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Macromolecular prodrugs. VI. Kinetic study of poly[α,β-(*N*-2-hydroxyethyl-DL-aspartamide)]-ketoprofen hydrolysis

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Abstract

The kinetics of hydrolysis of ketoprofen from poly[α,β-(*N*-2-hydroxyethyl-DL-aspartamide)]-ketoprofen conjugate (PHEA-Ket) in aqueous buffer solutions in the pH range 1.0–10.6 at 37°C was studied. The hydrolysis of PHEA-Ket conjugate followed the pseudofirst-order kinetic. The following rate constants were obtained: $k = 1.542 \times 10^{-2} h^{-1}$ at pH = 1.0, $k = 6.260 \times 10^{-3} h^{-1}$ at pH = 9.2, and $k = 0.155 h^{-1}$ at pH = 10.6. At pH = 5.0 and 7.0 no significant hydrolysis was observed. The PHEA-Ket was also stable after exposure to several enzymatic systems in neutral medium. Determination of ketoprofen was performed by high performance liquid chromatography using diclofenac as the internal standard.

Keywords: Polymeric prodrug; Ketoprofen; Poly[α,β-(*N*-2-hydroxyethyl-DL-aspartamide)]; Poly[α,β-(*N*-2-hydroxyethyl-DL-aspartamide)]-ketoprofen conjugate; Hydrolysis; Enzyme; High performance liquid chromatography

1. Introduction

Ketoprofen, a potent analgesic and non-steroidal anti-inflammatory drug (NSAID), has been widely used in modern therapy. Its main disadvantage is the relatively short plasma half-life (1.5–4 h) and irritation of gastro-enteric mucosa (Cuthbert, 1974). Prolonged therapy with ketoprofen may cause gastrointestinal ulceration and hemorrhage. Since it is known that esterification of acidic anti-inflammatory drugs suppresses their gastrotoxicity (Whitehouse and Rainsford, 1980) numerous esters of ketoprofen have been synthesized and tested for their analgesic/anti-inflammatory activity and gastrointestinal toxicity (see for example: Ferruti et al., 1983; Komoto et al., 1986; Bundgaard and Nielsen, 1987; Metz et al., 1990a,b; Larsen and Jensen, 1991; Larsen et al., 1991; Schwenker and Stiefvater, 1991). One of ketoprofen ester derivatives is poly[α,β-(*N*-2-hydroxyethyl-DL-aspartamide)]-ketoprofen conjugate (PHEA-Ket), a polymeric prodrug in which

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ketoprofen is bond to the macromolecular backbone by ester linkages (Giammona et al., 1991; Zorc et al., 1993). The hydrolysis of PHEA-Ket in alkaline medium (pH = 11.0) has been previously described (Zorc et al., 1993). Giammona et al. (1991) described the release of ketoprofen from the same conjugate in simulated gastric juice. In this paper we wanted to complete the data of chemical hydrolysis PHEA-Ket conjugate in the wide pH range and in neutral medium in the presence of different enzymes. The present study was undertaken as a part of our evaluation of the potential utility of PHEA prodrugs as systems for controlled release of drugs.

2. Materials and methods

2.1. Materials

Ultraviolet spectral measurements were performed on a Pye Unicam SP-100 spectrophotometer. The following high performance liquid chromatography (HPLC) system was employed: a constant-flow pump, Model 6000 A, a wavelength detector at 254 nm, Model 440, an injector valve with a 20-μm loop, Model U6K (all Waters Assoc., Milford), and an integrator LKB 2220, LKB-Produkter AB (Pharmacia LKB, Uppsala). The column, 250 × 4.6 mm, was packed with Supelcosil LC-18-DB (3 μm particles) (Supelco, Bellefonte) and was equipped with a small precolumn containing Lichrosorb RP-18 particles ($\approx$ 10 μm) (Waters Assoc.). The eluent consisted of acetonitrile and 0.01 mol l^{-1} phosphate buffer (30:70 v/v) and the flow was 1.6 ml min^{-1}. Prior to the chromatography the samples were filtered, if necessary, over the SEP-PAK™ C18 (Waters). Reversed phase thin layer chromatography was performed on RP18 sheets (Merck, Darmstadt).

