

Characterization of Mefenamic Acid-Guaiacol Ester: Stability and Transport across Caco-2 Cell Monolayers

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Purpose. Prodrug of non-steroidal anti-inflammatory drugs (NSAIDs) or NSAIDs linked with guaiacol have been reported to suppress gastrointestinal (GI) toxicity or induce GI protective effect. In this study, mefenamic-guaiacol ester was synthesized and its physicochemical properties, stability, and transport across Caco-2 monolayers were investigated.

Methods. Synthesis of the ester was carried out using mefenamic acid, guaiacol, N, N'-dimethylaminopyridine, and N, N'-dicyclohexylcarbodiimide. The hydrolysis of the ester was investigated in aqueous buffer solutions pH 1-12 as well as in Caco-2 homogenate, human plasma, and porcine liver esterase. Caco-2 cell monolayers were utilized for transport studies. Due to the high lipophilicity of the ester with a calculated logP of 6.15, bovine serum albumin (BSA, 4%) was included in the receiver compartment to obtain a good *in vitro-in vivo* correlation. Permeation of the ester was assessed with or without the exposure of cells to PMSF, an inhibitor of esterase.

Results. The ester was stable at a wide pH range from 1-10. However, it was hydrolyzed by enzymes from porcine liver esterase and Caco-2 homogenate. With the PMSF exposure on the apical (AP) side and in the presence of 4% BSA on the basolateral (BL) side, the transported amount of the ester from AP-to-BL direction was 14.63% after 3 hr with a lag time of 23 min. The Papp for the ester was $4.72 \times 10^{-6} \text{ cm s}^{-1}$.

Conclusion. The results from hydrolysis studies indicate that this ester is a prodrug. The Papp value suggests the moderate absorption characteristic of the compound. The accumulation of this highly lipophilic ester in Caco-2 cells is reduced in the presence of BSA.

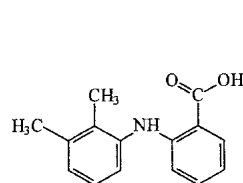
KEY WORDS: mefenamic acid; guaiacol; prodrug; caco-2 cells; hydrolysis; transport.

INTRODUCTION

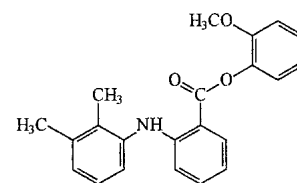
Non-steroidal anti-inflammatory drugs (NSAIDs) are widely used for the treatment of inflammatory diseases. The mechanism of action of NSAIDs involves the inhibition of cyclooxygenase (COX), the rate-limiting enzyme responsible

for the conversion of arachidonic acid into prostaglandins. NSAIDs are frequently associated with high incidences of gastroenteropathy, ranging from mild gastric upset to life-threatening ulceration and hemorrhage (1). GI injury produced by NSAIDs is generally believed to be a result of two different mechanisms. The first mechanism involves a local irritant produced by acidic group of the NSAIDs. The second effect is attributed to blockage of prostaglandin biosynthesis in the GI tract, which inhibits its cytoprotective effect.

Prodrugs that temporarily mask the acidic group of NSAIDs have been reported to suppress GI toxicity due to the local action mechanism (2). However, esterified NSAIDs and some related molecules have proven to retain anti-inflammatory activities. It has been reported that guaiacol is able to inhibit prostaglandin biosynthesis like the classic NSAID but it does not induce gastric damage (3). The guaiacol-esterified ibuprofen (methoxybutropate) has been available for sometimes as analgesic (4), and its water-soluble formulations have recently been developed (5-6). Gastric damage caused by this drug has been reported to be very low (3). Amtolmetin guacyl is another analgesic derived from an amidation of tolmetin by glycine, which is in turn linked to guaiacol by an ester bond (7). Amtolmetin guacyl has been reported to exhibit a good gastric tolerability and produce a gastroprotective effect (7-8). These studies provide the basis for combining guaiacol with other molecules of NSAIDs to obtain an analgesic with low GI toxicity. In this study, mefenamic-guaiacol ester was synthesized and its physicochemical properties, stability, and transport across Caco-2 monolayers were investigated.



