Drug Delivery Studies in Caco-2 Monolayers. Synthesis, Hydrolysis, and Transport of O-Cyclopropane Carboxylic Acid Ester Prodrugs of Various β-Blocking Agents

Lars Hovgaard,[1,5] Helle Brøndsted,[1] Anders Buur,[2,3] and Hans Bundgaard[2,4]

Received June 14, 1994; accepted October 12, 1994

A series of O-cyclopropane carboxylic acid ester prodrugs of various β-blocking agents was synthesized. All prodrugs were hydrolyzed to give their parent compounds in aqueous phosphate buffer of pH 7.4 and in 80% human plasma. The half-lives in buffer solutions varied from 4 hours for the timolol prodrug to about 1 day for the prodrug of alprenolol. In human plasma the half-lives were shorter, ranging from 1 to 7 hours. The formation of the O-cyclopropane carboxylic acid ester derivatives significantly increased the lipophilicities of the β-blockers as measured by the distribution coefficient between n-octanol and aqueous phosphate buffer of pH 7.4. To characterize the biomembrane permeability characteristics of the β-blockers, transport properties across Caco-2 cell monolayers were investigated. An increase in lipophilicity resulted in a higher permeability of the prodrugs as compared to the parent compounds. Hence, acebutolol experienced an increment of a factor 17 on the apparent permeability coefficient, Papp, whereas Papp for the more lipophilic drug propranolol was increased by a factor of only 1.26. Some conversion of the prodrugs to their parent compounds was observed during the transport and appeared to be due to enzymatic intracellular metabolism.

KEY WORDS: Caco-2; cell culture; mucosal transport; drug absorption; beta-blocking agents; prodrug.

INTRODUCTION

Beta adrenergic receptor blockers, β-blockers, are being widely used in the clinic for the treatment of diseases related to the cardiovascular system and ocular hypertension, glaucoma (1,2). They represent a family of compounds with a wide range of lipophilic properties. Therefore, the transport properties and thus the bioavailabilities of the compounds may vary. Although propranolol is well absorbed from the gastrointestinal tract, its bioavailability is low and unpredictable due to first-pass metabolism (3). Timolol has been studied for its ability to reduce the ocular hypertension experienced in glaucoma. The compound, however, is fairly hydrophilic and penetrates poorly through the corneal membrane into the body of the eye (4). Therefore, a series of β-blocking agents with various lipophilicities have previously been derivatized in order to form prodrugs with improved transport properties of the drug (3,5,6).

To study the transport properties of drugs across mucosal barriers, the adenocarcinoma cell line, Caco-2, has been shown to be a good *in vitro* model for passive diffusion of drugs (7,8). Once grown to confluence, the cell monolayers expresses a variety of morphological, cytochemical and transport features characteristic for the human intestinal enterocytes (9–11).

The aim of the present study was first to synthesize O-cyclopropane carboxylic acid ester prodrugs of a series of β-blocking agents in order to improve their transport characteristics, then to characterize the prodrugs with respect to hydrolysis and transport properties. As a part of this investigation the feasibility of using the Caco-2 cell monolayers as a screening model for a series of passively absorbed drugs and prodrugs was evaluated.

MATERIALS AND METHODS

Chemicals

Timolol maleate, betaxolol and propranolol were obtained from Sigma Chemicals Co., USA. Alprenolol, acebutolol and oxprenolol was kindly donated by Hässle, Sweden, Rhône Poulenc, Switzerland and Ciba-Geigy, Switzerland, respectively. The β-blockers were used as received. All buffer substances and solvents were of reagent grade.

Preparation of O-Cyclopropane Carboxylic Acid Ester Prodrugs

The bioreversible derivatization of the β-blocking agents was performed by reacting the acid chloride of cyclopropane carboxylic acid with the β-blockers in an appropriate solvent as previously described for timolol (5) and propranolol (3). The esters were synthesized with yields from 60 to 80%. Their purity determined by elemental analysis after the synthesis and again by HPLC at the time of experimental use was found to range from 95 to 99%. Table I lists the prodrugs synthesized and summarizes some analytical data.

