, and esterase activity differs among various tissues. It was reported that oxazepam succinate was stereoselectively hydrolyzed and the selectivity differed between brain and liver.[37] This study clearly demonstrates that hydrolysis of diastereomeric PL prodrugs by plasma, liver, and intestine is stereoselective.

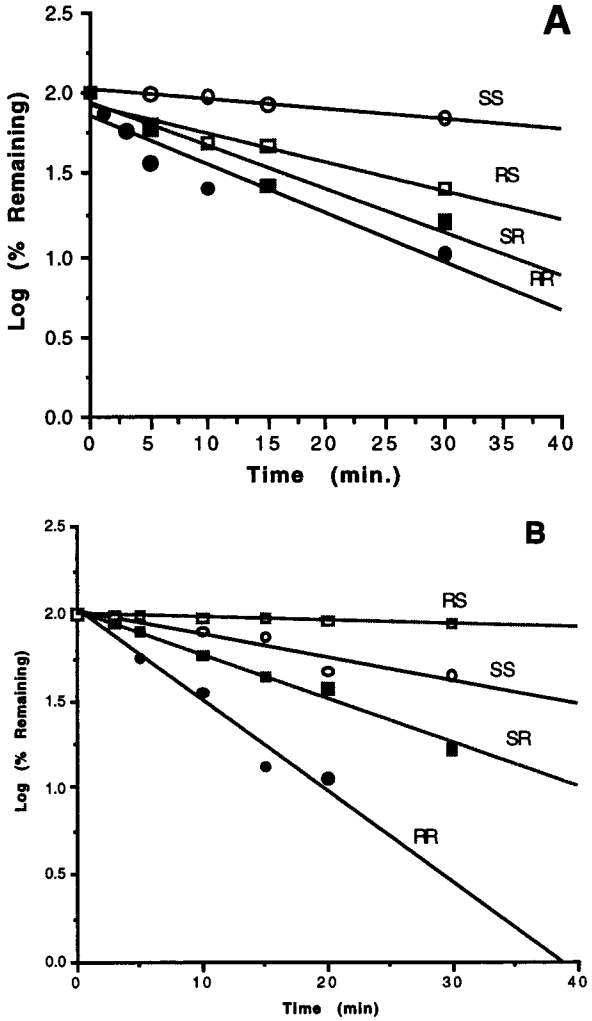


Figure 2—First-order plots for hydrolysis of diastereomeric propranolol ester prodrugs in plasma (A) and liver (B) homogenates.

In an attempt to characterize these differences, hydrolysis of these prodrugs was carried out with pure plasma esterases such as acetyl cholinesterase (EC 3.1.17) and butyryl cholinesterase (EC 3.1.1.8). The hydrolytic rate constants by these esterases were expressed in min^{-1} mg^{-1} and are tabulated in Table 2. It was found that the hydrolysis of all four diastereomeric ester prodrugs was faster by acetyl cholinesterase than by butyryl cholinesterase and was stereoselective.

The hydrolysis of these four diastereomeric ester prodrugs has also been studied in rat brain tissue homogenate, and results are shown in Table 2. Though there is no significant difference in the hydrolytic rate constants among the four diastereomeric ester prodrugs in the rat brain tissue homogenate, the plasma rate constants suggest significantly higher stability of the all diastereomers. In fact, when these rate constants were compared with those obtained in plasma, 1*S*2*S* prodrug was found to be 27 times

Table 2—Stereoselective Hydrolysis Rate Constants of Diastereomeric Ester Prodrugs of Propranolol in Different Rat Tissue Homogenates at 37 °C

	rate constants[a] ($\times 10^4$, min^{-1} mg^{-1} protein)							
prodrug	plasma	liver	intestine	brain	cytosol[c]	microsomes[c]	EC 3.1.1.7[b]	EC 3.1.1.8[b]
1*S*2*S*	1.89 ± 0.03	30.12 ± 0.18	83.64 ± 0.16	52.59 ± 0.99	0.003 ± 0.000	0.189 ± 0.020	1.32 ± 0.02	50.31 ± 1.21
1*R*2*R*	10.79 ± 0.13	129.10 ± 1.62	236.9 ± 3.27	60.29 ± 1.54	0.042 ± 0.005	0.090 ± 0.008	0.72 ± 0.01	32.43 ± 0.78
1*R*2*S*	9.12 ± 0.20	4.59 ± 0.09	84.18 ± 3.44	55.39 ± 0.01	0.015 ± 0.001	0.860 ± 0.008	0.15 ± 0.01	33.02± 0.66
1*S*2*R*	24.52 ± 0.37	97.72 ± 2.25	334.5 ± 5.64	73.01 ± 1.44	0.020 ± 0.002	0.183 ± 0.016	0.11 ± 0.01	41.18 ± 0.92

[a] Each value is the mean ± SD (n = 3). [b] Expressed as min^{-1} $units^{-1}$. [c] Skin.

