Effect of Probenecid on the Enantioselective Pharmacokinetics of Oxprenolol and Its Glucuronides in the Rabbit

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Purpose. To study the effect of probenecid on the stereoselective pharmacokinetics of oxprenolol and its glucuronides in the rabbit.
Methods. An oral dose of 50 mg/kg racemic oxprenolol was given to nine rabbits twice, in random sequence with and without the concurrent administration of probenecid. Oxprenolol enantiomers were determined in plasma and urine by an enantioselective HPLC method. Oxprenolol glucuronides were measured in plasma and urine after enzymatic hydrolysis.
Results. The disposition of the oxprenolol enantiomers in rabbits is stereoselective, mainly due to a difference in metabolism. Renal excretion is only a minor elimination route for unchanged oxprenolol, and the renal clearances of the enantiomers are similar. Pretreatment with probenecid did not affect the plasma concentrations of the oxprenolol enantiomers, but there was a slight decrease in their urinary excretion. The plasma concentrations of the oxprenolol glucuronides are much higher than those of the parent enantiomers, and those of (S)-glucuronide are about twice those of its antipode. About 10% of the oxprenolol dose is excreted in the urine as glucuronides. The renal clearances of both glucuronides are similar, and markedly higher than the creatinine clearance. After probenecid, the mean glucuronide plasma levels were markedly higher, with for both glucuronides a more than twofold increase in mean AUC. Probenecid decreased the renal clearance of both glucuronides to about 30%. Moreover, it decreased slightly the formation clearance of (S)-glucuronide, while the formation clearance of (R)-glucuronide was not significantly influenced.
Conclusions. Our results show that in the rabbit, both oxprenolol glucuronide diastereomers are actively secreted by the kidney, and that this process is inhibited by probenecid.

KEY WORDS: enantioselectivity; pharmacokinetics; oxprenolol; oxprenolol glucuronides; probenecid; active renal secretion.

INTRODUCTION

The β-blocking agent oxprenolol is a chiral drug of which the (−)-S-enantiomer is 10 to 35 times more active than the (+)-R enantiomer. The drug is extensively metabolized by oxidation and glucuronidation[1]. We found after oral administration of racemic oxprenolol in humans small differences in the plasma concentrations between the oxprenolol enantiomers, but marked differences between the plasma concentrations of the glucuronide diastereomers[2]. This latter difference is mainly related to the enantioselectivity of the renal excretion of the glucuronides: the renal clearance of (R)-oxprenolol glucuronide is in humans markedly higher than the creatinine clearance, suggesting active tubular secretion, whereas for the (S)-glucuronide the opposite is found. Active secretion has already been described for other glucuronides[3-5] but the finding that one of the glucuronides is actively secreted while its antipode is not, has not been reported for other drugs. More insight about this unusual finding could possibly be obtained by studying the effect of probenecid on the disposition of oxprenolol glucuronides. Probenecid is, indeed, a potent inhibitor of the renal tubular secretion of organic acids, including glucuronides[3-5]. As a first step we wanted to see in how far an animal model could be of help, and therefore studied in the rabbit the disposition of oxprenolol, and the influence of probenecid administration thereupon.

METHODS

Materials

rac-Oxprenolol.HCl, β-glucuronidase (from Helix pomatia) and probenecid (4-(dipropylaminosulfonyl)benzoic acid) from Sigma Chemical Company (St.-Louis, Missouri). [C^{14}]-Oxprenolol (spec. activity 65.3 μCi/mg; radiochemical purity more than 99.0%) was a gift from Ciba Geigy (Basel, Switzerland). (+)-(S)-NEIC [(+)-S-1-(1-naphthyl)-ethylisocyanate] was purchased from Aldrich (Bornem, Belgium). Solvents of HPLC-grade and other analytical reagents were obtained from Merck (Darmstadt, Germany).