Ketoprofen was supplied from Lek (Ljubljana) and diclofenac from Pliva (Zagreb). Acetonitrile used in the mobile phases was of HPLC grade (Aldrich, Milwaukee). All buffer substances were of analytical grade. The following buffer solutions were used: KCl/HCl buffer, pH = 1.0; phosphate buffer (KH_2PO_4/Na_2HPO_4), pH = 5.0 and 7.0; carbonate buffer ($Na_2CO_3/NaHCO_3$), pH = 9.2 and 10.6, and 0.1 mol l^{-1} Tris—HCl buffer, pH = 7.0.

Lipase from pig pancreas (EC 3.1.1.3), lipase from *Mucor javanicus* (EC 3.1.1.3) and esterase immobilized on Eupergit C, from pig liver (EC 3.1.1.1) were purchased from Fluka (Buchs). Lipase from *Candida cylindracea*, Type VII + lactose (EC 3.1.1.3) was bought from Sigma (St. Louis) and chymotrypsin from bovine pancreas (EC 3.4.21.1) from Boehringer (Mannheim). Yeast was manufactured in Pliva.

2.2. Chemistry

2.2.1. *Poly[α,β-(N-2-hydroxyethyl-DL-aspartamide)]-ketoprofen (PHEA-Ket)*

The outline of the procedure for the preparation of PHEA-Ket was the same as the method previously described (Zorc et al., 1993). The ketoprofen benzotriazolide and PHEA's aspartamide units were in molar ratio 1:1. The drug load in the product was 45.6%.

2.2.2. *Release of ketoprofen from PHEA-Ket conjugate. Chemical hydrolysis*

A methanolic solution of diclofenac[2] (final concentration $\gamma = 60$ μg ml^{-1}) was added to a solution of PHEA-Ket conjugate ($\gamma = 180–300$ μg ml^{-1}) in an appropriate buffer solution. The solution was thermostated at 37 ± 0.1°C. The amount of the released ketoprofen was determined in 10-μl aliquots at appropriate time intervals by HPLC. Each determination was carried out in triplicate. Rate constants were computed using a non-linear least-square fitting program.

2.2.3. *Release of ketoprofen from PHEA-Ket conjugate. Enzymatic hydrolysis*

(i) One millilitre of the enzyme suspension containing 17.2 mg of the enzyme and 0.5 ml of methanolic solution of diclofenac (final concentration $\gamma = 60$ μg ml^{-1}) were added to a solution of 25.4 mg PHEA-Ket conjugate in 3.5 ml 0.1 mol l^{-1} Tris—HCl buffer solution. The reaction mix-

[2] Except in experiments at pH = 1.0.

ture was thermostated for 5 days at 37 ± 0.1°C. The amount of the released ketoprofen was determined in 10-μl aliquots (previously filtered over the cartridge, if necessary) at appropriate time intervals by HPLC. Each determination was carried out in triplicate.

(ii) Five grams of backing yeast and 6.0 g of sacharose in 20 ml phosphate buffer pH = 7.0 were incubated at room temperature. After 30 min, the mixture was filtered. PHEA-Ket conjugate (33.6 mg) was dissolved in 4 ml of the filtrate. The reaction mixture was thermostated for 5 days at 37 ± 0.1°C. The amount of the released ketoprofen was determined in 10-μl samples (previously filtered over the cartridge, if necessary) at appropriate time intervals by HPLC. Each determination was carried out in triplicate.

3. Results and discussion

The preparation of PHEA-Ket has been described in our previous paper (Zorc et al., 1993). In short, ketoprofen was first transformed in the reactive benzotriazolide which readily reacted with poly[α,β-(*N*-2-hydroxyethyl-DL-aspartamide)] giving the final product PHEA-Ket conjugate. The drug loading in the prepared polymer-drug conjugate was 45.6%. That means that almost every second hydroxyl group was esterificated with ketoprofen and the remaining hydroxyl groups were still free. Such ratio of ester and hydroxyl groups assures hydrosolubility of the PHEA-Ket conjugate in contrast to ketoprofen which is not water-soluble. The structure of PHEA-Ket conjugate is shown in Fig. 1.

We have studied the kinetics of ketoprofen release from PHEA-Ket conjugate in aqueous buffer solutions in the pH range 1.0–10.6 at 37 ± 0.1°C. The results are presented in Figs. 2–4.