Mefenamic acid



Mefenamic acid-guaiacol ester

MATERIALS AND METHODS

Materials

Caco-2 cells were obtained from the American Tissue Culture Collection (Rockville, Maryland) at passage 18. All chemicals used for culturing the Caco-2 cells were obtained from Gibco BRL (Life Technologies, New York). Porcine liver esterase (#E-3128) and phenylmethyl sulfonylfluoride (PMSF) were obtained from Sigma (St. Louis, Missouri). Other reagents and solvents were purchased from common suppliers and were used as received.

Melting point was determined on a MEL-TEMP II capillary melting point apparatus and is uncorrected. Proton magnetic resonance spectra were recorded on a Bruker Spectrospin 300 NMR. Chemical shifts are reported in parts per million (ppm, δ units) and peak multiplicities are expressed as follows: s, singlet; d, doublet; m, multiplet. The IR spectra were recorded on a Perkin Elmer Model 1600 FT-IR spec-

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trometer. Mass spectrum was recorded on a Micromass Platform II mass spectrometer. Yield is of purified product and was not optimized. The purity of the ester was assessed by analytical HPLC as described below.

Synthesis of Mefenamic Acid - Guaiacol Ester

To a solution of mefenamic acid (3.0 g, 12.43 Mmol) in dry CH_2Cl_2 (150 ml) was added guaiacol (1.39 g, 11.19 Mmol), the mixture was cooled at 0°C , followed by the addition of N, N'-dimethylaminopyridine (0.06 g, 0.5 Mmol), and N, N'-dicyclohexylcarbodiimide (3.10 g, 15.08 Mmol). The reaction mixture was stirred at room temperature overnight. The precipitated N, N'-dicyclohexylurea was removed by filtration. The filtrate was concentrated in vacuo, and the residue was purified by column chromatography using CH_2Cl_2 :hexane (1:1) as the eluent. The product was recrystallized from CH_2Cl_2 /MeOH to afford 1.9 g (51%) of mefenamic-guaiacol ester as yellow crystals: >99% purity; mp $111.5\text{--}111.9^\circ\text{C}$; IR (KBr) $3337, 1693\text{ cm}^{-1}$; ^1H NMR (300 MHz, CDCl_3) δ 2.08 (s, 3H, ArCH_3), 2.23 (s, 3H, ArCH_3), 3.76 (s, 3H, ArOCH_3), 6.69 (m, 2H, aromatic), 7.25–6.90 (m, 8H, aromatic), 8.15 (d, $J = 8\text{ Hz}$, 1H, aromatic), 9.06 (s, 1H, NH, broad); MS: (IE, 70 eV) m/z 347 [M].

Analysis of Samples

For the detection and quantitation of mefenamic acid and its ester, a high-performance liquid chromatography (HPLC) system capable of separating mefenamic acid and guaiacol from the ester was used. The HPLC system (Waters, Milford, Massachusetts) consisted of Waters 600 Pump, Waters 717 plus Autosampler, Waters 486 Tunable Absorbance Detector, and Waters 746 Data Module. The analytical column was Rexchrom ODS, $5\mu\text{m}$, $15\text{ cm} \times 4.6\text{ mm}$, (Regis Technologies, IL, USA) equipped with a precolumn packed with Novapak RP-8, $5\mu\text{m}$, $3.9 \times 20\text{ mm}$ (Waters, Milford, Massachusetts). The mobile phase was a mixture of 60% acetonitrile and 40% phosphate buffer pH 3. The flow rate was 1.0 ml min^{-1} . The eluents were detected at 280 nm. The retention times were 6.7 and 19.8 min for mefenamic acid and the ester, respectively.

For determination in biologic media, samples were deproteinized by adding $500\mu\text{ l}$ of acetonitrile. After mixing and, in the case of determination of accumulated amount of the ester in Caco-2 cell monolayers, sonication for 5 min, the samples were centrifuged for 10 min at 12,000 rpm at 4°C . Aliquots of clear supernatant were then injected into the HPLC system and analyzed as described above.

Protein content was determined using a Coomassie[®] protein assay reagent (Pierce, Rockford, Illinois) according to the method of Bradford (9). BSA was used as a standard.

