Esterase Activity Toward the Diastereomers of Cefuroxime Axetil in the Rat and Dog

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INTRODUCTION

Cefuroxime axetil, an orally active prodrug form of cefuroxime, is a 50:50 mixture of the two diastereomers (1′*R*,6*R*,7*R* and 1′*S*,6*R*,7*R*) formed by the esterification of the C-4 carboxylic acid with racemic 1-bromoethyl acetate (Fig. 1). When administered orally to humans, the parent drug cefuroxime is unabsorbed (1), whereas the prodrug shows absolute bioavailabilities of 36 and 52% in the fasted and fed states, respectively (2). Similar results in rats led to the discovery and isolation of a cefuroxime axetil esterase from rat, human, and dog intestinal washings (3). The esterase can hydrolyze cefuroxime axetil to the nonabsorbable cefuroxime in the gut lumen and is therefore suspected as a possible cause of incomplete bioavailability.

This paper further describes the oral absorption process of cefuroxime axetil by evaluating hydrolysis of the individual diastereomers by enzymes found in the brush border/mucosal epithelial area of the duodenum and small intestine. Serum esterases are also studied as any cefuroxime axetil absorbed intact will face hydrolysis in the circulation and complicate the interpretation of blood level assays. Two animal species are evaluated, rat and dog, and comparisons are made between rates of hydrolysis of the two diastereomers in each of the tissue systems.

MATERIALS AND METHODS

Materials. The 1′*R*,6*R*,7*R* and 1′*S*,6*R*,7*R* diastereomers and the 50:50 diastereomeric mixture of cefuroxime axetil were used as received (Eli Lilly and Company). HPLC-grade acetonitrile and methanol (Mallinckrodt) were used. Dye reagent (Bio-Rad) and electrophoresis grade Fraction V bovine serum albumin (Calbiochem) were used for the protein content assay. All other chemicals were reagent grade and all solutions and buffers were prepared using sterile water for injection.

Drug Solution Preparation. Saturated solutions of the individual diastereomers and the 50:50 diastereomeric mixture were prepared by adding excess solid material to isotonic phosphate buffer (0.067 *M*, pH 7.4) and stirring for 15 min. The solutions were filtered through 0.45-μm Acrodisc syringe filters (Gelman Science) and assayed. Initial concentrations ranged from 2 to 4 μg/ml for the 1′*S*,6*R*,7*R* isomer and from 2 to 6 μg/ml for the 1′*R*,6*R*,7*R* isomer.

Esterase Preparation—Rat. Each nonfasted male Wistar rat (200–250 g) was anesthetized with Metofane (Pittman-Moore) inhalation anesthetic and approximately 5 ml of blood was obtained by cardiac puncture and allowed to clot for 20 min at room temperature. The rat was sacrificed with intracardiac sodium pentobarbital (The Butler Company), the abdomen opened, and the first 30 cm of intestine distal to the pyloris removed. The intestine was rinsed with buffer, opened longitudinally, and flattened. The mucosal surface was scraped with a glass microscope slide and the collected material weighed and diluted 1:5 with cold buffer. A mechanical tissue grinder (Tekmar) was used to homogenize the mixture for 30 sec while kept on ice. The resulting homogenate and clotted blood were centrifuged (Beckman GPR tabletop centrifuge) at 4°C, 2800*g* for 15 min, then kept on ice until used. The serum obtained was diluted 1:9 with cold buffer prior to the experiment. Both the diluted serum and the intestinal supernatant were assayed for protein content using the Bio-Rad protein assay with bovine serum albumin as the protein standard. For studies using attenuated enzymes, the preparations were placed in boiling water for 10 min.

Esterase Preparation—Dog. Nonfasted beagle dogs of mixed sex and age (average weight, 10.4 ± 3.9 kg) sacrificed for other studies were used. A 5-ml blood sample was obtained from a forepaw and treated as above to obtain the diluted serum prep. Approximately 45 cm of duodenum was obtained and rinsed with cold buffer. Mucosal tissue was scraped and treated as described above.