Analytical Procedures

For detection and quantitation of the β-blockers and their prodrugs, a high-performance liquid chromatography (HPLC) system capable of separating the parent drug from its prodrug was used. The HPLC system was PC controlled and consisted of a Hitachi-Merck gradient controller pump (Model L6200), a Hitachi-Merck UV-detector (Model L4000) and a Hitachi-Merck autosampler (Model A-655). Data acquisition and processing were performed using the Hitachi-Merck HPLC-manager (Model 6000). The analytical column was a reversed-phase Knauer column (4.6 · 120 mm) packed with Spherisorb ODS1 C-18 (5 μm particles, PhaseSep UK) and protected with a Knauer precolumn (4.6 · 40 mm) packed with SynChropak Bulk Support. Mobile phase systems of acetonitrile-methanol-acetate buffer of pH 4.5 were used and the flow was 1.0 ml/min. Each β-blocking

[1] The Royal Danish School of Pharmacy, Department of Pharmaceutics, 2 Universitetsparken, DK-2100 Copenhagen Ø, Denmark.
[2] The Royal Danish School of Pharmacy, Department of Pharmaceutical Chemistry.
[3] Present address: H. Lundbeck A/S., 7-9 Ottiliavej, DK-2500 Valby, Denmark.
[4] Deceased.
[5] To whom correspondence should be addressed.

0724-8741/95/0300-0387$07.50/0

Table I. Physicochemical Data of Various β-Blockers and Their O-Cyclopropane Carboxylic Acid Ester Prodrugs

Compound	ID#	Mw	λ max (nm)	Rt (min)	A/Ao (%)
Acebutolol		372,93	233	4,68	
Acebutolol ester	I	520,53	233	6,73	97.3
Alprenolol		285,84	213	5,51	
Alprenolol ester	II	433,44	213	6,66	94.6
Betaxolol		343,94	222	5,02	
Betaxolol ester	III	491,54	222	6,15	nd
Oxprenolol		301,84	204	4,56	
Oxprenolol ester	IV	485,94	204	5,9	96.7
Propranolol		295,84	232	6,2	
Propranolol ester	V	411,94	232	8,04	97.8
Timolol		432,52	294	4,66	
Timolol ester	VI	500,54	294	5,66	98.7

Rt is retention time observed in HPLC analysis, A/Ao is the purity of prodrug, nd = not determined.

agent and its prodrug was detected at its UV absorbance maximum (Table I). Quantitation of the esters as well as the parent β-blockers was done by measuring the peak areas or peak heights in relation to those of standards chromatographed under the same conditions.

Determination of Distribution Coefficients

The distribution coefficients of the β-blockers and their esters were determined in a system consisting of n-octanol and 0.02M aqueous phosphate buffer of pH 7.4 at 22°C as previously described (4) or obtained from the literature (1,2).

Hydrolysis of O-Cyclopropane Carboxylic Acid Ester Prodrugs

The hydrolysis of the esters was studied in aqueous buffer solutions at 37 ± 0.2°C. The buffers used were hydrochloric acid (pH ≤2), acetate (pH 4–5), phosphate (pH 2–3 and 6–8), borate (pH 8.3–10), carbonate (pH 10.5–11) and sodium hydroxide (pH > 11.5). A constant ionic strength (μ) of 0.5 was maintained for each buffer by adding a calculated amount of potassium chloride. The rates of hydrolysis were determined by using the reversed-phase HPLC procedure previously described. The reactions were initiated by adding 50μl of a stock solution of the esters in an acetonitrile-water mixture to 10.0ml of buffer solution, pre-equilibrated to 37°C, in screw-capped test tubes. The initial concentration was $0.2–2.0 \cdot 10^{-4}$M. At appropriate times samples were taken and immediately chromatographed. Further, the kinetics of hydrolysis of the O-cyclopropane carboxylic acid esters was studied in 0.05M phosphate buffer of pH 7.4 containing 80% human plasma at 37°C, the initial ester concentration being $0.3–2.0 \cdot 10^{-4}$M. Samples of 250μl were taken at appropriate times and deproteinized by mixing with 500μl of a solution of 2% w/v $ZnSO_4$ in 50% v/v methanol-water. After centrifugation for 5 min at 13,000 rpm the clear supernatant fraction was analyzed by HPLC as described previously. Pseudo-first-order rate constants were determined from the slopes of linear plots of the logarithm of residual ester against time.

