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COMMUNICATION

Evaluation of Hydroxyethyl Esters of Mefenamic Acid and Diclofenac as Prodrugs

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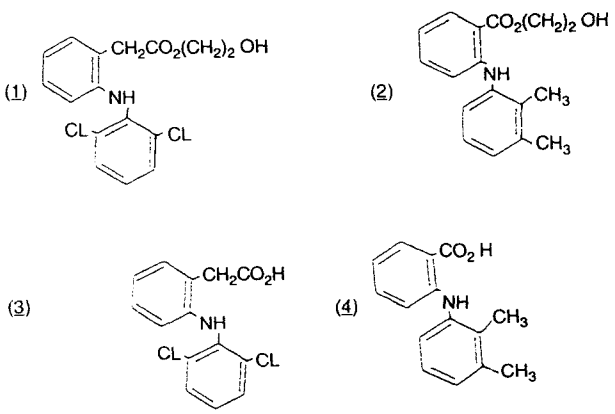
ABSTRACT

Hydroxyethyl esters of diclofenac and mefenamic acid were prepared with the aim of obtaining enzymatically labile prodrugs, and their stability was evaluated in aqueous buffer solutions of pH 7.4, 1 N HCl, and also in human plasma. The hydrolytic degradation of diclofenac ester in aqueous buffer solutions was slow, as shown by $t_{1/2}$ values greater than 22 hr, while rapid enzymatic hydrolysis occurred in the plasma ($t_{1/2}$ = 1.12 hr). However, the mefenamic acid ester showed a relatively higher stability in buffer solutions ($t_{1/2}$ greater than 38 hr at pH 10) as well as in the plasma ($t_{1/2}$ = 7.28 hr) compared with the diclofenac ester. Therefore, the mefenamic acid ester would not be considered suitable as a prodrug.

INTRODUCTION

Diclofenac (3) and mefenamic acid (4) are nonsteroidal anti-inflammatory drugs (NSAIDs) widely used to relieve the pain and stiffness associated with a variety of inflammatory disease including active inflammatory arthritis (1–5). As with other NSAIDs, these drugs are liable to cause gastrointestinal side effects such as gastric irritation, abdominal pain, erosive lesions of the gastroduodenal mucosa, and bleeding (6–10). Prodrug formation through masking of the carboxylate moiety of these drugs has been considered as an approach to minimize such side effects as well as to improve their delivery characteristics (11–13). Among the many possible prodrugs, bioreversible esters have received considerable attention due to the presence of enzymes in the living systems capable of hydrolyzing them. Hydroxyethyl esters of some penicillins showed bioconversion half-lives in the plasma much lower compared to that in aqueous solutions of pH 7.4 (14).

The present study aimed at evaluating the kinetics of degradation under different conditions of the



hydroxyethyl esters of diclofenac (1) and mefenamic acid (2), and their feasibility as prodrugs. These esters not only mask the carboxylate moiety but can also be used as intermediates for further chemical modifications, through the free hydroxyl group, to form acyloxy diester prodrugs.

MATERIALS AND METHODS

Apparatus

The hydroxyethyl esters of diclofenac and mefenamic acid (1 and 2) were characterized by a variety of analytical techniques. Nuclear magnetic resonance, ^{1}H-NMR, spectra were recorded in $CDCl_3$ solution with TMS as internal standard at 300 MHz by means of a Brucker spectrometer. Mass spectra were obtained using 707-E VG spectrometer. Infrared (IR) spectral data were recorded using a Shimadzu IR-435 instrument and KBr disk. Melting points were determined using a Gallenkamp Electrothermal melting point apparatus and were uncorrected. High-performance liquid chromatography (HPLC) was carried out using a system consisting of a Beckman 114M HPLC pump, a variable wavelength UV (Jasco-875 UV) detector and a 20-µl Rheodyne loop injection valve. The column used was an ODS-2 (phase separator).

Chemicals

Samples of diclofenac sodium, mefenamic acid, and benzoylmetronidazole were obtained from Al Hikma Pharmaceutical Company (Jordan). All solvents used were HPLC grade and other chemicals were analytical or reagent grade.

Preparation of Hydroxyethyl Ester of Diclofenac (1)

Diclofenac sodium (3 g, 0.0094 mol) suspended in 10 ml of dry DMF was reacted with bromoethanol (1.3 g, 0.01 mol) by stirring under dry conditions for 24 hr at room temperature. Ethylacetate (30 ml) was added, and the solid was filtered off. The solution was washed with (3 × 20 ml) of aqueous solution saturated with NaCl, dried over anhydrous $MgSO_4$, and filtered. The solvent was evaporated in vacuo to yield a colorless oil which was solidified upon cooling. Recrystallization from methanol gave 2 g (63%) of (1): mp 70–71°C; IR (KBr) 1715 cm^{-1} (ester carbonyl) ^{1}H NMR ($CDCl_3$) δ 2.2 (br, 1H, OH), 3.8 (m, 4H, CH_2 and CH_2CO), 4.2 (t, 2H, CH_2), 6.5 (d, 1H, ArH, j = 8), 6.8 (s, 1H, NH), 6.9–7.2 (m, 4H, ArH), 7.3 (d, 2H, ArH, j = 8); MS m/z 340 (MH^+).