Chemical Stability

The hydrolysis of ester was carried out in aqueous buffer solutions, pH 1–12, at 37°C . The buffer systems used were HCl (pH 1–2), acetate (pH 3–5), phosphate (pH 6–8), and NaOH (10–12). The ionic strength of 0.04 was maintained for each buffer by adding an appropriate amount of KCl. To check for buffer catalysis, three different buffer concentrations were used (0.01, 0.02, and 0.05 M). The reaction was initiated by adding $75\mu\text{ l}$ of a stock solution of ester in ace-

tonitrile to 10 ml of buffer solutions, pre-equilibrated at 37°C , in screw-capped test tubes. The initial concentration was $2 \times 10^{-4}\text{ M}$. At appropriate times, the reaction samples were withdrawn and analyzed by HPLC as described above. Pseudo-first order rate constants (k) were calculated from the slopes of linear plots of the logarithm of residual ester concentration against time, and the corresponding half-life obtained from the equation: $t_{1/2} = 0.693/k$. Triplicate samples were analyzed, and the mean value of the rate constant was calculated.

Enzymatic Stability

The degradation of the ester was studied at 37°C in Caco-2 homogenate, human plasma, and porcine liver esterase preparations. Caco-2 homogenate was prepared according to the method of Augustijns *et al.* (10) with slight modifications. Briefly, Caco-2 cells grown in 75-cm^2 culture flask for 21–25 days were washed with ice-cold phosphate-buffered saline (PBS), pH 7.4. The cells were scraped off, homogenized in 4 ml ice-cold PBS using a glass-teflon polytron homogenizer (Thomas, Pennsylvania), and centrifuged at 12,000 rpm for 10 min at 4°C . The resultant supernatant was used as Caco-2 homogenate (protein content = 0.63 mg ml^{-1}). Human plasma was obtained from the Songklanakarin Hospital Blood Bank (Hat-Yai, Songkhla). For stability studies, human plasma was diluted to 80% (v/v) with phosphate buffer, pH 7.4 (protein content = 49.57 mg ml^{-1}). Porcine liver esterase was used at 0.06 unit ml^{-1} .

The ester was incubated at the concentration of $12\mu\text{M}$ (containing 0.6% acetonitrile), $8\mu\text{M}$ (containing 0.6% acetonitrile), and $50\mu\text{M}$ (containing 4% DMSO) in prewarmed 80% human plasma in phosphate buffer pH 7.4, porcine liver esterase and Caco-2 homogenate, respectively. The mixtures were kept in a water bath at 37°C , and at appropriate intervals, samples ($250\mu\text{ l}$) of the reaction solution were withdrawn and analyzed by HPLC as described above. Reactions were followed for at least two half-lives. Apparent half-lives for the disappearance of ester and pseudo-first order rate constants were calculated as previously described.

Solubility Determination

The solubility of the ester and mefenamic acid were determined at room temperature by adding an excess amount of ester to the solvent in screw-capped test tubes. The mixture was sonicated for 15 min and then rotated for 48–72 h to ensure equilibrium. Saturated solution of the ester was filtered through a $0.5\text{-}\mu\text{m}$ membrane filter (Millipore Co., Bedford, Massachusetts), and an aliquot of the filtrate was diluted with a mobile phase and analyzed by HPLC. Experiments were performed in triplicate.

Transport Studies

For transport studies, cells were seeded at a density of $60,000\text{ cells cm}^{-2}$ on Transwell[®] (Costar, USA) polycarbonate membrane (24 mm diameter, 4.7 cm^2 , $3.0\mu\text{m}$ pore size). Cells were grown at 37°C in an atmosphere of 5% CO_2 and 90% relative humidity. Cells were maintained in Dulbecco's Modified Eagles Medium (DMEM) containing 10% fetal calf

serum, 1% nonessential amino acids, 1% L-glutamine, 1% antibiotic-antimycotic mixture (10,000 U ml⁻¹ benzylpenicillin, 10,000 µg ml⁻¹ streptomycin, and 25 µg ml⁻¹ amphotericin B). The medium was changed every other day. The cells from a passage number between 42 and 48 were used in the experiments. After 21–23 days in culture, the Caco-2 cell monolayers were used for transport studies. Transport medium (TM) consisted of Hanks Balanced Salt Solution (HBSS) supplemented with 25 mM glucose, adjusting to pH 6.5 for the apical (AP) side and 7.4 for the basolateral (BL) side.

The integrity of each batch of cells was tested by measuring the transepithelial electrical resistance (TEER) using the Millicell[®]-ERS system (Millipore Co., Bedford, Massachusetts) before and after the experiments. Before the experiments, cells were equilibrated with HBSS for 20 min at 37°C, and the TEER was determined. The TEER value corrected for the blank filter resistance was in the range of 250–300 Ω.cm².