Caco-2 Cell Culture

The Caco-2 cells were obtained from American Tissue Culture Collection, MD, USA.

They were cultivated in a clean room facility under aseptic conditions at 37°C in an atmosphere of 90% relative humidity, 90% air and 10% CO_2 using Dulbecco's Modified Eagles Medium (DMEM) containing 9% Fetal Calf Serum (FCS), 1.0% nonessential amino acids, 1.0% of L-glutamine, 100U/ml benzylpenicillin and 10U/ml streptomycin. All medium components were obtained from In-Vitro, Gibco, UK. The cells were maintained in 75cm^2 culture flasks (MEDA, Greiner, Austria), the medium was changed every other day and the cells were trypsinized once a week.

For transport studies the cells were cultivated on porous polycarbonate filter membranes with a pore size of 0.4μm and a surface area of 4.7cm^2 (Transwell, Costar, USA). At the time of passage approximately 10^5cells/ml were seeded onto filters. The cells were supplied fresh medium every other day until the time of usage, which was between 21st and 28th day after seeding. For this series of studies passage numbers from 30 to 40 was used. To ensure the absence of mycoplasma in the cell culture regular testing by Gene-Probe was performed. The culture was found to be negative for mycoplasma.

Transport Studies

Transport of the β-blocking agents and their prodrugs was studied across Caco-2 cell monolayers. This was done in an atmosphere of 90% air and 10% CO_2 at 37°C. As shown previously, agitation of the wells during the penetration studies was of utmost importance to control the thickness of the unstirred water layer, UWL, above the cell monolayer (12,13). In this series of experiments the angle of a thermostated plate shaker was 2.5° and the rotational speed was set to 300 rpm. This had previously been shown to minimize the thickness of UWL in the wells used for this study (13). The transport studies were performed in Hanks Balanced Salt Solution, HBSS. The β-blockers or prodrugs were dissolved in HBSS in concentrations of 6×10^{-4}M–8.3×10^{-4}M, except for propranolol, which was 5.5×10^{-5}M, and 2.5ml was added to the apical side of the monolayers. Then 3.0ml HBSS was added to the basolateral side. At time intervals varying from 3 to 5 minutes, 100μl samples were automatically collected as described elsewhere for up to 35 min (13). Before and after each experiment the transepithelial electrical resistance, TEER, was measured (Millicell, Millipore, USA) as an indicator for the integrity of the cell monolayer. TEER was in the range of 250 to 300 ohms equivalent to 1175 to 1410 ohms · cm^2 for filters with areas of 4.7cm^2. No decrease in TEER was observed. Finally the permeability of the monolayers towards the aqueous pore marker ^{14}H-PEG-4000 (DuPharma, New England Nuclear, USA) was determined. Permeation rates of ^{14}H-PEG-4000 of up to 0.1%/hr were accepted. After transport the samples were analyzed by HPLC.

Data Handling

The apparent permeability coefficients were calculated

from plots of total amount transported (drug and prodrug) versus time according to the following equation (Eq. (1)).

$$\text{Papp} = \frac{dQ}{dt \cdot A \cdot Co \cdot 60} \quad (1)$$

Where dQ/dt is the slope of the penetration profiles across the Caco-2 cell monolayer (mol/min). A is the diffusional area of the inserts (4,7cm^2) and Co is the initial concentration on the apical side. 60 converts from minutes to seconds. All permeabilities reported are averages of 3 to 6 experiments.