Experimental Protocol. Borosillicate 13 × 100-mm culture tubes were suspended in a controlled-temperature water bath (Haake D3) at 37°C. A magnetic stir bar and 2.75 ml of drug solution were added to each tube and allowed to equilibrate. Continuous stirring was initiated and 0.25 ml of esterase preparation was added to each tube. Aliquots of 0.2 ml were removed at time 0 and every 30 min for 2 hr and added to small tubes containing 0.1 ml 10% trichloroacetic acid (TCA) and 0.1 ml acetonitrile. This procedure precipitated any proteins present. The small tubes were vortexed, then centrifuged at 4°C, 2000*g* for 5 min. The supernatant was decanted and assayed for the cefuroxime axetil isomers by reverse-phase HPLC.

Stability of cefuroxime axetil in the TCA/acetonitrile preparation was confirmed and recovery studies of the diastereomers from the precipitation procedure were conducted. Recovery was 101 ± 4.2 and 99.3 ± 4.9% for the 1′*R*,6*R*,7*R* and 1′*S*,6*R*,7*R* diastereomers, respectively. Error is coefficient of variation with $n = 48$ for each.

HPLC Analysis. Cefuroxime and the two diastereomers of cefuroxime axetil were separated isocratically on a DuPont Zorbax C_{18} column (4.6 mm × 15 cm) at ambient temperature. The mobile phase consisted of methanol:water (38:62) delivered at a flow rate of 1.5 ml/min with a Beckman Model 110B pump. Injections were made using a Bio-Rad

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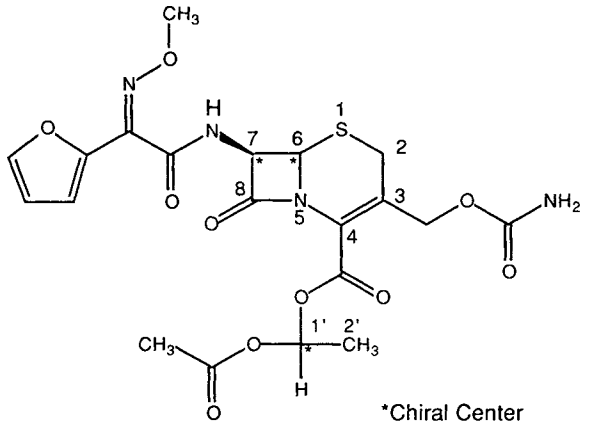

Fig. 1. Structure of cefuroxime axetil, [(1′*R*,*S*)-acetoxyethyl (6*R*,7*R*)-3-carbamoyloxymethyl-7-[(*z*)-2-(fur-2-yl)-2-methoxyiminoacetamido] ceph-3-em-4-carboxylate].

autosampler (Model AS-48) with a 100-μl fixed loop operated at 4°C. Effluent was monitored with a Kratos Spectraflow (Model 783) uv detector at 280 nm. Quantitation was based on peak areas calculated using a Model 3390A Hewlett Packard integrator. Capacity factors for the 1′*R*,6*R*,7*R* and 1′*S*,6*R*,7*R* diastereomers were 6.6 and 8.0, respectively. External standard curves were constructed using the TCA–acetonitrile centrifugation procedure described above. The data are linear over the range 0.05–8.0 μg/ml.

RESULTS AND DISCUSSION

When the diastereomers of cefuroxime axetil are exposed to the esterases present in intestinal mucosa and serum, hydrolysis to the biologically active cefuroxime occurs. An example of the observed hydrolysis is shown in Fig. 2 as a first-order decline in initial concentration over time. All studies gave apparent first-order kinetics, with observed rate constants being determined from the slopes of these plots. The enzymatic contribution to the rate constant was calculated by subtracting values for the hydrolytic rate constants in the presence of attenuated tissue from the observed values. These corrected rate constants were normalized by dividing by the protein content in each experiment to give enzymatic rate constants with units of milliliters per minute per milligram of protein. As a result of the limited aqueous solubilities of the isomers, apparent k_m and V_{max} values for the tissue preparations could not be determined.

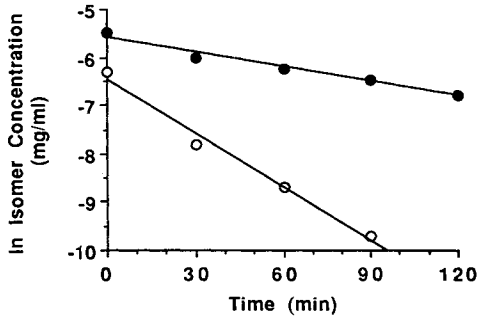

Fig. 2. Concentration of the (●) 1′*R*,6*R*,7*R* and the (○) 1′*S*,6*R*,7*R* diastereomers of cefuroxime axetil over time in the presence of dog intestinal esterases.