Preparation of Hydroxyethyl Ester of Mefenamic Acid (2)

Bromoethanol (1.5 g, 0.012 mol) was added dropwise to a stirred suspension of sodium mefenamate (3.0 g, 0.011 mol); and KI (0.18 g, 0.0012 mol) in dry DMF (20 ml). After the addition was completed, the reaction mixture was stirred for 8 hr at 70°C. The reaction mixture was treated with EtOAc (40 ml) and the ppt formed was filtered off. The filtrate was washed with 5% solution of sodium thiosulfate (3 × 20 ml). Evaporation of the organic layer gave an oily liquid which was poured into ice water to yield a solid ppt. The solid ppt was taken by filtration and purified by recrystallization from aqueous EtOH to yield 2.5 g (77%) of (2): mp 80°–81°C; IR (KBr) 1680 cm^{-1} (ester carbonyl) ^{1}H NMR, ($CDCl_3$), δ 2.1 (s,3H, $-CH_3$), 2.2 (s, 3H, CH_3), 3.9 (t, 2H, CH_2), 4.4 (t, 2H, CH_2), 6.6–8 (m, 7H, aromatic), 9.2 (s, 1H, NH): MS m/z 286 (MH^+).

Chromatographic Conditions

Diclofenac and its ester were analyzed on an ODS-2 column and a mobile phase consisting of acetonitrile:water:acetic acid (172:125:1), adjusted to pH 4.5 with acetic acid. The mobile phase for mefenamic acid and its ester was acetonitrile:water (50:50) adjusted to pH 6 with acetic acid. The flow rate of the mobile phase was 1 ml/min and the detector wavelength was set at 280 nm and at a sensitivity of 0.01 AUFS.

Calibration Curve Data

Aqueous Solution

From a stock solution of the diclofenac ester (1) in methanol (25 mg/25 ml), dilutions were made with methanol so as to obtain 20, 40, 60, 80, and 100 μg/ml. To each of these solutions were added 250 μl of the internal standard (benzoylmetronidazole, 240 μg/ml); and after vortex mixing, an appropriate volume of aliquot was injected on to the HPLC column. Peak height ratios (prodrug/internal standard) were measured and plotted against concentration of the prodrug. Triplicate analysis of each concentration was carried out and the average peak height ratios were used to construct the calibration curve by least square regression analysis.

Calibration curve data for diclofenac were obtained as described above, with concentrations of diclofenac solutions ranging from 20 to 100 μg/ml in methanol. Calibration curve data for mefenamic acid and its ester (2) were obtained in a similar way, with the same internal standard.

Human Plasma

Standard solutions of either diclofenac or its ester were used to spike blank human plasma (100 μl) to provide calibration standards of 20, 40, 60, 80, and 100 μg/ml. To each of these samples were added 100 μl of the internal standard solution and 200 μl of acetonitrile. After vortex mixing, these samples were centrifuged at 11,500 rpm for 5 min, and an appropriate volume of the clear supernatant was injected onto the HPLC column. Calibration curve data for mefenamic acid and its ester in plasma were also obtained in a similar way.

Kinetic Measurements

The decomposition of the hydroxyethyl esters of diclofenac and mefenamic acid was studied in aqueous buffer solutions of 1 N HCl and pH 7.4, and also in human plasma. An accurately weighed amount of each ester (5 mg) was added to 100 ml of the buffer solutions (or 3 mg to 10 ml of human plasma), sonicated for 2 min, filtered through a Millipore filter, and kept in a shaking water bath at 37° ± 2°C. Samples (0.5 ml) were withdrawn at hourly intervals for 6 hr in the case of the aqueous buffer solutions and at 15-min intervals for 300 min in the case of plasma. A reversed-phase HPLC method capable of separating the ester prodrug from the drug was employed to follow the degradation profile. Internal standard, 250 μl, was added to the buffer solutions and vortex mixed for 2 min; 20 μl of the aliquot were analyzed by HPLC. In the case of plasma, 100 μl were withdrawn at the indicated time intervals, and vortex mixed with 100 μl of internal standard and 200 μl of acetonitrile. The mixture was centrifuged at 11,500 rpm for 1 min, and 20 μl of the clear supernatant was analyzed by HPLC.

The first-order rate constants for degradation of the prodrugs were determined from the slope of the linear plots of log of the amount of ester remaining versus time.

