Stability of Cephalosporin Prodrug Esters in Human Intestinal Juice: Implications for Oral Bioavailability

KLAUS STOECKEL,1,4 WERNER HOFHEINZ,2 JEAN PAUL LANEURY,3 PATRICK DUCHENE,3 STEVE SLEDLOFSKY,4 AND ROBERT A. BLOUIN5

CLINPHARM SUPPORT GmbH, CH-4051 Basel,1 and Talmattweg 7, CH-4103 Basel, Bottmingen,2 Switzerland; ADME BIOANALYSES, F-06250 Mougins, France; and College of Medicine3 and College of Pharmacy, University of Kentucky, Lexington, Kentucky 40536

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The levels of degradation of cefetamet pivoxil (CAT), cefuroxime axetil (CAE), and cefpodoxime proxetil (CPD) in 0.6 M phosphate buffer (pH 7.4) and human intestinal juice (pH 7.4) at 37°C over 24 h were compared. Significant differences in the time courses of degradation and in the patterns of degradation products were observed. (i) The relative proportions of the Δ2- and Δ3-cephalosporins were roughly reversed in the two incubation media. In phosphate buffer, the major degradation product was the Δ2-cephalosporin (CAT = 61%; CAE = 74%; CPD = 85%), while in intestinal juice it was the Δ3-cephalosporin (CAT = 86%; CAE = 75%; CPD = 87%). (ii) Generally, the degradation of the prodrug esters progressed faster in intestinal juice than in phosphate buffer (e.g., for CAT the half-lives \( t_{1/2} \) were 0.78 and 4.3 h, respectively). (iii) The two diastereoisomers of CAE and CPD were degraded at different rates in intestinal juice (for the CAE diastereoisomers, \( t_{1/2} \) = 0.37 and 0.93 h; for the CPD diastereoisomers, \( t_{1/2} \) = 0.18 and 0.98 h) but were degraded at similar rates in phosphate buffer (for the CAE diastereoisomers, \( t_{1/2} \) = 1.6 h; for the CPD \( t_{1/2} \) diastereoisomers, = 2.2 h). It is concluded that (i) the Δ2 isomerization does not significantly affect the bioavailability of prodrug esters since enzymatic hydrolysis in the intestinal fluid proceeds mainly to the active Δ3-cephalosporin and (ii) the high degree of stereoselectivity of the enzymatic ester hydrolysis should make it possible to increase the bioavailabilities of certain prodrug esters (CAE, CPD) by using the more stable diastereoisomer.

Cephalosporins usually exhibit poor bioavailabilities when they are given orally. Higher values are obtained only with cephalosporins that are taken up by carrier systems or when the polarity of the carboxylic acid group in the 4 position is reduced by esterification (10). Esterification of the carboxylic acid group produces derivatives which can be absorbed by passive diffusion. Therapeutically useful compounds can, however, be obtained only if the absorbed prodrug ester is readily converted back to the active drug. The success of the prodrug ester approach depends vitally on the solubility and lipophilicity of the prodrug ester as well as its stability to chemical and enzymatic ester cleavage.

Cephalosporin prodrug esters exhibit oral bioavailabilities of approximately 50%. Premature hydrolysis in the intestine before absorption has been discussed as a possible reason for their incomplete bioavailability (4, 6, 8). Hydrolysis can proceed either by enzyme-catalyzed direct hydrolysis to the parent cephalosporin (6) or via a reversible base-catalyzed isomerization yielding the Δ2-cephalosporin ester which is rapidly cleaved to give the biologically inactive Δ2-cephalosporin (7). Both pathways will remove some of the cephalosporin available for absorption. The result is incomplete bioavailability of the oral prodrug ester. However, direct hydrolysis to the nonabsorbable but biologically active cephalosporin in the gut lumen could affect the gut flora, causing undesirable intestinal side effects (3).

To date, no studies have assessed the relative importance of the two pathways. Acquiring a better understanding of these pathways would be beneficial in designing cephalosporin prodrug esters with improved bioavailabilities and intestinal tolerability characteristics. Therefore, we studied the degradation of three clinically relevant cephalosporin prodrug esters (cefetamet pivoxil, cefuroxime axetil, and cefpodoxime proxetil) in human intestinal juice and phosphate buffer (a well-understood model for chemical hydrolysis [5, 7–9]).

MATERIALS AND METHODS

Chemicals and reference compounds. All chemicals and solvents were of analytical grade. They were all purchased from Carlo Erba, Nanterre, France.