The data fit pseudofirst-order kinetics and the following rate constants were obtained: $k = 1.542 \times 10^{-2}$ h^{-1} (0.90%), $t_{1/2} = 126.72$ h at pH = 1.0; $k = 6.260 \times 10^{-3}$ h^{-1} (2.48%), $t_{1/2} =$ 110.70 h at pH = 9.2; $k = 0.155$ h^{-1} (2.51%), $t_{1/2} = 4.47$ h at pH = 10.6. The results were average of three runs. The numbers in fences are relative standard deviations of the mean values. In all experiments $r = 0.945$–0.999. In weak acidic (pH = 5.0) and neutral medium no significant hydrolysis was observed. Less than 1% of ketoprofen was released in a period of 5 days. The results show that ketoprofen can be released from PHEA-Ket conjugate after chemical hydrolysis of ester bonds both in acid and basic conditions. The rate of hydrolysis is highly pH dependent and it is the highest in basic solutions.

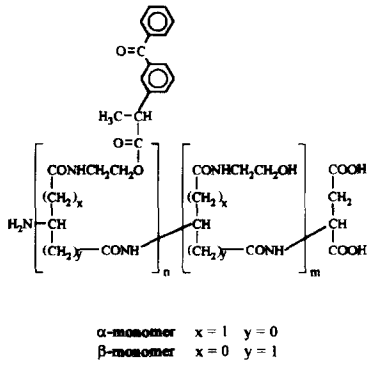

Fig. 1. The structure of PHEA-Ket conjugate.

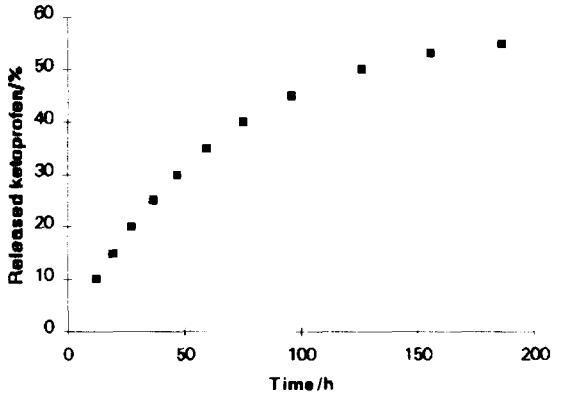

Fig. 2. Hydrolysis of PHEA-Ket conjugate in HCl/KCl buffer (pH = 1.0; t = 37 ± 0.1°C; $k = 1.542 \times 10^{-2}$ h^{-1}).

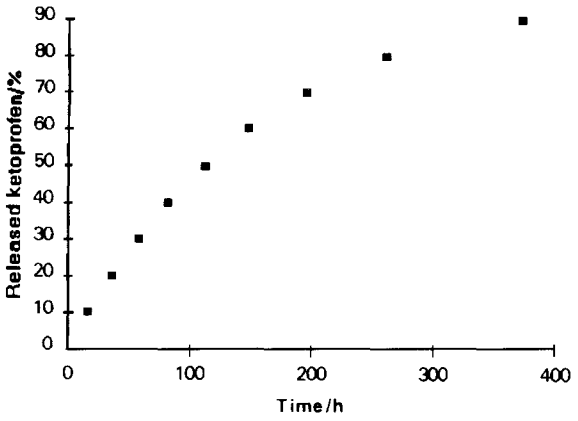

Fig. 3. Hydrolysis of PHEA-Ket conjugate in carbonate buffer (pH = 9.2; t = 37 ± 0.1°C; $k = 6.260 \times 10^{-3}$ h^{-1}).

Table 1
Enzymes used for enzymatic hydrolysis of PHEA-Ket conjugate

Enzyme	EC number	Producer
Lipase from pig pancreas	3.1.1.3	Fluka
Lipase from *Mucor javanicus*	3.1.1.3	Fluka
Lipase from *Candida cylindracea*, Type VII + lactose	3.1.1.3	Sigma
Esterase from pig liver immobilized on Eupergit C	3.1.1.1	Fluka
Chymotrypsin from bovine pancreas	3.4.21.1	Boehringer