Table 3—Calculated Flux (J_s) and Permeability (K_p) Values for Propranolol and the Prodrugs

compound	solubility in donor phase (μg/mL)[d]	J_s (μg cm^{-2} h^{-1})	$K_p \times 10^3$ (cm h^{-1})
(*R*)-propranolol	–	94.48 ± 11.09	1.40 ± 0.30
(*S*)-propranolol	–	95.61 ± 14.08	1.41 ± 0.27
1*S*2*S*	702 ± 48	29.63 ± 0.26	42.20 ± 1.24[b,c]
1*R*2*S*	450 ± 27	13.17 ± 0.16	29.26 ± 3.41[b,c]
1*S*2*R*	650 ± 39	10.58 ± 0.34	16.27 ± 3.12[b]
1*R*2*R*	628 ± 40	8.16 ± 1.24	12.99 ± 2.84[b]

[a] Each value is the mean ± SD ($n = 3$). [b] $p < 0.05$ compared to propranolol. [c] $p < 0.05$ compared to the other prodrugs. [d] Determined as described in Materials and Methods.

more stable in plasma (rate constant $0.189 \pm 0.03 \times 10^{-3}$ min^{-1} mg^{-1}), compared to the brain tissue (rate constant $5.259 \pm 0.99 \times 10^{-3}$ min^{-1} mg^{-1}), making it a likely prodrug candidate if brain targeting is needed for this drug.

Percutaneous Permeation—Topically applied drugs may be subject to considerable metabolic conversion. Simultaneous metabolism may affect the overall transport and disposition of the active species. It is possible to synthesize prodrugs, which permeate the skin more rapidly than the parent molecule, but are metabolized to the active form at the site of action. Simultaneous transport and metabolism after topical application of a drug is of interest to rationalize and develop a topical dosage form. Studies with propranolol have been conducted in the past using hairless mouse skin on the premise that there is no stereoselective penetration of propranolol.[38] In this study, shaved skin of male Sprague Dawley rats has been used. No stereoselective permeation of propranolol was observed. This is consistent with the findings of Heard et al., where no stereoselective permeation of propranolol through human skin was reported.[39]

All the diastereomeric ester prodrugs were hydrolyzed simultaneously during permeation and permeated as both intact prodrug and PL. To make the comparison simple only the total permeated amount (sum of intact prodrug and converted PL) of each diastereomeric prodrug was considered. The permeation parameters are shown in Table 3. The highest flux of the prodrugs was obtained with the 1*S*2*S* diastereomeric prodrug. The target rate of delivery (R_o) for propranolol and the prodrugs was calculated from reported values of the pharmacokinetic parameters for propranolol,[40] namely, clearance (Cl) and desired therapeutic steady-state concentration (C_{ss}) by:

$$R_o = C_{ss}\mathrm{Cl}$$

The clearance of (*S*)-propranolol is 16 mL min^{-1} kg^{-1}, and the required steady-state concentration for control of resistant ventricular arryhthmias is 1 μg mL^{-1}. Using these values, the target delivery rate for (*S*)-propranolol and its prodrugs is 0.672 mg h^{-1} and for (*R*)-propranolol and its prodrugs 67.2 mg h^{-1} for a 70 kg individual. This difference in delivery rate arises because the *S* isomer of propranolol is 100 times more potent than the *R* isomer. On the basis of this and the comparatively lower flux values obtained with the other prodrugs, the 1*S*2*S* prodrug is by far the best candidate for transdermal delivery. Taking into consideration the size of a patch for delivery, a patch size of 22.68 cm^2 is required for the 1*S*2*S* prodrug, and a size of 7.07 cm^2 is required for (*S*)-propranolol. Delivery of (*R*)-propranolol and its prodrugs is, however, problematic because of the relatively higher target for delivery and constraints in patch size.

From the in vitro diffusion studies, it appears that there is a 3- to 12-fold higher flux value associated with propranolol than with the prodrugs. However, it should be noted that the donor concentration of the 1*S*2*S* prodrug in the diffusion studies was approximately 1.6 mM and that of propranolol was 230 mM. The permeability coefficient (K_p) is independent of donor concentration and serves as an important parameter in comparing permeation potential. The K_p value was higher for all prodrugs compared to that of propranolol. From the flux and K_p values, it can be concluded that the 1*S*2*S* prodrug is promising in that it allows greater permeation with a lower concentration of the drug. This is preferable, since chances of skin irritation may be minimized.[38]

Hydrolysis of prodrugs in the cytosol and microsomal fraction of the skin were conducted to determine the location of the esterases. Stereoselective hydrolysis was observed in both fractions. The hydrolytic rate constants of the four diastereomeric ester PL prodrugs are summarized in Table 2. The prodrugs were hydrolyzed to a lesser extent in the cytosol as compared to microsomes. An interesting observation was that the prodrugs made with (*S*)-2-phenylbutyryl chloride were hydrolyzed to a lesser