The chemical structures of the cephalosporin prodrug esters used in this study are shown in Fig. 1. All cephalosporins used in this study were obtained from the sample collection of F. Hoffmann-La Roche Ltd., Basel, Switzerland. They included cefetamet sodium salt (Ro 15-8074/001), the Δ2 isomer of cefetamet (Ro 19-4295/000), cefetamet pivoxil-methyl ester (cefetamet pivoxil; Ro 15-8075/000), cefpodoxime sodium salt (Ro 43-1652/001), cefpodoxime 1-isoproploxy-carboxylic acid ester (cefetamet pivoxil; Ro 15-8075/000), cefpodoxime pivoxil (Ro 24-2238/000), and cefuroxime sodium salt (Ro 12-6341/001), and cefuroxime 1-acetyloxy)ethyl ester (cefuroxime axetil; Ro 24-2238/000).

Instrumentation. Nuclear magnetic resonance (1H NMR) spectra were recorded on a Bruker AM 400 spectrometer (400 MHz) in D_2O. The high-performance liquid chromatography (HPLC) system consisted of a Shimadzu LC-6A pump and a Shimadzu SIL-6A autoinjector (Shimadzu Corporation, Kyoto, Japan), a Beckman DAD 168 detector, and a UV spectrophotometer (DU-64; Beckman). In the case of the Cefuroxime study, a Beckman DAD 168 detector was used. The optical rotation in the 1H NMR spectra were recorded on a Bruker AM 400 spectrometer (400 MHz) in D_2O. The high-performance liquid chromatography (HPLC) system consisted of a Shimadzu LC-6A pump and a Shimadzu SIL-6A autoinjector (Shimadzu Corporation, Kyoto, Japan), a Beckman DAD 168 detector, and a UV spectrophotometer (DU-64; Beckman).

Intestinal juice collection. Four male subjects (average age, 28 years) were recruited from the medical personnel of the University of Kentucky. They were healthy as evidenced by a complete medical history. Informed consent was obtained from all study participants. After a 12-h fasting period each subject received with the help of a radiologist a 14 French Bilboa-Dotter tube (Cook Inc., Indianapolis, Ind.). The proximal holes of the tube were taped shut through the nose. The tube was guided into the duodenum by standard fluoroscopy. After the tube had reached the duodenum, gallbladder contraction was stimulated by a 10-min intravenous infusion of 20 mg of cholecystokinin octapeptide (Sinalic, Kinevac; Squibb, Princeton, N.J.) per kg of body weight. During this period and the following 15 min, all duodenal secretions were collected. A volume of 5 to 10 ml of intestinal juice (pH, approximately 7.0, as determined with pH paper) was collected from each patient. Immediately after sample collection, the tube was removed and a standard breakfast was provided to the volunteers.

* Corresponding author. Mailing address: CLINPHARM SUPPORT GmbH, Leimenstrasse 57, CH-4051 Basel, Switzerland. Phone: 41 61 274 07 47, Fax: 41 61 274 07 48, E-mail: 100271,1114@compuserve.com.
The intestinal juice samples from each subject were stored separately at ~80°C until further use.

Analytical scale incubation with phosphate buffer (pH 7.4). The following were added sequentially to a test tube: 1.5 ml of deionized water, 300 μl of 0.6 M phosphate buffer (pH 7.4), and 20 μl of an acetonitrile solution of either cefetamet pivoxil, cefuroxime axetil, or cefpodoxime proxetil at a concentration of 3 mg/ml. The mixture was stirred well and was incubated for 24 h in a temperature-controlled water bath at 37°C. At 0, 10, and 30 min as well as at 1, 2, 4, 6, 10, and 24 h, samples of 150 μl were withdrawn from the incubation solution, mixed with 150 μl of 0.5 M perchloric acid, and centrifuged at 3,000 × g for 10 min. A 50-μl sample of the supernatant was then injected onto an analytical HPLC column for analytical separation (see below). All analytical incubations were performed in duplicate.

Analytical scale incubation with intestinal juice (pH 7.4). The following were added sequentially to a test tube: 300 μl of freshly thawed intestinal juice, 60 μl of 0.6 M phosphate buffer (pH 7.4); the pH of the mixture of intestinal juice and phosphate buffer was 7.4), and 20 μl of an acetonitrile solution of either cefetamet pivoxil, cefuroxime axetil, or cefpodoxime proxetil at a concentration of 1 mg/ml. The following procedures were performed exactly as described above for the phosphate buffer study.