RESULTS AND DISCUSSION

Lipophilicity of O-Cyclopropane Carboxylic Acid Ester Prodrugs of β-Blockers

The logarithmic distribution coefficients (logD) for the β-blockers and prodrugs between n-octanol and aqueous buffer pH 7.4 are listed in Table II. As expected derivatization of the β-blocking agents to their O-cyclopropane carboxylic acid esters increased the lipophilicity in all cases. The lipophilicities expressed as logD was increased by 1.5 or more. The increase in lipophilicity is partly due to the conversion of the hydroxy group to a more lipophilic ester group, and partly due to a decrease in pKa of the amino group after prodrug formation. The electron withdrawing effect of the ester as compared to the hydroxy group (3,4) affords a greater proportion of the lipophilic free base form at pH 7.4. The large increment in lipophilicity is expected to be followed by an increase in the transport properties across Caco-2 monolayers. This was indeed found to be the case and will be discussed in detail later.

Kinetics of Hydrolysis of O-Cyclopropane Carboxylic Acid Ester Prodrugs

The kinetics of hydrolytic break-down of the various O-cyclopropane carboxylic acid β-blocker esters was studied in aqueous solution at 37°C over a wide range of pH values (pH 1–12). Under the conditions used, the reactions displayed first-order kinetics for several half-lives. The influence of pH on the rate of degradation for the esters has previously (3,5) been described by the following rate expression (Eq. (2)).

$$k_{obs} = (k_H a_H + k_o + k_{OH} a_{OH}) \frac{a_H}{a_H + K_a} + k'_{OH} a_{OH} \frac{K_a}{a_H + K_a} \quad (2)$$

where a_H and a_{OH} refer to the hydrogen ion and hydroxide ion activities, respectively, $a_H/(a_H + K_a)$ and $K_a/(a_H + K_a)$ are the fractions of total ester in protonated and free base forms, respectively, and K_a is the apparent ionization constant of the protonated NH-group in the esters. The rate constant k_H refers to the specific acid-catalyzed reaction of the protonated ester, and k_{OH} and k'_{OH} are the second-order rate constants for the apparent specific base-catalyzed reactions of the protonated and neutral species, respectively. The rate constant k_0 refers to the spontaneous or water-catalyzed hydrolysis of the protonated species.

This was also found to be the case for the O-cyclopropane carboxylic acid esters investigated in this study, and the various rate constants derived from the pH-rate relationship are listed in Table III together with the kinetically derived pK_a values.

As revealed by HPLC analysis, the disappearance of the esters in aqueous solutions at pH 1 to 7 was accompanied by the progressive appearance of free β-blockers. At pH values higher than 7, however, the parent β-blocker except for timolol was not formed in stoichiometric amounts, however, it was the major product. It has previously been reported for O-acyl esters of propranolol (3,14), that intramolecular aminolysis yielding the corresponding N-acyl analogue is taking place in basic solutions. The relative importance of hydrolytic degradation and intramolecular O to N acyl transfer is highly dependent on pH of the medium and on steric properties within the β-blocker. The bulky tertiary butylamino group on timolol prevents the aminolysis reaction to occur, in that timolol is formed in stoichiometric amounts in the pH-range of 1 to 12 (5). On the other hand O-acyl propranolol esters indeed form the corresponding N-acyl derivative at pH-values higher than 7 (3,14). The latter was also found for the O-cyclopropane carboxylic acid esters investigated in this study. In every case an unknown peak possessing higher retention time as compared to the O-cyclopropane carboxylic acid ester appeared in the chromatogram. In fact, this peak was the only one formed in highly basic solutions, i.e. pH 12. The steric properties of the β-blockers examined in this study are, except for timolol, expected to be equal and similar to that of propranolol, since the side-chain contains the same secondary propylamino group. This group does, apparently not prevent the O to N acyl transfer reaction.

Table II. Logarithmic Distribution Coefficients, logD, of Various β-Blockers and Their O-Cyclopropane Carboxylic Acid Ester Prodrugs

β-Blocker	Log D for β-blocker	Log D for ester
Acebutolol	−0.04[a]	1.59
Alprenolol	0.31[b]	2.78
Betaxolol	0.28	0.63
Oxprenolol	0.45[a]	1.98
Propranolol	1.38[a]	3.02
Timolol	−0.04[c]	1.74[c]

The distribution coefficients, D, are all averages of at least 3 measurements. The standard deviations were found to be no higher than 5%.

[a] From 1.