RESULTS AND DISCUSSION

The degradation profiles of the hydroxyethyl esters of diclofenac (1) and mefenamic acid (2) were followed by a specific and sensitive HPLC procedure. A representative chromatogram of diclofenac (3) and its ester (1), along with an internal standard (IS) is shown in Fig. 1(a); and that of mefenamic acid (4), its ester (2), and

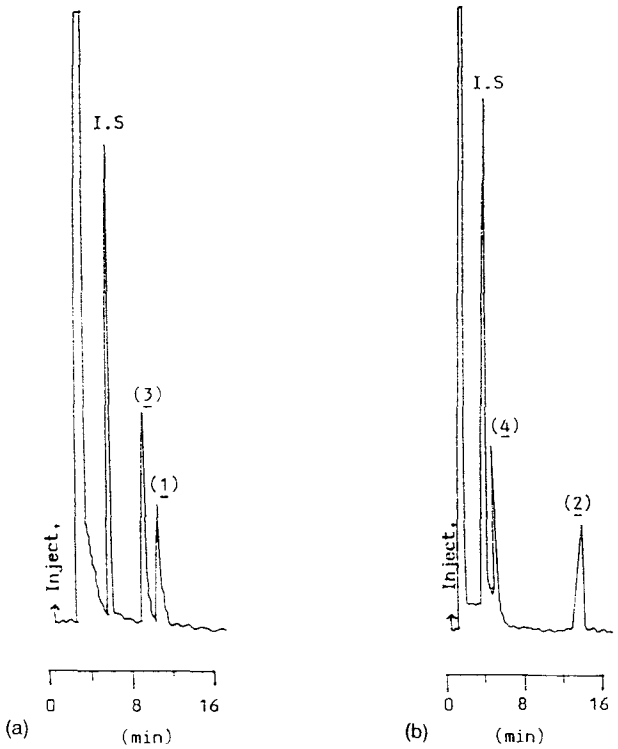


Figure 1. Representative chromatograms of: (a) diclofenac (3), hydroxyethyl ester of diclofenac (1), and the internal standard (IS); (b) mefenamic acid (4), hydroxyethyl ester of mefenamic acid (2), and the internal standard (IS).

an internal standard (IS) in Fig. 1(b). The retention times of diclofenac, its ester, and internal standard were 9, 11, and 6 min, respectively; and for mefenamic acid, its ester, and internal standard were 5, 14, and 4 min, respectively. No additional peaks were observed in the chromatogram throughout the study period.

Figure 2 shows a plot of the logarithm of percent remaining of the diclofenac ester (1) versus time in aqueous buffer solutions of pH 7.4, 1 N HCl and in human plasma. The correlation coefficient, r, for these plots was always higher than that of linear plots of percent remaining versus time, and therefore the degradation was assumed to follow a pseudo-first-order process. Table 1 shows the first-order rate constant and the corresponding half-life of degradation of the esters in three different media. The kinetic data (k, $t_{1/2}$) in the table indicate that the prodrug of diclofenac relatively resists hydrolysis in 1 N HCl ($t_{1/2}$ = 22.07 ± 1.67 hr) and at pH 7.4 ($t_{1/2}$ = 36.66 ± 8.79 hr), while rapid and complete hydrolysis occurs in plasma ($t_{1/2}$ = 1.12 ± 0.24 hr). The prodrug of mefenamic acid was more resistant to hydrolysis in aqueous solutions of different pHs ($t_{1/2}$ of 347.59 ± 12.9, 333.17 ± 7.51, and 36.86 ± 0.83 hr at 1 N HCl, pH 7.4 and pH 10, respectively), while in plasma the hydrolysis $t_{1/2}$ was 7.11 ± 0.23 hr. This relatively slow enzymatic hydrolysis of mefenamic acid ester to the parent drug is not considered a desirable property of prodrug, and it therefore does not seem to warrant further kinetic studies. However, the possibility that the ester has intrinsic activity remains to be checked. The diclofenac ester, on the other hand, appears to survive the GIT conditions, at least for the normal transit period. Even if complete absorption does occur with minimal undesirable effects, there may be a delay in achieving therapeutic concentration and therefore response, since the generation of the parent drug in the plasma is not instantaneous. In vivo absorption study would be necessary to assess the practical utility of the presumed prodrug of diclofenac.

