Solubility of Mefenamic Acid Under Simulated Fed- and Fasted-State Conditions

Christopher N. TenHoor,[1] Vassiliki Bakatselou,[1] and Jennifer Dressman[1,2]

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INTRODUCTION

In the presence of food, drug absorption can be reduced, delayed, increased, or remain unchanged (1,2). Although for some drugs the effects of food on drug absorption are due to interactions with specific food components (3–5), for the majority of drugs the changes in GI physiological parameters from the fasted to the fed state are likely to be the main source of differences in availability. Three physiological functions seem to be of primary importance. These are the secretory responses to meal intake (gastric, pancreatic, and biliary), changes in the motility patterns of the GI tract, and the increase in portal blood flow.

The objective of the current study was to investigate the degree to which normal physiological variations in bile salt concentrations and pH between the fed and the fasted state can influence the solubility and subsequently the absorption of a poorly soluble drug. In the intestine, the bile salts act as endogenous surfactants, solubilizing and emulsifying hydrophobic fatty acids and monoglycerides. Drug dissolution can also be affected by bile in the intestine. At concentrations below the critical micelle concentration (CMC), the bile salts can bind to the drug surface, increasing its wettability in the aqueous environment and thus the effective surface area (SA_t). At higher concentrations of bile salts (>CMC), the intrinsic solubility (C_s) of hydrophobic compound can be increased by micellar solubilization. Applying the Noyes–Whitney equation [Eq. (1)], increases in the effective surface area and intrinsic solubility are both predicted to result in an increased dissolution rate.

$$-\frac{dX}{dt} = \frac{DSA_t}{h}\left(C_s - \frac{X_d}{V}\right) \quad (1)$$

where X is the mass of the drug dissolved, D is the drug diffusivity, SA_t is the surface area of the particles at time t, h is the boundary layer thickness, and C_s is the intrinsic solubility of the drug.

Tangerman *et al.* (6) studied the concentration of conjugated bile acids in the upper jejunum in the fasted state and at 30 and 60 min after a standard meal. The results showed a distinct peak in the upper jejunal bile acid concentration at 30 min after the test meal. The mean values for the fasting, 30-min postprandial, and 60-min postprandial concentrations were 5, 15, and 8 m*M*, respectively.

In the case of ionizable drugs, the luminal pH and buffer composition will be additional important determinants of the solubility. Intestinal pH is known to vary both on an interindividual basis and between the fed and the fasted states. Dressman *et al.* (7) determined the overall median fasting pH in the duodenum to be 6.1, with an interquartile range of pH 5.8–6.5. During ingestion of a standard meal, duodenal pH fluctuated randomly around a grand median value of 6.3. The overall postprandial median pH was 5.4, with an interquartile range of 5.0–5.7.

The model compound chosen for these studies was the anthranilic acid nonsteroidal antiinflammatory drug (NSAID) mefenamic acid (Ponstel, Parke-Davis). Mefenamic acid has an apparent pK_a of 4.2, a value fairly typical for the anthranilic and indole acetic acid-type NSAIDs (8). It is reported to be almost insoluble in water (40 μg/ml at 25°C, pH 7.1, and 80 μg/ml at 37°C, pH 7.1) and only slightly soluble in ethanol, chloroform, and ether (8). Taking the average dose to be 250 mg, the amount of liquid required to dissolve a single dose is over 6 liters. Studies by Dillard and Fordtran have shown that the volume of the jejunum and ileum combined are less than 1 liter (9). These simple calculations strongly suggest that dissolution will be a key factor in determining the bioavailability of mefenamic acid. Although mefenamic acid itself is almost always administered in the fed state to avoid GI irritation, it is a useful model compound to represent the behavior of poorly soluble, weakly acidic drugs.

MATERIALS AND METHODS

Mefenamic acid (2-[(2,3-dimethylphenyl)amino]-benzoic acid) was donated by Warner-Lambert/Parke Davis (Ann Arbor, MI). Five concentrations of sodium taurocholate were used to determine the solubility of mefenamic acid. Sodium taurocholate was chosen because of its stability, the consistency of the CMC over the pH range used, and its importance as an endogenous component of bile. The CMC of sodium taurocholate was determined to be 4.0 m*M* at 40°C and ionic strength 0.15 (10). Concentrations of 0.10 and 1.0 m*M* sodium taurocholate represent sub-CMC levels, 4.0 m*M* corresponds to average CMC values and 10.0 and 20.0 m*M* sodium taurocholate represent values above the CMC. This range of values encompasses the range found in the small intestine under normal circumstances.