[b] From 5.

[c] From 2.

Hydrolysis in Human Plasma

The hydrolysis of the esters to the parent β-blockers was studied in aqueous phosphate buffer pH 7.4 containing 80% human plasma and was found to follow first-order kinetics over several half-lives. The rate of hydrolysis was found to be higher in plasma than in buffer for all prodrugs, except for the ester of timolol (Figure 1). Previous findings by Bundgaard *et al.* (4,5) indicate that various esters of ti-

Table III. Rate Data and Ionization Constants for the Hydrolysis of Various O-Cyclopropane Carboxylic Acid β-Blocker Esters (μ = 0.5; 37°C)

Compound	k_H $M^{-1} * min^{-1}$	k_0 min^{-1}	k_{OH} $M^{-1} * min^{-1}$	k'_{OH} $M^{-1} * min^{-1}$	pKa
I	n.d.	n.d.	$1.9 * 10^3$	115	8.5
II	n.d.	n.d.	$7.5 * 10^2$	50	8.7
III	n.d.	n.d.	n.d.	n.d.	n.d.
IV	n.d.	n.d.	$1.4 * 10^3$	105	8.5
V	n.d.	$1.5 * 10^{-5}$	$1.5 * 10^3$	66	8.3
VI[a]	$1.3 * 10^{-4}$	$5.3 * 10^{-6}$	$4.9 * 10^3$	7.6	8.4

[a] Data from ref. 5.
n.d. = not determined.

molol were hydrolyzed slower in plasma than in buffer. It was argued, that the enzymatic hydrolysis rate in plasma was reduced due to increased protein binding, as the lipophilicity of the prodrugs was increased. However, the O-cycloacyl ester prodrugs were found to be on the border line and might not be as affected by protein binding as other prodrugs. The degree of hydrolysis in buffer and in plasma varied for the different prodrugs. No correlation was observed between the lipophilicity and the hydrolysis rate in neither media.

Penetration Studies

It has been shown, that the type of filter support is very important when transport of lipophilic compounds across Caco-2 cells are studied, as the lipophilic compounds adsorb onto the filter membrane (15). For lipophilic β-blockers polycarbonate filters have been reported to be suitable supports (8). Therefore, this filter type was used for the present experiments.

As all the compounds penetrated relatively easy, the penetration studies were performed for maximally 35 minutes. In Figure 2 an example of the penetration profiles is given. The amount of acebutolol transported is compared to the amount of the corresponding O-cyclopropane carboxylic acid ester transported. From the distribution coefficient measurements it was found, that the logD values of acebutolol and its ester were −0.04 and 1.59 respectively. This difference in the lipophilicities explains the large difference in the transported amounts across the Caco-2 monolayers. The more lipophilic prodrug is transported faster. An estimation of the lag-time for the transport yields 5 and 2.5 minutes for the parent compound and prodrug, respectively. Based on the slope of amount transported versus time after steady state flux has been achieved, the apparent permeability coefficient can be found.

Table IV summarizes the apparent permeability coefficients for the various β-blockers and their prodrugs. Papp for the parent compounds was found to range from 4.5×10^{-6} to 8.2×10^{-5}cm/s. For the prodrugs Papp was shifted upwards to 7.8×10^{-5} to 1.1×10^{-4}cm/s. The least permeable β-blocker, acebutolol (Papp = 4.49×10^{-6}cm/s), experienced an increase in permeability to 7.80×10^{-5}cm/s by the prodrug formation. However, the prodrug was still the least permeable compound amongst the prodrugs. This was found to be a general trend. Moreover, acebutolol, being the least permeable β-blocker, gained the most by the prodrug formation. This is explained by the fact, that compounds that penetrate mucosal barriers poorly are those with low lipophilicities. Acebutolol has a low logD value of −0.04, and it is expected, that this compound is transported very slowly. By forming the O-cyclopropane carboxylic acid ester prodrug, and thus increasing its logD value to 1.59, acebutolol is expected to gain a great deal transport wise. In contrast hereto, a compound such as propranolol, which was found to be very lipophilic having a logD of 1.38, is expected to show very good transport characteristics. Indeed the Papp was found to be 8.24×10^{-5}cm/s. The formation of a O-cyclopropane carboxylic acid ester prodrug on propranolol increased the lipophilicity in terms of logD to 3.02. This is a