Transport studies were performed in triplicate for a compound in the AP-to-BL or BL-to-AP direction at 37°C in a shaking water bath (85 strokes min⁻¹). Permeation of the ester through Caco-2 cell monolayers was assessed with or without the exposure of cells to PMSF (0.5 mM), an inhibitor of serine protease/mammalian esterase, for 15 min on the AP side prior to transport experiments.

TM containing the ester at the concentration of about 30 µM (containing 4% DMSO) was added to the donor compartment (AP, 1.5 ml or BL, 2.6 ml). TM in the presence or absence of BSA (4%) was added to the receiver compartment (BL or AP), and samples were removed at various times (i.e., 30, 60, 90, 120, 150, and 180 min) from both sides (receiver, 200 µl; donor 50 µl). The sample volume removed from the receiver side was replaced with fresh, prewarmed TM in the presence or absence of BSA (4%). Samples were kept at -80°C until HPLC analysis. The total amount of parent compound and ester was used for the calculations. The permeability [apparent permeability coefficient, Papp (cm s⁻¹)] of the compound was calculated according to the equation:

$$P_{app} = \frac{1}{AC_0} \frac{dQ}{dt}$$

Where dQ/dt is the rate of appearance of drug in the receiver compartment (µmole s⁻¹), C_0 is the initial drug concentration in the donor compartment (mM), and A is the surface area of the monolayer (cm²).

The accumulation of the ester in Caco-2 cells was also measured. After performing transport studies, the transport medium was removed and the cells were washed with HBSS, pH 7.4. The cells were then removed from the polycarbonate filters, diluted using 100 µl PBS, and kept in Eppendorf tubes at -80°C until HPLC analysis.

RESULTS AND DISCUSSION

Chemical and Enzymatic Stability

The chemical and enzymatic degradation of ester was followed for at least two half-lives. The hydrolysis follows first-order kinetics. The degradation products of ester were mefenamic acid and guaiacol. By analyzing for the presence

of remaining ester and mefenamic acid, mass balance (>91%) was achieved in all experiments. The observed first order rate constants and apparent half-lives in aqueous buffer are shown in Table I. There is no buffer catalysis in buffer systems used. The pH-rate profile of ester hydrolysis is shown in Fig. 1. The total rate equation for hydrolysis can be written as:

$$\frac{dc}{dt} = k_{obs}c$$

$$k_{obs} = k_0 + k_{OH^-}[OH^-]$$

Where k_{obs} is the apparent first-order rate constants, $[OH^-]$ is the hydroxide ion concentration, k_{OH^-} is the rate constant for specific base-catalyzed hydrolysis, and k_0 is the apparent first-order rate constant for spontaneous or water-catalyzed hydrolysis. As seen in Fig. 1, the minimum rate of hydrolysis occurs at pH below 10, where the rate of reaction is apparently pH-independent. The average of k_{obs} (or k_0) at this pH range is 2.65×10^{-4} min⁻¹ (Table I). With increasing pH above 10, the ester degrades progressively faster to yield mefenamic acid and guaiacol. The plot of log k_{obs} vs. pH (10–12) gave a straight line with a slope of +1.11, indicating that the hydroxide ion concentration is the major factor that influences the hydrolysis rate of ester. The rate equation at these pHs can be written as: $k_{obs} = k_{OH^-}[OH^-]$. The k_{OH^-} value determined from this kinetic model is 4.78×10^{-2} min⁻¹.

The susceptibility of the ester to undergo potential enzymatic hydrolysis, was studied *in vitro* at 37°C in phosphate buffer, pH 7.4, containing either 80% human plasma, porcine liver esterase, or Caco-2 homogenate. The observed half-lives for hydrolysis in these biologic media are listed in Table I. The data indicate that enzymes from Caco-2 homogenate and porcine liver esterase markedly increase the rate of hydrolysis as compared to that from human plasma. Because the protein content in human plasma is higher, it is possible that the observed slow rate of hydrolysis in plasma may be due to binding of the ester to plasma proteins, which in effect protects some of the substrate molecules from hydrolysis. This result is consistent with previous findings by Brunner-Guenat *et al.* (11) and Bundgaard *et al.* (12). A typical concentration-time profile for the degradation of ester in Caco-2 homogenate is shown in Fig. 2. The result shows the rapid degradation of ester and the release of free mefenamic acid with a half-life of 22.4 min. In the presence of 0.06 unit ml⁻¹ of porcine liver esterase and at the initial ester concentration of 8 µM, the apparent half-life is reduced to 3.65 min.