Animals

Male rabbits (New Zealand) were purchased from Roscam (Merelbeke, Belgium). Oxprenolol was administered to the conscious rabbits by oral gavage after an overnight fast. The nine animals were given a single oral dose of 50 mg/kg oxprenolol on two occasions, with and without concurrent administration of probenecid in random sequence. There was at least a one week interval between the two administrations. On one of these occasions, probenecid (50 mg/kg) was given to the animals also by oral gavage, once 1 hour before, and once simultaneously with the oxprenolol administration[6]. On the control day, water was administered instead of probenecid.

Blood Sampling and Analytical Methods

One ml blood samples were withdrawn from a right ear vein before oxprenolol dosing and 0.15, 0.5, 1, 1.5, 2, 3, 4 and 6 hours thereafter. Blood was collected in heparinized tubes and after centrifugation at 3000 rpm; the plasma samples were stored at −20°C until analysis. Urine was collected from 0 to 24, from 24 to 48, and from 48 to 72 hours, using rabbit metabolic cages (Technoplast, Italy). After centrifugation and measuring the total volume, aliquots were stored at −20°C.

Plasma and urine samples were assayed for oxprenolol and oxprenolol glucuronides by a HPLC method as described before[2]. The glucuronides are hydrolyzed to their parent enantiomers which are then measured. Plasma (50 μl) and urine (5–200 μl)samples are incubated for 24 hours at

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37°C with 250μl acetate buffer (0.2M,pH 4.8) and 500 units β-glucuronidase containing 50 units sulfatase. In preliminary experiments the hydrolysis conditions were optimized and it was ascertained that the results obtained with pure glucuronidase not containing sulfatase, were similar. The enantiomer concentrations are calculated from calibration curves, constructed by least-squares regression analysis of the peak height ratios enantiomer/internal standard. The glucuronide concentrations are expressed as oxprenolol equivalents. For the determination of the plasma oxprenolol concentrations, all samples from one rabbit, without and with probenecid, are analyzed in two runs: one run for unhydrolyzed samples, one run for hydrolyzed samples. With each run of unhydrolyzed samples, two quality control samples (0.1 and 1.0 μg/ml rac-oxprenolol, made from a different stock solution, by another person) were analyzed, together with the calibration curve (0.02 to 1.5 μg/ml rac-oxprenolol). For the determination of the plasma oxprenolol concentrations after hydrolysis, three quality control samples (1, 4 and 8 μg/ml) were analyzed with each run, together with the calibration curve (0.1 to 10 μg/ml rac-oxprenolol). Accuracy and reproducibility measured on these plasma quality control samples were acceptable, with coefficients of variation and deviations of the nomimal value below 15% (n=9).

The urine samples of all rabbits, without and with probenecid, were assayed in two runs, one without and one with hydrolysis. With each run two quality samples were assayed (0.25 μg/ml and 1.25 μg/ml without and 10 μg/ml and 75 μg/ml with hydrolysis) together with the calibration curve (0.05 μg/ml to 1.5 μg/ml without and 5 μg/ml to 200 μg/ml with hydrolysis).

Protein binding of the oxprenolol enantiomers was measured by equilibrium dialysis on blank samples as described before[7].

Creatinine was measured in plasma and urine with the Jaffe colorimetric method, using a Merckotest kit (Merck, Darmstadt, Germany).

Data Analysis and Statistics

Kinetic parameters were calculated by non-compartmental analysis. The areas under the plasma concentration-time curve(AUC) values were determined from 0 h to the time of the last measured plasma concentration, using the linear trapezoidal rule . For the oxprenolol enantiomers, the oral clearance (Cl_o) was calculated from $Cl_o = D/AUC$ where D is the dose of each enantiomer i.e. 25 mg/kg. The intrinsic clearance (Cl_{intr}) was calculated from Cl_o/f_u, where f_u is the free fraction in plasma. The renal clearances of the oxprenolol enantiomers and of the glucuronide diastereomers were calculated by dividing the amount of substance in the urine recovered over 72 hours, by the plasma AUC value of the corresponding product. The formation clearances of the glucuronide diastereomers were calculated by dividing the amount of each glucuronide recovered over 72 hours in urine, by the plasma AUC value of the corresponding oxprenolol enantiomer. The creatinine clearance was calculated from the average of the values obtained for the three consecutive 24 hours. All results are given as means ± SEM. For all parameters the (R)/(S) ratios were calculated. Comparison between (R)-and (S)-enantiomers, and between control state and probenecid pretreatment was done with the Wilcoxon rank test. Significance was assumed when $p < 0.05$.