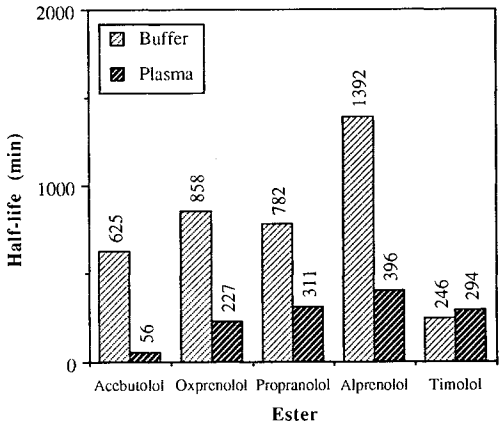

Fig. 1. Half-lives for O-cyclopropane carboxylic acid esters of various β-blockers in aqueous buffer and 80% human plasma at pH 7.4 and 37°C. Data for the O-cyclopropane carboxylic acid ester of timolol are obtained from Bundgaard *et al.* (5).

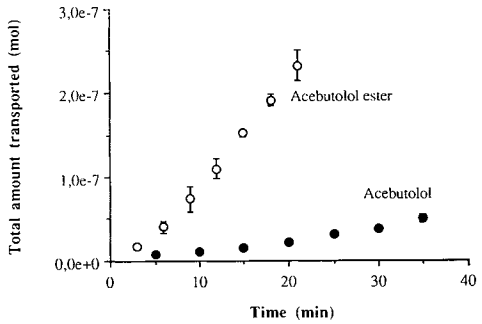

Fig. 2. Transport profiles of acebutolol and its O-cyclopropane carboxylic acid ester.

Table IV. Apparent Permeability Coefficients, Papp, for Various β-Blockers and Their O-Cyclopropane Carboxylic Acid Ester Prodrugs

β-Blocker	Papp (cm/s) $\times 10^6$ for β-blocker	Papp (cm/s) $\times 10^6$ for ester
Acebutolol	4.49 ± 0.45	78.0 ± 1.1
Alprenolol	75.0 ± 7.7	108 ± 4.7
Betaxolol	48.5 ± 8.75	96.5 ± 9.23
Oxprenolol	65.5 ± 2.21	97.2 ± 13.7
Propranolol	82.4 ± 5.1	104 ± 6.13
Timolol	44.3 ± 2.8	78.9 ± 4.50

The apparent permeability coefficients are averages of 3 to 6 diffusion experiments ± SD.

large increment, but the significance of this would not be expected to be reflected much in the Papp value. In fact this was only found to increase to 1.04×10^{-4}cm/s. The relative increments in Papp were 17 times for the most hydrophilic compound and only 1.26 times for the lipophilic compound.

Figure 3 shows the correlation between the lipophilicities and the permeability coefficients of the various β-blockers and their prodrugs. In the range from a logD of 0.5 to 3, a linear correlation was found. In this region an increased logD automatically reflects an increased permeability. This is in full agreement with the literature, where a series of β-blocking agents were shown to follow similar patterns (8,16,17). In the region below 0.5 the compounds became too hydrophilic and no longer follow this linear relationship. This suggests, that the transport mechanism possibly changes from being mainly transcellular to paracellular. The total area of the monolayers available to transcellular transport has been estimated to be over 99% and the area available to paracellular transport to 0.1% (18). Therefore a change in transport mechanism from transcellular to paracellular may result in a dramatic decrease in the amount transported across the Caco-2 monolayers.