Table I. Hydrolysis Rates and Half-Lives of the Ester in Buffer Solution, 80% Human Plasma, Porcine Liver Esterase and Caco-2 Homogenate at 37°C

Incubation mixture	K _{obs} (min ⁻¹)	Half-life
Buffer pH 1.21–10.59	$2.65 \times 10^{-4} \pm 6.82 \times 10^{-5a}$	46.91 ± 1.61 h
Buffer pH 11.23	$1.20 \times 10^{-3} \pm 3.16 \times 10^{-5b}$	29.41 ± 0.94 h
Buffer pH 11.75	$6.53 \times 10^{-3} \pm 7.44 \times 10^{-4b}$	4.46 ± 0.53 h
Buffer pH 12.00	$1.02 \times 10^{-2} \pm 1.70 \times 10^{-4b}$	2.82 ± 0.05 h
80% human plasma	$1.90 \times 10^{-3} \pm 1.00 \times 10^{-4b}$	15.23 ± 0.80 h
Porcine liver esterase	$1.92 \times 10^{-1} \pm 2.83 \times 10^{-2b}$	3.65 ± 0.54 min
Caco-2 homogenate	$3.13 \times 10^{-2} \pm 4.32 \times 10^{-3b}$	22.40 ± 2.87 min

^a Value represents mean ± S.D. for Kobs of 10 buffer pHs.

^b Value represents mean ± S.D. of three determinations.

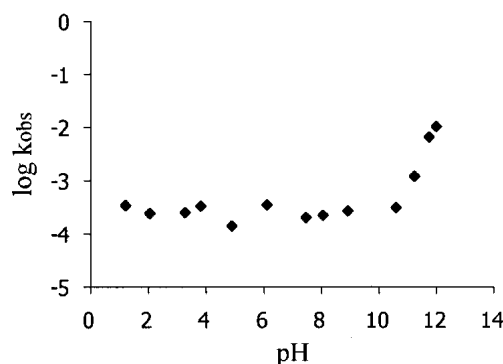


Fig. 1. pH-rate profile for the chemical degradation of ester at 37°C.

Solubility and Partition Coefficient

The ester of mefenamic acid-guaiacol is poorly soluble in water ($\approx 0.56 \mu\text{g ml}^{-1}$). However, it is readily soluble in organic solvents such as ethanol, benzyl alcohol and methyl benzoate. For mefenamic acid, a weak organic acid with pKa of 4.2, its solubility is dependent on the solution pH; the solubility increases with increasing pH. Its solubilities at pH 1.16, 4.96, 5.92, 6.93, and 7.62 are 0.05, 0.24, 3.02, 33.62, and $329.75 \mu\text{g ml}^{-1}$, respectively.

The octanol-water partition (logP) of the ester could not be determined since its value is too high to obtain a reliable result by experimentation (13). The logP value was therefore estimated using the ClogP program (Biobyte, California), which calculates the value directly from its molecular structure. The calculated logP value for the ester is 6.15.

Transport across Caco-2 Cell Monolayers

Previous *in vivo* data suggest that intestinal absorption of drugs may decline when their lipophilicity becomes too high (14). Recent *in vitro* studies in HT29-18-C1 and Caco-2 cell lines showed that when the log $D_{o/b}$ (octanol/buffer distribution coefficient) value was lower than 3.5, the permeability increased with the lipophilicity, but when the log $D_{o/b}$ value was between 3.5 and 5.2, the permeability decreased with increasing lipophilicity (15). However, there has been an argument that the *in vitro* cell monolayer systems do not adequately mimic the *in vivo* situation by underestimating the *in vivo* bioavailability of highly lipophilic compounds, unless ac-

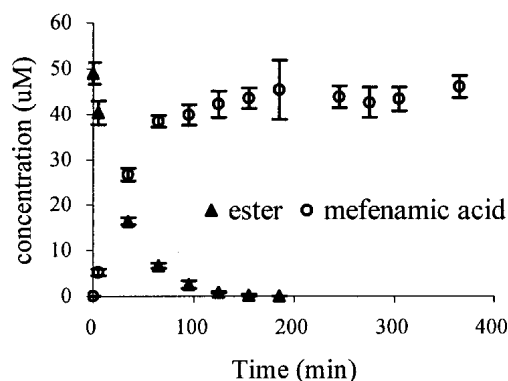


Fig. 2. Stability of ester at 37°C in Caco-2 homogenate, showing the time course of the disappearance of ester and the appearance of mefenamic acid.