RESULTS

The mean plasma concentration-time curves of the oxprenolol enantiomers and their glucuronides in control and in probenecid-pretreated rabbits are shown in Figure 1. The

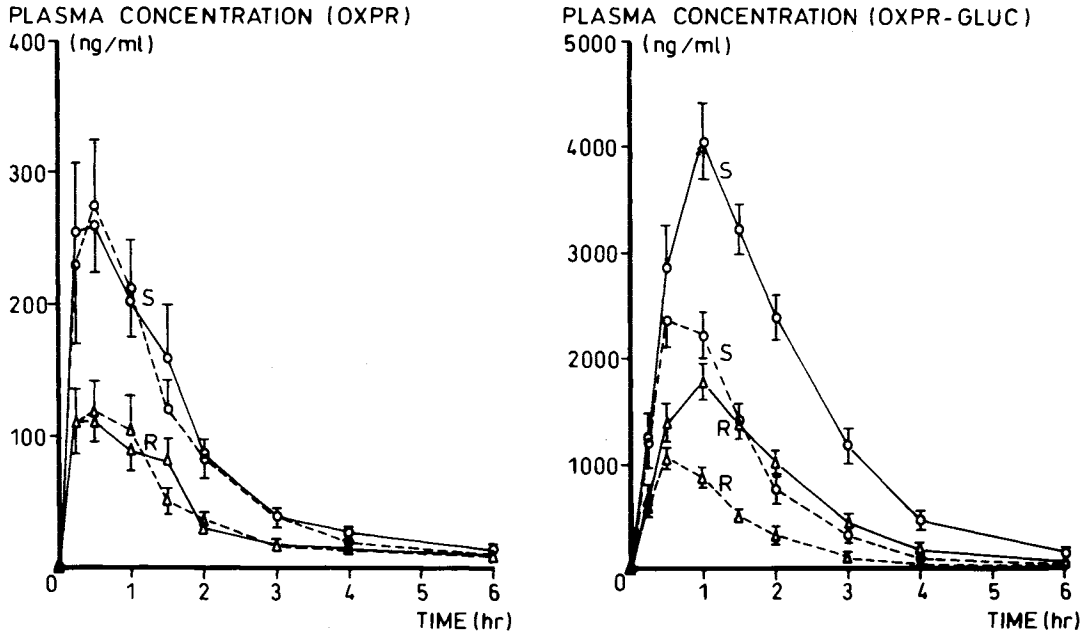

Fig. 1. Plasma concentration-time curves of the oxprenolol enantiomers and their glucuronides (expressed as oxprenolol equivalents) after oral administration of 50 mg/kg racemic oxprenolol,in nine rabbits, in control state (----) and after probenecid pretreatment (——).

corresponding pharmacokinetic parameters are given in Tables I and II.

Control Experiments

In the control state, without probenecid pretreatment, the plasma concentrations of (S)-oxprenolol are about twofold higher than those of (R)-oxprenolol, resulting in a marked difference in AUC. (S)-oxprenolol is 5% more protein bound than (R)-oxprenolol. The Cl_{intr} calculated from these data is much lower for (S)- than for (R)-oxprenolol. The amount of unchanged oxprenolol in the urine over 72 hours is less than 1%. The renal clearance values of both enantiomers differ slightly, but not significantly, and are somewhat higher than the creatinine clearance. The plasma concentrations of the oxprenolol glucuronides are much higher than those of the parent enantiomers, with AUC values about 7 to 8-fold higher. For (S)-glucuronide, the AUC and the amount recovered in 72 hours urine are about twice those of (R)-glucuronide. The renal clearances for both glucuronides are similar, and markedly higher than the creatinine clearance. The formation clearance of (S)-glucuronide is significantly higher than that of (R)-glucuronide.