The significant difference in the stability profiles of the esters (1) and (2) can be explained on the basis of stearic and electronic differences in the two compounds. However, the polarity of the carbonyl group seems to be the major factor compared to the stearic effect. The

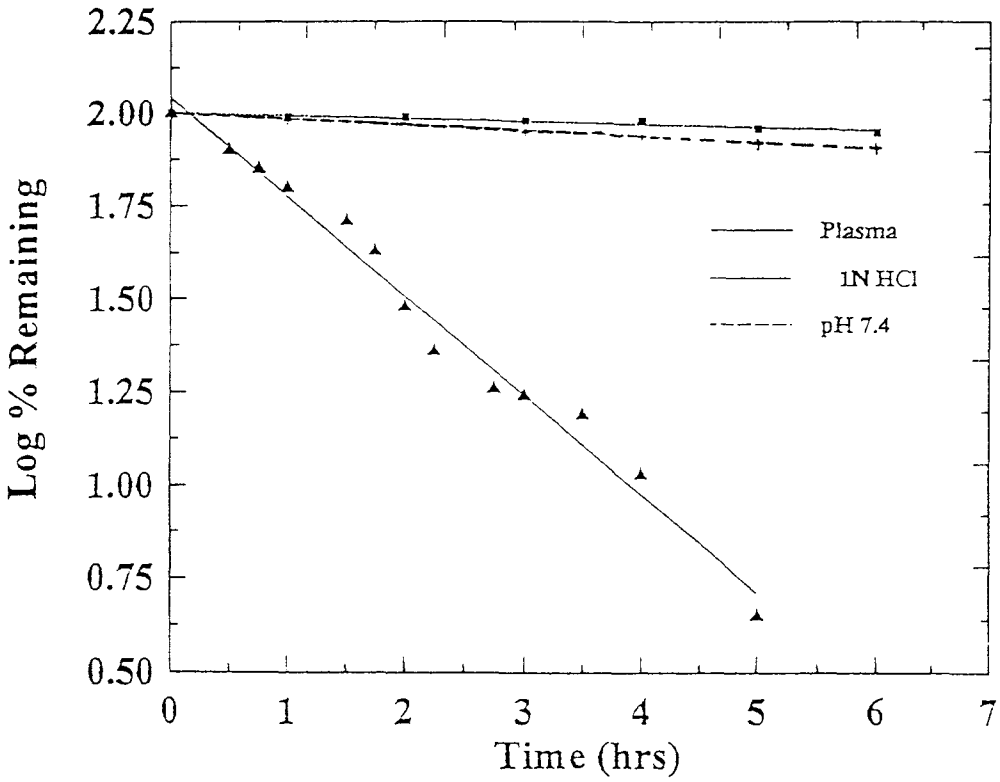


Figure 2. Plot of log percent remaining versus time for hydroxyethyl ester of diclofenac (1) at different conditions.

Table 1

Kinetic Parameters for Degradation of Hydroxyethyl Esters of Diclofenac (1) and Mefenamic Acid (2)

Compounds	Conditions	K (hr^{-1})	$t_{1/2}$ (hr)
1	1 N HCl	$0.0314 \pm 2.4 \times 10^{-3}$	22.07 ± 1.67
	pH 7.4	$0.0189 \pm 4.3 \times 10^{-3}$	36.66 ± 8.79
	pH 10	NT[a]	NT[a]
	Plasma	0.634 ± 0.125	1.123 ± 0.247
2	1 N HCl	$0.00185 \pm 5 \times 10^{-5}$	374.59 ± 12.9
	pH 7.4	$0.00208 \pm 1.89 \times 10^{-4}$	333.17 ± 7.51
	pH 10	$0.0188 \pm 1.03 \times 10^{-3}$	36.86 ± 0.83
	Plasma	$0.0974 \pm 8.33 \times 10^{-3}$	7.11 ± 0.236

[a]Not tested.

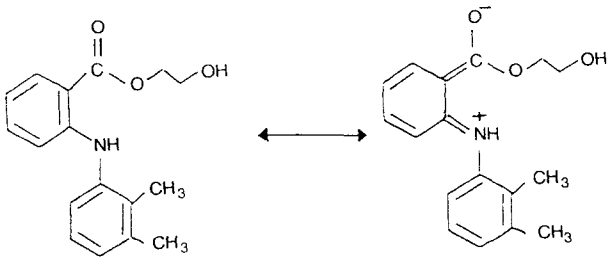


Figure 3. Resonance structrue of hydroxyethyl ester of mefenamic acid (2).

nitrogen atom in mefenamic acid can decrease the polarity of carbonyl by resonance effect (Fig. 3); this cannot take place in diclofenac ester due to the presence of a methylene group that separates the carbonyl from the aromatic ring.

CONCLUSION

The stability of hydroxyethyl esters of carboxylic acids depends on the aroyl moiety of the compound. The stability of mefenamic acid ester in plasma, reflected by its long $t_{1/2}$, makes this ester unsuitable for examination as a prodrug. However, it should not be assumed that it has no intrinsic anti-inflammatory activity. Concerning the diclofenac ester, it is obvious that the compound can survive GIT conditions and if absorbed would deliver the parent drug. The moderate $t_{1/2}$ of diclofenac ester may reflect its ability to deliver the parent drug in a sustained-release fashion. Further evaluation including in vivo study is required.

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