ceptors such as serum proteins are added to the receiving medium (16). To obtain a good *in vitro-in vivo* correlation, BSA was included in the BL side to provide an absorptive driving force, resembling the *in vivo* sink condition (17–18). BSA can reduce the amount of lipophilic compounds absorbed into the cells and non-specific binding to the AP side, thus increasing their diffusion out of the cells (17–19). Besides, protein binding might also prevent non-specific adsorption of drugs to experimental apparatus such as supporting filter or diffusion chamber in the BL side (20). These interactions result in an enhanced transport of lipophilic compounds and provide a reasonable estimate of drug permeability under physiological conditions (17–18). Since the serosal side is perfused with the blood that has about 4% albumin, the inclusion of 4% BSA in the BL side was used to optimize the *in vitro* experimental condition in this study.

Due to the short biologic half-life of the ester (22.4 min) in Caco-2 homogenate, studies of ester transport across Caco-2 cell monolayers were carried out in the presence or absence of PMSF esterase inhibitor. And to increase the solubility of poorly soluble compounds, DMSO as high as 5% was used in the presence of 4% BSA in the BL side (17). The TEER value obtained under this condition was lowered but remained within an acceptable range. In this study, 4% of DMSO was used to facilitate the solubilization of the ester. After the transport experiments, the TEER values were

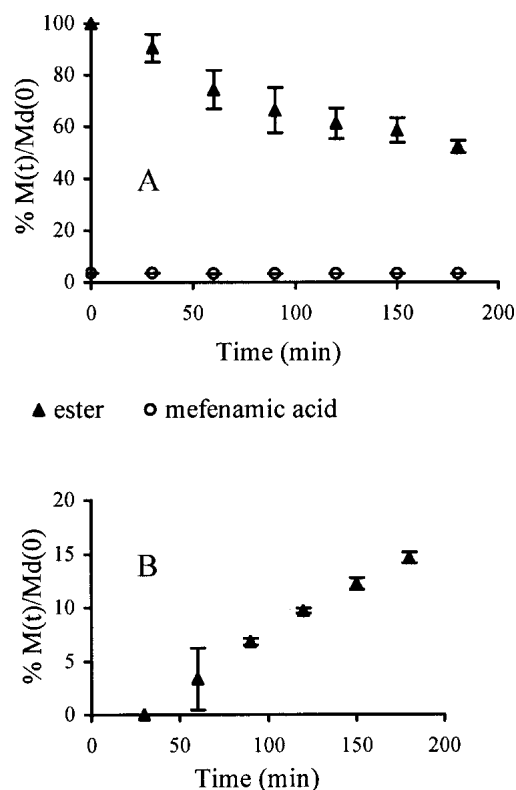


Fig. 3. A, Time course for the disappearance of ester and appearance of mefenamic acid on the AP side when ester was applied to the AP side of the Caco-2 cell monolayers, in the presence of 4% BSA in the BL side and incubated up to 3 hours at 37°C after the exposure of cells with PMSF; B, shows the ester transported to the BL side. The results were plotted as mass fractions, $M(t)$, relative to the mass on the AP side (donor compartment) at time zero, $M_d(0)$. Experiments were performed in triplicate (average $\pm$ SD).