During the transport studies the metabolic activity of the cells towards the prodrugs was followed. Initially, the purity of the prodrugs was estimated to range between 95 and 99%. The cells were found to hydrolyze the prodrugs to various degrees. Table V summarizes the findings. On the apical side of the monolayers, all compounds were degraded to some extent, ranging from 5 to 32%. However, the percentage of degraded prodrug after transport across the cells was significantly higher. The degradation was increased by a factor of 2 to 3. This suggests, that the transport intracellularly through the cells is accompanied by some enzymatic metabolism. The prodrug of propranolol shows a much higher degree of metabolism as compared to the other prodrugs. This is accounted for by the fact, that propranolol was used in a concentration ten times lower than the other concentrations due to toxic effects at high concentrations. Thus, the enzymatic degradation process is saturated when high concentrations of drug are used. As the concentration decreases, the enzymatic process becomes more dominating. It is worth noting, that the degree of degradation on the apical side is above the degradation expected in the same time period in pure buffer solution. Thus, it can be concluded that brush-border enzymes also take part in the degradation. During analysis of the samples, no other peaks appeared in the chromatograms, suggesting that intramolecular aminolysis, previously observed for *e.g.* propranolol esters (3), did not take place during the course of the experiment. Therefore, the only degradation products observed were the parent β-blockers. Narawane *et al.* (19) investigated the differences in the *in vitro* permeability of various parts of the gastrointestinal tract to β-blockers and timolol prodrugs. They found, that the O-cyclopropane carboxylic acid ester of timolol was completely hydrolyzed after passage. This was not observed in the present study possibly because of experimental differences. Furthermore, Narawane *et al.* (19) used tissue from rats in Ussing chambers, whereas Caco-2 cells are of human origin.

In conclusion, this study shows, that the formation of O-cyclopropane carboxylic acid ester prodrug on a series of β-blocking agents increases the lipophilicity and hence the transport characteristics of the compounds across Caco-2 monolayers. The lipophilicity increment was found to be similar for all β-blockers despite their intrinsic lipophilicity. However, the increment in permeability was found to increase more markedly for the least lipophilic β-blockers in the family of compounds studied. This was attributed to a change in transport mechanism from paracellular to transcellular transport. The lipophilicity and the apparent perme-

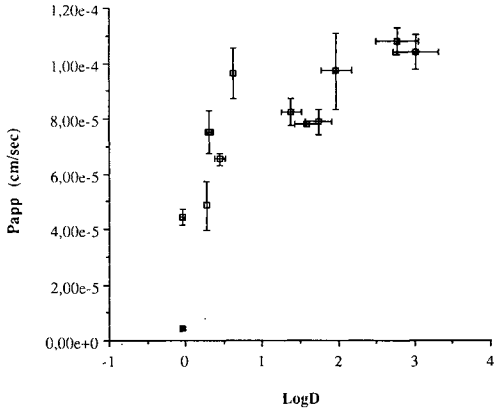

Fig. 3. The apparent permeability coefficient, Papp, as a function of the logarithmic distribution coefficient, logD, for various β-blockers and their O-cyclopropane carboxylic acid ester prodrugs.

Table V. Metabolism of O-Cyclopropane Carboxylic Acid Ester Prodrugs of Various β-Blockers During Transport Studies in Caco-2 Monolayers

Ester	Purity of prodrug (%)	% Hydrolyzed on apical side	% Hydrolyzed on basolateral side
Acebutolol	n.d.	n.d.	n.d.
Alprenolol	96.7	9,1	18,9
Betaxolol	94.6	8,4	15,9
Oxprenolol	97.8	5,5	13,3
Propranolol	98.7	32,6	76,7
Timolol	97.3	5,9	9,3

n.d. = not determined.

ability coefficients of the compounds across Caco-2 monolayers was found to be correlated. The Caco-2 monolayers has been shown to be a very useful model for screening of transport of prodrugs across mucosal barriers.