Since it is well known that ester bonds, besides chemical reaction, readily undergo enzymatic cleavage, hydrolysis of PHEA-Ket conjugate was carried out with several enzymes. The reactions were run in 0.1 mol l^{-1} Tris—HCl pH = 7.0 buffer at 37 ± 0.1°C following the procedure described for ketoprofen ethyl ester lipase hydrolysis (Hernáiz et al., 1994). The enzymes employed are listed in Table 1. All the experiments were performed with the same amount of enzyme and the same concentration of the substrate. The stability of PHEA-Ket conjugate to yeast enzymes was also tested. The reaction was carried out in phosphate buffer pH = 7.0 at the same temperature. After 5 days, in all experiments less than 1% of ketoprofen was released. That means that PHEA-Ket conjugate was stable after exposure to the used enzymatic systems. These results could be expected since it was reported that ketoprofen ethyl ester and other esters of (*R,S*)-2-aryl propionic acid hydrolyses were only partial (20–40%) due to the steric restrictions in the interaction with the active site of lipase (Gu et al., 1986a,b, Hernáiz et al., 1994). Having ketoprofen bound to the polymeric backbone the ester bonds are even more sterically hindered. That makes enzyme-substrate interaction almost impossible.

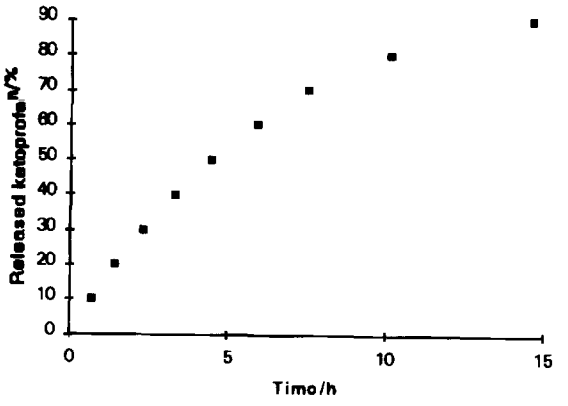

Fig. 4. Hydrolysis of PHEA-Ket conjugate in carbonate buffer (pH = 10.6; t = 37 ± 0.1°C; $k = 0.155$ h^{-1}).

HPLC was chosen for ketoprofen determination. This method is commonly used for ketoprofen analysis since it is precise, sensitive and selective (Bannier et al., 1978; Farinotti and Mahuzier, 1979; Jefferies et al., 1979; Upton et al., 1980). In order to chose the optimal mobile phase and internal standard the reverse phase thin layer chromatography was employed. Between several aryl propionic acid derivatives, diclofenac was chosen as the internal standard. The best resolution between ketoprofen and diclofenac was

achieved using acetonitrile and 0.01 mol l^{-1} phosphate buffer (30:70 v/v) as mobile phase (Fig. 5). Since diclofenac is not stable in solutions at low pH, concentration of the released ketoprofen at pH = 1.0 was determined from a calibration curve. The use of the appropriate precolumn enabled direct HPLC sample analysis. The sample pretreatment (filtration over the cartridge) was necessary only in some cases (see *Materials and methods*).

PHEA-Ket conjugate is a hydrosoluble ketoprofen derivative. The kinetic evidence obtained show that the hydrolysis of ketoprofen from the conjugate proceeds by both acid- and base-catalyzed reaction pathways and that the conjugate is stable in neutral solutions with or without enzymes. It can be considered as a typical example of 'drug reservoir' that might sustain a drug to be available in the body for longer time periods after chemical hydrolysis in gastro-intestinal system.

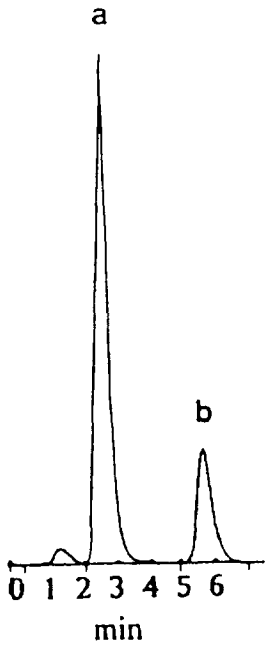

Fig. 5. Chromatogram of ketoprofen (a) and diclofenac (b). Column: 250 × 4.6 mm packed with 3 μm Supelcosil particles. Mobile phase: acetonitrile:0.1 mol l^{-1} phosphate buffer (30:70 v/v). Flow-rate: 1.6 ml min^{-1}.

Acknowledgements

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