Influence of Probenecid

For the unconjugated enantiomers, probenecid pretreatment causes a slight decrease in renal clearance but AUC, oral clearance, free fraction and intrinsic clearance are not affected. In contrast, the plasma concentrations of the glucuronide diastereomers are increased markedly after probenecid, with AUC values which are about twofold higher. The amount of the glucuronide diastereomers excreted in the urine is not changed, but the renal clearances are markedly reduced by probenecid. The formation clearance of (S)-oxprenolol glucuronide is significantly decreased after probenecid, but this is not the case for (R)-oxprenolol glucuronide.

Table I. Pharmacokinetic Parameters of the Oxprenolol Enantiomers, After Oral Administration of 50 mg/kg Rac-oxprenolol in Nine Rabbits in Control State and After Probenecid Pretreatment

	Control n = 9	Probenecid n = 9
Weight (kg)	2.9 ± 0.1	2.9 ± 0.1
Cl_{creat} (ml/min)	11.48 ± 0.68	13.19 ± 0.94
(R)-oxpr		
AUC (μg · min/ml)[b]	13.6 ± 1.9	13.9 ± 2.1
Cl_o (ml/min)	2132 ± 274	2187 ± 342
F_u	0.78 ± 0.004	0.77 ± 0.01
Cl_{intr} (ml/min)	2749 ± 358	2849 ± 461
Cl_{ren} (ml/min)	15.9 ± 2.9	10.3 ± 1.5
% of dose excr	0.31 ± 0.05	0.22 ± 0.04
(S)-oxpr		
AUC (μg · min/ml)	28.1 ± 4.4[a]	30.1 ± 4.0[a]
Cl_o (ml/min)	1074 ± 153[a]	986 ± 153[a]
F_u	0.73 ± 0.01[a]	0.73 ± 0.01[a]
Cl_{intr} (ml/min)	1486 ± 222[a]	1367 ± 224[a]
Cl_{ren} (ml/min)	18.7 ± 3.7	11.0 ± 1.8
% of dose excr	0.72 ± 0.13[a]	0.52 ± 0.09[a]

[a] Significantly different from (R) ($p < 0.001$, Wilcoxon test).
[b] AUC, area under the plasma concentration-time curve; Cl_o, oral clearance; F_u, unbound fraction; Cl_{intr}, intrinsic clearance; Cl_{ren}, renal clearance; Cl_{creat}, creatinine clearance.

Table II. Pharmacokinetic Parameters of the Oxprenolol Glucuronides (Expressed as Oxprenolol Equivalents), After Oral Administration of 50 mg/kg Rac-oxprenolol in Nine Rabbits in Control State and After Probenecid Pretreatment

	Control	Probenecid
(R)-oxpr-gluc		
AUC (μg · min/ml)[d]	100.0 ± 10.3	228.1 ± 20.1[b]
Cl_{ren} (ml/min)	32.2 ± 4.2	11.9 ± 0.81[b]
% of dose excr	5.0 ± 0.7	4.4 ± 0.5
Cl_{form} (ml/min)	290.5 ± 73.4	262.3 ± 64.6
(S)-oxpr-gluc		
AUC (μg · min/ml)	224.6 ± 22.7[a]	529.7 ± 39.3[ab]
Cl_{ren} (ml/min)	34.2 ± 3.8	12.33 ± 0.61[b]
% of dose excr	12.8 ± 1.4[a]	10.4 ± 0.9[a]
Cl_{form} (ml/min)	373.8 ± 90.2[a]	270.4 ± 56.5[c]

[a] Significantly different from (R) ($p < 0.001$, Wilcoxon test).
[b] Significantly different from control state ($p < 0.001$, Wilcoxon test).
[c] Significantly different from control state ($p < 0.05$, Wilcoxon test).
[d] AUC, area under the plasma concentration-time curve; Cl_{ren}, renal clearance; Cl_{form}, formation clearance.