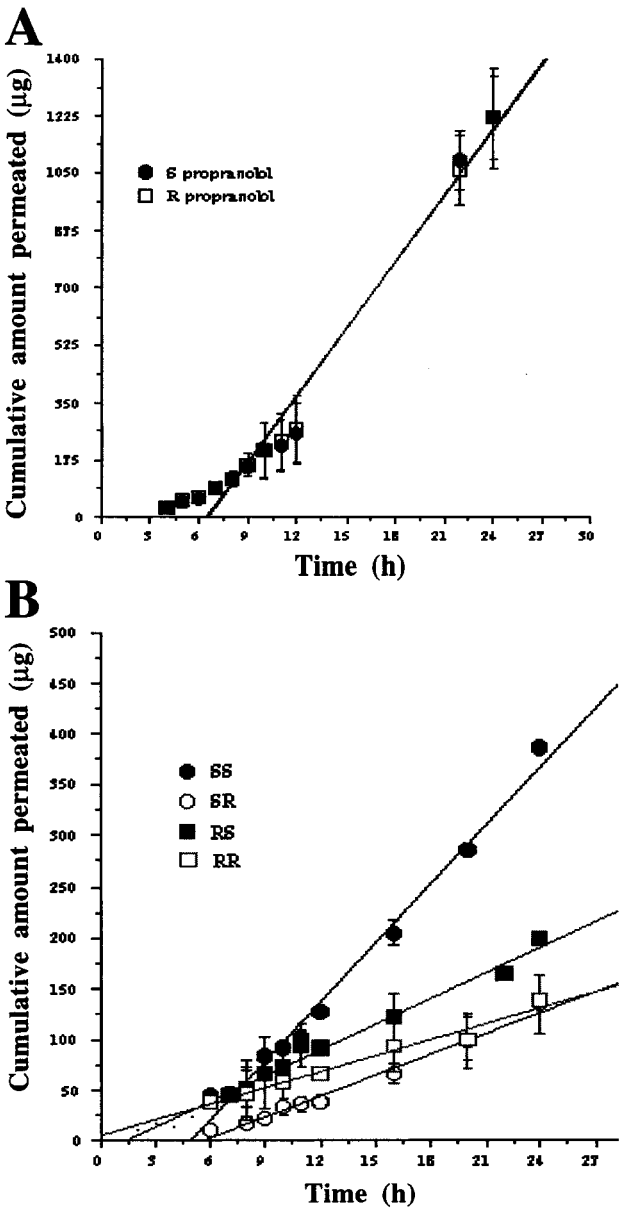


Figure 3—Penetration of propranolol enantiomers (A) and diastereomeric prodrugs (intact prodrug and transported propranolol) (B) across full thickness rat skin at 37 °C.

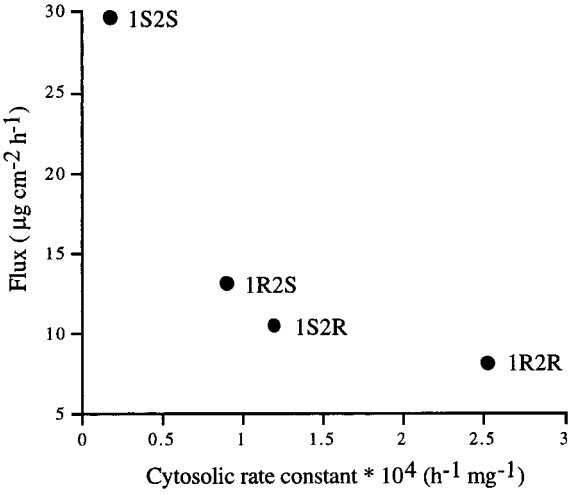


Figure 4—Plot showing inverse relationship between flux and skin cytosolic rate constants.

extent in the cytosol than those made with (*R*)-2-phenylbutyryl chloride. This assumes significance in light of the fact that the *S* isomer of propranolol is 100 times more potent than the *R* isomer. These prodrugs being more efficient at avoiding metabolism further help in improving the skin permeation of propranolol.

In Figure 3, the cumulative amount of propranolol and the diastereomeric ester prodrugs (sum of intact prodrug and regenerated propranolol) transported through the full thickness rat skin was plotted against time. It appears that all four drugs impart increase in lipophilicity as compared to parent PL as indicated by their PC data. The hydrolysis data in conjunction with the flux data clearly suggests that the prodrug, which has optimum stratum corneum partitioning and exhibits the most resistance to enzymatic hydrolysis, is able to cause the highest permeation. As shown (Figure 4), the transdermal fluxes may be inversely related to their skin cytosolic enzymatic rate constants. Therefore, a design of prodrugs should not only involve optimization of physicochemical parameters, i.e., lipophilicity and aqueous solubility, but must also include optimum evaluation of biochemical stability by membranes containing degradative enzymes.

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