Preparative scale incubation with intestinal juice (pH 7.4). The following were added sequentially to a test tube: 600 μl of freshly thawed intestinal juice, 120 μl of 0.6 M phosphate buffer (pH 7.4), and 50 μl of an acetonitrile solution of prodrug ester at a concentration of 20 mg/ml. The mixture was stirred well and was incubated for 15 h in a temperature-controlled water bath at 37°C. The incubation was stopped by the addition of 80 μl of 5 M perchloric acid. After centrifugation at 3,000 × g for 15 min, the supernatant was evaporated with nitrogen at 40°C to a volume of approximately 100 μl. The material from two incubations in 150 μl of fluid was subjected to HPLC for preparative separation of the metabolites of cefpodoxime proxetil, a slightly different eluent composition was used: the eluent was 86% mobile phase I for the first 6.5 min, and this was reduced to 60% mobile phase I between 6.5 and 9 min. The washing (up to 30 min after the start) was performed with the starting eluent of 86% mobile phase I.

Preparative separation after incubation with intestinal juice. The chromatographic conditions for the preparative separation were the same as those described above in the analytical section except that trifluoroacetic acid was used in place of perchloric acid in mobile phase I. The two major peaks that formed during the incubation (for cefetamet pivoxil, ~6.1 and 7 min; for cefuroxime axetil, ~11 and 12.2 min; for cefpodoxime proxetil, ~7.3 and 7.7 min) were collected, evaporated under nitrogen at 40°C to ~150 ml, and further purified by HPLC on the same C18 Nucleosil (5 μm, 150 by 4.6 mm) column by using an isocratic mobile phase separation (flow rate, 1 ml/min with a mobile phase of 88% water and 12% acetonitrile for cefuroxime axetil and cefpodoxime proxetil). For cefetamet pivoxil, a mixture of 90% water and 10% acetonitrile was used. The fraction containing major acidic metabolites was collected, concentrated with a stream of nitrogen to ~100 μl, and stored at ~18°C. Immediately before initiating 1H NMR analysis in D2O, each fraction was evaporated to dryness by freeze-drying.

Quantification of the incubation results. At specific time points during the in vitro incubation with phosphate buffer and intestinal juice, samples were withdrawn and separated on an analytical HPLC column (for a description of the procedure, see above). For the quantification of the metabolites, the relative percentage of the areas under the main peaks at 265 nm was measured. Quan- tification of the prodrug esters and their metabolites neglects the small differences in their molar absorption at 265 nm (for cefetamet, ε [265 nm] = 0.99 × 10^-4 for the Δ3 isomer and 1.19 × 10^-4 for the Δ3 isomer).

Kinetic rate constants and t1/2s. The kinetic degradation rate constants (kD) of the different prodrug esters were determined as ~1 times the slope. The slope was determined by linear regression of the linear portion of the ln (prodrug ester concentration)-versus-time curves. The half-life t1/2 degradation values were determined by ln 2 divided by kD.

RESULTS

The time courses and the patterns of the products of degradation differed significantly between phosphate buffer and intestinal juice at pH values of 7.4. Similar differences were
observed with all three prodrug esters. The time course of disappearance of the prodrug esters and the appearance of the $\Delta_3$ and $\Delta_2$ acids for the hydrolysis in phosphate buffer is shown in Fig. 3a, 4a, and 5a, and those for the hydrolysis in intestinal juice are shown in Fig. 3b, 4b, and 5b. All degradation studies were performed over a 24-h period. The pathways of degradation of prodrug esters following enzymatic and weak aqueous base hydrolysis are shown in Fig. 2.

**Cefetamet pivoxil.** Degradation of cefetamet pivoxil progressed much faster in intestinal juice than in phosphate buffer. Cefetamet pivoxil degraded in intestinal juice with a $K_{DEG}$ of $8.86 \times 10^{-2}$ h$^{-1}$, while in phosphate buffer the $K_{DEG}$ was only $1.62 \times 10^{-1}$ h$^{-1}$. The corresponding $t_{1/2}$s were 0.78 h in intestinal juice and 4.3 h in phosphate buffer.

Four product peaks (peaks A1, A2, A3, and A4) with retention times of approximately 6, 7, 15, and 16 min, respectively, were formed in phosphate buffer. After 24 h, the relative areas under the main peaks at 265 nm of these products were 61% for A1, 5% for A2, 8% for A3, and 22% for A4.