REFERENCES

1. R. D. Schoenwald and H.-S. Huang. Corneal penetration behavior of β-blocking agents I: Physicochemical factors. *J. Pharm. Sci.* 72:1266–1272 (1983).
2. J. M. Cruickshank. The clinical importance of cardioselectivity and lipophilicity in beta blockers. *Am. Heart J.* **100**:160–178 (1980).
3. A. Buur, H. Bundgaard and V. H. L. Lee. Prodrugs of propranolol: Hydrolysis and intramolecular aminolysis of various propranolol esters and an oxazolidin-2-one derivative. *Int. J. Pharm.* 42:51–60 (1988).
4. H. Bundgaard, A. Buur, S.-C. Chang and V. H. L. Lee. Prodrugs of timolol for improved ocular delivery: Synthesis, hydrolysis kinetics and lipophilicity of various timolol esters. *Int. J. Pharm.* 33:15–26 (1986).
5. H. Bundgaard, A. Buur, S.-C. Chang and V. H. L. Lee. Timolol prodrugs: synthesis and lipophilicity of various alkyl, cycloalkyl and aromatic esters of timolol. *Int. J. Pharm.* **46**:77–88 (1988).
6. N. Bodor and A. Elkoussi. Improved delivery through biological membranes. LVI. Pharmacological evaluation of alprenoxime—a new potential antiglaucoma agent. *Pharm. Res.* **8**:1389–1395 (1991).
7. A. R. Hilgers, R. A. Conradi and P. S. Burton. Caco-2 cell monolayers as a model for drug transport across the intestinal mucosa. *Pharm. Res.* 7:902–910 (1990).
8. P. Artursson. Epithelial transport of drugs in cell culture. I. A model for studying the passive diffusion of drugs over intestinal absorptive (Caco-2) cells. *J. Pharm. Sci.* 79:476–482 (1990).
9. M. Pinto, S. Robine Leon, M.-D. Appay, M. Kedinger, N. Triadou, E. Dussaulux, B. Lacroix, P. Simon-Assmann, K. Haffen, J. Fogh and A. Zweibaum. Enterocyte-like differentiation and polarization of the human colon carcinoma cell line Caco-2 in culture. *Bio. Cell.* 47:323–330 (1983).
10. I. H. Hidalgo, T. J. Raub and R. Borchardt. Characterization of the human colon carcinoma cell line (Caco-2) as a model system for intestinal epithelial permeability. *Gastroenterology.* **96**:736–749 (1989).
11. G. Wilson, I. F. Hassan, C. J. Dix, I. Williamson, R. Shah, M. Mackay and P. Artursson. Transport and permeability properties of human Caco-2 cells: In-vitro model of the intestinal epithelial cell barrier. *J. Controlled Release.* 11:25–40 (1990).
12. I. J. Hidalgo, K. M. Hillgren, G. M. Grass and R. T. Borchardt. Characterization of the unstirred water layer in Caco-2 cell monolayers using a novel diffusion apparatus. *Pharm. Res.* 8:222–227 (1991).
13. A. Buur and N. Mørk. Metabolism of testosteron during in-vitro transport across Caco-2 cell monolayers: Evidence for β-hydroxysteroid dehydrogenase activity in differentiated Caco-2 cells. *Pharm. Res.* 9:1290–1294 (1992).
14. J. M. Quigley, C. G. M. Jordan and R. F. Timoney. The synthesis, hydrolysis kinetics and lipophilicity of O-acyl esters of propranolol. *Int. J. Pharm.* **101**:145–163 (1993).
15. P. Nicklin, B. Irwin, I. Hassan, I. Williamson and M. Mackay. Permeable support type influences the transport of compounds across Caco-2 cells. *Int. J. Pharm.* **83**:197–209 (1992).
16. D. C. Taylor, R. Pownall and W. Burke. The absorption of β-adrenoreceptor antagonists in rat in-situ small intestine; the effect of lipophilicity. *J. Pharm. Pharmacol.* 37:280–283 (1985).
17. P. Artursson and J. Karlsson. Correlation between oral drug absorption in humans and apparent drug permeability coefficients in human intestinal epithelial (Caco-2) cells. *Biochem. Biophys. Res. Comm.* 175:880–885 (1991).
18. J. R. Pappenheimer. Physiological regulation of transepithelial impedance in the intestinal mucosa of rats and hamsters. *J. Membr. Biol.* **100**:137–148 (1987).
19. M. Narawane, S. K. Podder, H. Bundgaard and V. H. L. Lee. Segmental differences in drug permeability, esterase activity and ketone reductase activity in the albino rabbit intestine. *J. Drug Targ.* 1:29–39 (1993).