Enzymatic rate constant data for the individual isomer in the absence and presence of the other isomer are summarized in Table I. Each number represents the average of 6 to 19 individual values. Statistical analysis of the data (Student *t* test, $\alpha = 0.05$) indicates that neither isomer alters the rate of hydrolysis of the other, so the data for each isomer in the presence and absence of the other isomer are combined. These data are given in Fig. 3 and show stereoselective hydrolysis, the 1′*S*,6*R*,7*R* isomer being hydrolyzed faster than the 1′*R*,6*R*,7*R* isomer in all cases. The 1′*S*,6*R*,7*R*/1′*R*,6*R*,7*R* ratios of enzymatic rate constants for dog serum and intestine are 14 and 2.5, respectively, and those for rat serum and intestine are 13 and 3.4. The rate constants for the rat tissues are larger that those for the corresponding dog tissue in all cases ($\alpha = 0.005$). It is unknown whether the rat tissue contains quantitatively more esterase per amount of protein or whether different esterases are present which have higher specific activities.

There are numerous examples in the literature of stereoselective hydrolyses of esters by tissue esterases (4–8) and of the implications of such selective metabolism in absorption (9) and distribution (10). Reports of other studies with ester-containing drugs indicate that, in general, rates of ester hydrolysis are greater in the rat than in the dog for both intestine (11) and blood (12). Our data with the cefuroxime axetil ester are in agreement. Using these data to predict hydrolysis in humans, however, is difficult. The presence of a cefuroxime axetil esterase in the human intestine has been demonstrated (3), but its specific activity toward the diastereomers has not been determined. It has been classified as a carboxylesterase, but unlike most, it is quite specific for cefuroxime axetil, with minimal activity toward α-naphthyl acetate and *p*-nitrophenyl acetate (3). In human blood a half-life of 3.5 min has been reported for the 50:50 diastereomeric mixture (13) but it is unknown if the esterase involved is the same as that in the intestine. The specificity of the cefuroxime axetil esterase in the intestine (and possible specificity of esterases in the blood) prohibits the unconditional use of published rank order esterase activities showing rat > human > dog in the intestine (11) or rat > dog > human in blood (12), as they were determined for esters probably susceptible to general esterases of low specificity.

Table I. Enzymatic Rate Constants for the Hydrolysis of Cefuroxime Axetil Isomers in the Absence and Presence of the Other Isomer[a]

		Isomer 1′*R*, 6*R*, 7*R*		Isomer 1′*S*, 6*R*, 7*R*	
Species	Tissue	Alone	50:50 mix[b]	Alone	50:50 mix[b]
Dog	Serum	0.00276	0.00161	0.0308	0.0280
	Intestine	0.0250	0.0275	0.0918	0.0900
Rat	Serum	0.00715	0.00762	0.111	0.0849
	Intestine	0.0250	0.0275	0.0918	0.0900

[a] Units are ml/min/mg protein.
[b] In the presence of an equal initial concentration of the other isomer.

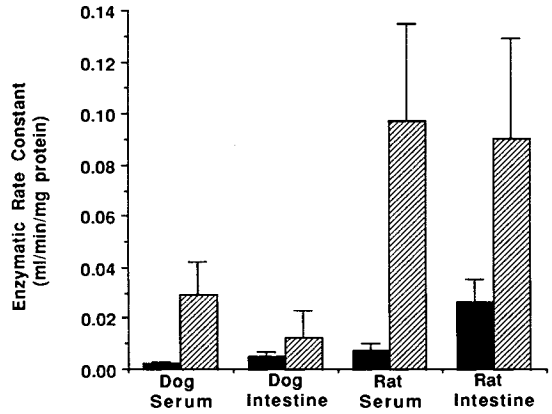

Fig. 3. Enzymatic rate constants for the hydrolysis of the (■) 1′*R*,6*R*,7*R* and the (▨) 1′*S*,6*R*,7*R* diastereomers of cefuroxime axetil in animal tissue. Error is SD.

The data presented here assist in interpreting preclinical animal bioavailability studies. The relative contribution of intestinal metabolism of the isomers to the observed oral bioavailability can now be assessed, providing a better understanding of the absorption process of cefuroxime axetil.

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