Ester cleavage in intestinal juice was completed after 4 to 6 h. A1 and A2 could clearly be identified as the dominant products, while the formation of A3 and A4 could not be detected. However, small peaks of A3 and A4 could have been obscured by the abundance of interfering background peaks on the chromatogram from the HPLC. The relative percentages of A1 and A2 at the end of the reaction in intestinal juice were roughly reversed from that in phosphate buffer (approximately 86% for A2 and 14% for A1).

The major product, A1, that formed in phosphate buffer was the $\Delta_2$ isomer of cefetamet, whereas the minor product, A2, was cefetamet ($\Delta_3$ isomer). This assignment was confirmed by cochromatography with reference compounds and by $^1$H NMR spectroscopy of a fraction containing both A1 and A2 which was isolated by preparative chromatography after a 15-h incubation in intestinal juice (see above). A1 cochromatographed under analytical chromatographic conditions with the $\Delta_2$ isomer of cefetamet and A2 cochromatographed with cefetamet. The 400-MHz $^1$H NMR of the isolated fraction containing A1 and A2 showed two sets of signals which were assigned to cefetamet and its $\Delta_2$ isomer. Cefetamet ($\Delta_3$ isomer) was identified by the following signals: 1.88 ppm (s, 3$^9$-CH$_3$), 3.25 and 3.62 ppm (AB, $J = 5.18$ Hz, 2-CH$_2$), 3.99 ppm (s, N-O-CH$_3$), and 5.18 and 5.74 ppm (AB, $J = 4.8$ Hz, 6-CH and 7-CH). The $\Delta_2$ isomer was identified by signals at 1.92 ppm (s, 3$^9$-CH$_3$), 4.00 ppm (s, N-O-CH$_3$), 4.87 ppm (s, 4-CH), 5.38 ppm and 5.57 ppm (AB, $J = 4.5$ Hz, 6-CH and 7-CH), and 5.99 ppm (s, 2-CH).

The structures of the two products, A3 and A4, formed in phosphate buffer have not been identified.

**Cefuroxime axetil.** In line with the other two prodrug esters the diastereoisomers of cefuroxime axetil degraded faster in intestinal juice than in phosphate buffer. Hence, there were significant differences in $K_{DEG}$ and $t_{1/2}$ between the two incubation media (Table 1). Both diastereoisomers degraded in phosphate buffer with the same $K_{DEG}$ of approximately $4.2 \times 10^{-1}$ h$^{-1}$ ($t_{1/2} = 1.6$ h). However, in intestinal juice, one diastereoisomer declined with a $K_{DEG}$ of $7.45 \times 10^{-1}$ h$^{-1}$ ($t_{1/2} = 0.93$ h). The major product, A3, that formed in phosphate buffer was the $\Delta_2$ isomer of cefuroxime, whereas the minor product, A4, was cefuroxime ($\Delta_3$ isomer). This assignment was confirmed by cochromatography with reference compounds and by $^1$H NMR spectroscopy of a fraction containing both A3 and A4 which was isolated by preparative chromatography after a 15-h incubation in intestinal juice (see above). A3 cochromatographed under analytical chromatographic conditions with the $\Delta_2$ isomer of cefuroxime and A4 cochromatographed with cefuroxime. The 400-MHz $^1$H NMR of the isolated fraction containing A3 and A4 showed two sets of signals which were assigned to cefuroxime and its $\Delta_2$ isomer.
and the other declined with a $K_{\text{DEG}}$ of $18.8 \times 10^{-1}$ h$^{-1}$ ($t_{1/2} = 0.37$ h).

In phosphate buffer, cefuroxime axetil degraded to four product peaks (peaks B$_1$, B$_2$, B$_3$, and B$_4$) with retention times of approximately, 11, 12.2, 15.5, and 16.2 min, respectively. The relative areas under the main peaks at 265 nm after 10 h, by which time the 3 esters were completely hydrolyzed, were 7% for B$_1$, 54% for B$_2$, 11% for B$_3$, and 31% for B$_4$. Continued incubation led to increases for B$_2$ and B$_4$. After a 24-h incubation time, the relative areas under the main peaks at 265 nm were 4% for B$_1$, 54% for B$_2$, 11% for B$_3$, and 31% for B$_4$.