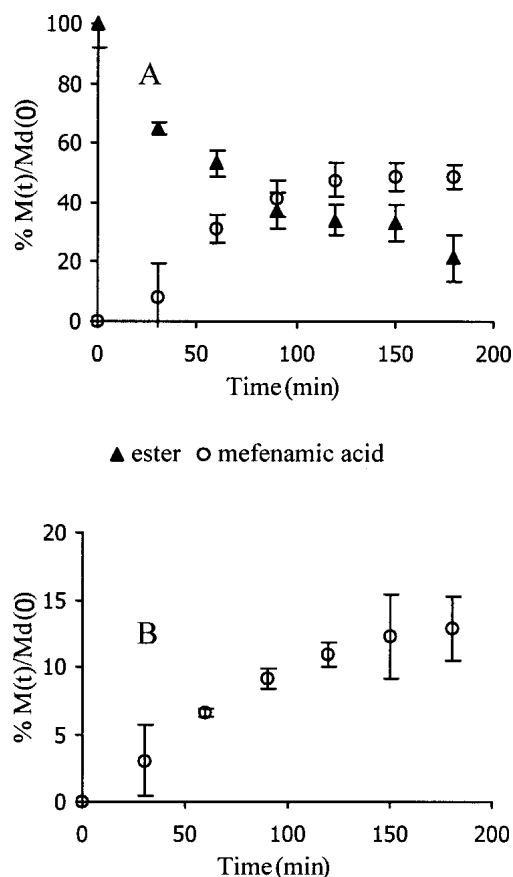


Fig. 4. A, Time course for the disappearance of ester and appearance of mefenamic acid on the AP side when ester was applied to the AP side of a Caco-2 cell monolayers, in the presence of 4% BSA in the BL side and incubated up to 3 hours at 37°C; B, shows mefenamic acid transported to the BL side. The results were plotted as mass fractions, $M(t)$, relative to the mass on the AP side (donor compartment) at time zero, $Md(0)$. Experiments were performed in triplicate (average $\pm$ SD).

found to be only slightly lower, i.e., 6% of the initial value, indicating that the integrity of the monolayer was preserved over a period of at least 3 h. In addition, the viability of the Caco-2 cells after exposure to PMSF was assessed with a MTT assay (21), which measured mitochondrial dehydrogenase activity of viable cells spectrophotometrically. This study revealed that PMSF did not affect the cell viability at the concentration used.

Time courses of the transport (from AP-to-BL) with and without PMSF exposure on the AP side are shown in Fig. 3 and Fig. 4, respectively. With the exposure to PMSF (15 min) and in the presence of 4% BSA on the BL side, the amount of ester transported from the AP-to-BL direction increased linearly with time after a lag time of 23 min (Fig. 3B). The amount of ester transported in 3 h from AP-to-BL was 14.63%. Bi-directional transport studies in which the transport of ester across the Caco-2 cell monolayer (AP-to-BL and BL-to-AP) was carried out to determine whether the ester is transported via an efflux mechanism. The apparent permeability coefficients of ester from AP-to-BL ($4.72 \times 10^{-6} \text{ cm s}^{-1}$) and BL-to-AP ($5.37 \times 10^{-6} \text{ cm s}^{-1}$) were found to be statistically indifferent ($P < 0.05$). Therefore, the ester is not a substrate for the polarized efflux systems in Caco-2 cell monolayer.

No permeation to the BL side was observed with PMSF exposure in the absence of BSA, although a significant amount of the ester was accumulated in Caco-2 cells. The accumulated amount was two-fold higher in the absence of BSA compared to its presence. This result suggests that the accumulated amount of this highly lipophilic compound depends on protein binding, since protein binding facilitates the permeation of the compound to the receiver side and cellular accumulation of the compound is decreased. A similar observation was reported for cosalane uptake which was reduced in the presence of BSA (22).

Without PMSF, the ester when applied to the AP side of Caco-2 cell monolayers was converted to mefenamic acid to some extent on the AP side and no ester was detected on the BL side, although 4% BSA was added to this side (Fig. 4). Due to the modest amount of initial ester concentration, the length of transport study, and the detection limit of analytical method, no permeation of the ester was observed.

Based on the chemical stability experiments, the ester has sufficient chemical stability under the acidic conditions encountered in the stomach with pH as low as 1 and in the intestine with pH of about 8. The ester can, however, be degraded to mefenamic acid and guaiacol by enzymes from human plasma, porcine liver esterase and Caco-2 homogenate. This suggests that the ester might be hydrolyzed *in vivo* before, during or after absorption, to release mefenamic acid and guaiacol which would then exert its characteristic pharmacological action. According to Yazdani *et al.* (23) and Yee (24), compounds with Papp of 0.4 or less than $1 \times 10^{-6} \text{ cm s}^{-1}$ will be poorly absorbed. Compounds with a Papp varied between $7 (23) - 10 (24) \times 10^{-6} \text{ cm s}^{-1}$ had well oral absorption. If the addition of BSA provides a good estimation of Papp, the ester can probably be moderately absorbed. Further studies are needed to investigate the protective effect of this ester on GI toxicity.

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