DISCUSSION

We previously found in humans that the renal clearance of (R)-oxprenolol glucuronide is markedly higher than the creatinine clearance, while for the (S)-glucuronide, the reverse is true[2]. We studied the disposition of oxprenolol and its glucuronides in rabbits, and the influence of probenecid therapy thereupon. Probenecid is, indeed, known to inhibit the renal secretion of organic acids, including glucuronides.[3–5]

Unchanged Oxprenolol

In the rabbit, the plasma concentrations of the oxprenolol enantiomers are different, and this is mainly due to a difference in metabolism. Renal excretion is only a minor elimination route for the enantiomers; the renal clearance values found are slightly, but not significantly higher than the creatinine clearance in the same animals. In humans, only small differences were found between the plasma concentrations of the oxprenolol enantiomers; as in the rabbit, renal excretion is only a minor elimination route but the renal clearances of the enantiomers are much lower than the creatinine clearance, probably due to the extensive plasma binding of the oxprenolol enantiomers in humans.[2] In rabbits, in contrast, the plasma binding is very low.

Probenecid pretreatment did not lead to an increase of the plasma concentrations of the oxprenolol enantiomers. There was, likewise, no effect of probenecid on their plasma protein binding. This latter finding is not unexpected as oxprenolol is only slightly protein bound in the rabbit, and, moreover, it binds mainly to α_1-acid glycoprotein[8], while probenecid binds mainly to albumin[9]. There was a small effect of probenecid on the renal clearance of both oxpre-

nolol enantiomers, suggesting that some active secretion is involved.

Oxprenolol Glucuronides

In the control experiments, the plasma concentrations of the (S)-glucuronide are about twice those of (R)-glucuronide. About 10% of the oxprenolol dose is found in the urine as glucuronides, with the amount of (S)-glucuronide being more than twice that of (R)-glucuronide. The renal clearances of both oxprenolol glucuronides are similar, and markedly higher than the creatinine clearance, indicating active secretion. In humans, only for the (S)-glucuronide did the renal clearance value suggest active secretion[2].

After probenecid pretreatment, the plasma concentrations of both glucuronides are markedly increased, while their renal clearances are significantly reduced. After probenecid the renal clearance of the glucuronides approach the creatinine clearance value, suggesting complete inhibition of the active secretion. Inhibition of active secretion by probenecid has also been described for glucuronides of other drugs[3–5]. In the absence of probenecid, the formation clearances of the glucuronide diastereomers are slightly different. This, together with the similarity of their renal clearance, suggests that the difference in plasma concentrations between the glucuronides does not result only from a difference in their formation or elimination, but is a consequence of the difference in concentrations of oxprenolol enantiomers available for glucuronidation. This finding is different from that in humans, where the stereoselective difference in plasma concentrations of the glucuronides was mainly related to the difference in their renal elimination. Probenecid reduces significantly the formation clearance of S-glucuronide, but not that of its antipode. Probenecid is known to inhibit urine diphosphate glucuronyltransferase in vitro[10]. Inhibition by probenecid of the formation clearance, probably by competition, was also reported for glucuronides of other drugs[4–5]. In these studies the inhibition found was, however, more pronounced than in our experiments. Vree et al found no inhibitory effect of probenecid on the formation of the acyl glucuronides of furosemide[11].

Our results show that in the rabbit the stereoselective disposition of oxprenolol and its glucuronides differs from that in humans. In the rabbit, for both glucuronides evidence for active secretion is seen, whereas in humans this was only true for (S)-glucuronide. In the rabbit probenecid inhibits the active secretion of both diastereomers. On the basis of our results, it would be of interest to study in humans how probenecid affects the renal clearance of both the actively secreted (S)-diastereomer and of its antipode.

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