In intestinal juice ester hydrolysis of the two diastereoisomers was completed at between 4 and 6 h. As with the two other prodrug esters, the ratio of peaks B$_1$ and B$_2$ was roughly reversed (i.e., 75% B$_1$ and 25% B$_2$). No peaks of the products B$_3$ and B$_4$ were observed, possibly because of interfering background peaks from the incubation medium.

As the major product formed in phosphate buffer, B$_2$ was assigned the structure of the 3 acid (active isomer of cefpodoxime) (5). Consequently, B$_1$ was cefuroxime. This assignment was proven by cochromatography of B1 and cefuroxime under analytical chromatographic conditions in which both compounds migrated with identical retention times. A chromatographic fraction containing B$_1$ was obtained after incubation with intestinal juice for 15 h. Its 400-MHz $^1$H NMR spectrum showed the typical signals for cefuroxime at 3.44 and 3.71 ppm (AB, $J = 18$ Hz, 2-CH$_2$), 4.02 ppm (s, N-O-CH$_3$), and 5.36 and 5.98 ppm (AB, 3'-CH$_2$-O-CH$_3$, partially obscured by the HOD signal), and 5.25 and 5.83 ppm (AB, $J = 4.8$ Hz, 6-CH and 7-CH) and with three multiplets at 6.65, 6.92, and 7.71 ppm (three hydrogens of the furane ring). In addition to the signals of cefuroxime, a second set of signals was observed at 3.77 and 3.95 ppm (AB, $J = 18$ Hz, 2-CH$_2$), 4.02 ppm (s, N-O-CH$_3$), and 5.36 and 5.98 ppm (AB, $J = 5$ Hz, 6-CH and 7-CH) and with three multiplets at 6.65, 6.92, and 7.71 ppm coinciding with the signals of the furane protons of the major component of cefuroxime. These signals are indicative of a compound which is very similar to cefuroxime. The compound differs from cefuroxime probably only by a different substituent at the 3' position. Cefpodoxime proxetil. Like the other two prodrug esters, the two diastereoisomers of cefpodoxime proxetil degraded considerably faster in intestinal juice than in phosphate buffer (Table 1). In phosphate buffer, both diastereoisomers degraded with approximately the same $K_{\text{DEG}}$ of $(2.74 \times 10^{-1})$ h$^{-1}$ ($t_{1/2} = 2.5$ h) and $(3.13 \times 10^{-1})$ h$^{-1}$ ($t_{1/2} = 2.2$ h). In intestinal juice, however, one diastereoisomer degraded much faster than the other one ($K_{\text{DEG}} = 38.7 \times 10^{-1}$ h$^{-1}$ [$t_{1/2} = 0.18$ h] versus $K_{\text{DEG}} = 7.10 \times 10^{-1}$ h$^{-1}$ [$t_{1/2} = 0.98$ h]).

After ester hydrolysis in phosphate buffer for 24 h there were three clearly identifiable product peaks (peaks C$_1$, C$_2$, and C$_3$) with retention times of approximately 6.3, 15.4, and 16.3 min, respectively. The relative areas of these three peaks were 85% for C$_1$, 7.5% for C$_2$, and 7.5% for C$_4$.

In intestinal juice, ester hydrolysis of the two diastereoisomers was completed within 6 h. It produced two identifiable peaks at approximately 7 and 7.5 min (C$_1$, C$_2$). The relative areas of the two peaks were 13 and 87%, respectively. As in the experiments with the other two prodrug esters, background peaks of the incubation medium did not allow the exclusion of minor amounts of C$_3$ and C$_4$.

C$_2$ could not be measured in the phosphate buffer experiment. This is apparently due to the poor separation of C$_1$ and C$_2$ and the low C$_2$ levels.

As the major product of the hydrolysis in phosphate buffer, C$_1$ was identified as the 3 acid of cefpodoxime (5). Thus, C$_1$ had to be assigned to cefpodoxime. This assignment was confirmed by $^1$H NMR. A fraction containing C$_1$ as a minor product and C$_2$ as a major product was isolated by chromatography after incubation with intestinal juice for 15 h. In its 400-MHz $^1$H NMR it contained a set of signals of the 3 acid of cefpodoxime at 3.50 ppm (s, C-O-CH$_3$), 3.40 and 3.64 ppm (AB, $J = 18$ Hz, 2-CH$_2$), 4.00 ppm (s, N-O-CH$_3$), 4.2 and 4.3 ppm (AB, $J = 6$ Hz, 3'-CH$_2$-O-CH$_3$, partially obscured

<table>
<thead>
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<th>Compound</th>
<th>0.6 M phosphate buffer, pH 7.4</th>
<th>Intestinal juice, pH 7.4</th>
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<td>$K_{\text{DEG}}$ (h$^{-1}$ 10$^{-1}$)</td>
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<td>4.3</td>
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</tr>
<tr>
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</tr>
<tr>
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</tr>
<tr>
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by the solvent signal), and 5.25 and 5.81 ppm (AB, J = 4.5 Hz; 6-CH and 7-CH). Besides these, the typical signals of the Δ2 isomer could be seen at approximately 4.9 ppm (s, 4-CH, largely obscured by the HOD signal), 5.42 and 5.61 ppm (AB, J = 4.5 Hz, 6-CH and 7-CH), 6.39 ppm (s, 2-CH). The ratio of the Δ3 isomer to the Δ2 isomer was approximately 8:1.

DISCUSSION

Hydrolysis of cephalosporin esters is a well-studied process. The C-4 ester group of Δ3 esters is rather resistant to hydrolysis, whereas the C-4 ester group of the Δ2 isomers is easily hydrolyzed. It is known that at a pH above 6, cleavage of the hydrolytically stable Δ3 prodrug ester occurs via isomerization to the hydrolytically unstable Δ2 ester. Isomerization is followed by rapid hydrolysis to the Δ2-cephalosporin (5, 7, 9). It is generally assumed that this degradation pathway is active in the intestine (2, 4). Whether or not active Δ3-cephalosporin will reach the blood following oral administration of the prodrug is believed to depend on the relative rate of the Δ3 to Δ2 isomerization versus the rate of cleavage of the C-4 ester group (9). Thus, the bioavailability of cephalosporin prodrug esters is seen simply as a function of the kinetics of the Δ3 to Δ2 isomerization whereby a high isomerization rate means low bioavailability. This model has actually been used for optimization of oral cephalosporin prodrug esters (4, 5, 8, 9).

Enzymatic hydrolysis of prodrug esters was not considered a critical factor influencing the bioavailability of the prodrug esters, although it is responsible for the release of active Δ3-cephalosporin in intestinal tissue once the prodrug esters are absorbed. Indeed, the hydrolytic activity of homogenates of intestinal tissue was high enough for prodrug esters to be completely hydrolyzed within minutes (4). In contrast, the content of the intestinal lumen of mice was shown to contain only about 1% of the hydrolytic activity of the intestinal tissue. This ester-cleaving activity was considered the source of fecal excretion of Δ3 acids, but it was not considered a critical factor for the bioavailability of prodrug esters (4).

Our studies have shown that the current theory has grossly overestimated the importance of the isomerization mechanism in the absorption process of oral cephalosporin prodrug esters. With all the compounds studied (cefetamet pivoxil, cefuroxime axetil, and cefpodoxime proxetil), rapid hydrolytic cleavage to the biologically active Δ3-cephalosporin was observed in intestinal juice without the concomitant formation of significant amounts of the Δ2 isomers. Spontaneous base-catalyzed isomerization to the Δ2 ester was significantly slower than hydrolytic cleavage to the Δ3 acid. Thus, the isomerization process is not efficient enough to compete with enzymatic ester cleavage. Therefore, the incomplete bioavailability of the prodrug esters is simply a consequence of the efficient enzymatic hydrolysis and not the result of the Δ3 to Δ2 isomerization and spontaneous hydrolysis of Δ2 esters.

A further relevant observation is the high stereoselectivity of the enzymatic cleavage reaction. With cefuroxime axetil and cefpodoxime proxetil, both of which are diastereoisomer mixtures, one of the diastereoisomers was hydrolyzed faster than the other one by factors of 2.5 and 5.5, respectively (Table 1). The presence of a cefuroxime axetil esterase in the intestinal tissue of dogs, rats, and humans has previously been demonstrated to be stereoselective. It was considered to contribute to the incomplete bioavailability of cefuroxime axetil (1, 6).

In view of the great difference in the hydrolytic rates of the diastereoisomers seen in our study with cefuroxime axetil and cefpodoxime proxetil, it can be concluded that the observed stereoselectivity of the intestinal enzyme activity is of general importance for the prodrug ester concept. By using the most stable diastereoisomer of the prodrug ester instead of a mixture, higher bioavailabilities can be achieved for these compounds. Furthermore, elimination of the more rapidly hydrolyzed isomer should reduce the amount of biologically active cephalosporin formed in the gut, leading to improved intestinal tolerability.

REFERENCES