The solubility measurements were conducted in solutions of varying pH (5.0, 5.5, 6.0, and 6.5) prepared with a disodium phosphate/citric acid buffer and at bile salt concentrations ranging from 0 to 20 m*M* sodium taurocholate. The total ionic strength of all solutions was held constant at 0.15 *N* by adjustment with sodium chloride. The final adjustment of the pH of each buffer solution was made prior to the addition of mefenamic acid, using a standardized pH meter (Orion Research Model 811, Orion Research, Cambridge,

[1] College of Pharmacy, University of Michigan, Ann Arbor, Michigan 48109-1065.
[2] To whom correspondence should be addressed at 2007 College of Pharmacy, University of Michigan, Ann Arbor, Michigan 48109-1065.

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MA). To ensure thorough mixing, the samples were shaken on an orbital rotating mixer (Adam's Nutator, Becton Dickinson, Parsippany, NJ) in an oven maintained at 37 ± 0.5°C (Thelco Precision Oven, Precision Scientific Co., Chicago, IL). All determinations were made in triplicate.

Samples were taken at intervals of 4, 8, 24, and 48 hr to ensure that the equilibrium solubility had been reached. The samples were filtered using a 0.4-μm polycarbonate syringe filter (Nucleopore, Pleasanton, CA). The concentration of mefenamic acid in the filtrate was measured using a UV spectrophotometer (Lambda-7, Perkin Elmer, Oak Brook, IL). For each set of samples, a standard curve for that bile salt/pH combination was constructed and the peak wavelength was determined. This varied from 284.2 to 289.1 nm depending on the buffer/surfactant composition of the medium. The absorbance of each sample was determined, in triplicate, at the appropriate peak wavelength. All standard and sample solutions were run against a blank consisting of the appropriate buffer/surfactant combination to eliminate any contributions from background absorption.

Two experiments were conducted at 4 and 24 μg/ml mefenamic acid in pH 6.5, 0 m*M* sodium taurocholate to determine whether material was lost on the filters. The absorbances of the solutions were unchanged after filtration, indicating that it is unlikely that any significant amount of material was adsorbed during filtration.

RESULTS AND DISCUSSION

Concentration vs time data for mefenamic acid solubility in pH 5.5, 10 m*M* sodium taurocholate (NaTC) are shown in Fig. 1. Equilibrium was reached within 24 hr. This was the case for all pH/NaTC combinations. Solubilities were subsequently reported as the mean concentration after 48 hr of equilibration. Results are shown in Table I. In the absence of bile salts, solubility increases as the pH is raised further above the pK_a (4.2) of mefenamic acid. There is an increase of almost 50-fold between the solubility at pH 5.0 and that at pH 6.5, which corresponds to the usual range of pH values observed in the small intestine. As the bile salt concentration is increased, the effect of increasing the pH becomes less dramatic. At 20 m*M* NaTC, there is an increase of approximately eightfold between pH 5.0 and pH 6.5 (Fig. 2). At any given pH, the bile salt concentration also has a major influence on the solubility of mefenamic acid. At pH 5.0, there is an increase of almost 20-fold between 0 and 20 m*M* NaTC, while at pH 6.5 the factor is approximately 3-fold over the same concentration range. Most of the increase occurs above the CMC of NaTC (Fig. 3).

The concentration range of 1 to 20 m*M* NaTC represents the range usually found in the small intestine, with 1 m*M* being the lower end of the fasted state and 20 m*M* the upper end of the fed state. Comparing pH/NaTC combinations representative of the fed and fasted state, it appears that there is a trade-off in pH and bile salt effects between the two conditions, which results in there being little difference in the drug's solubility. At pH 6.0, 4 m*M* bile salt (average fasted-state conditions), the solubility of mefenamic acid is 7.7 μg/ml, whereas at pH 5.5, 10 m*M* bile salt (average fed-state conditions), it is 5.2 μg/ml. As a result, one would not expect there to be a dramatic difference in dissolution when drugs with pK_a and solubility characteristics similar to those of mefenamic acid are administered before versus after meals.

Table I. Forty-Eight-Hour Mean Mefenamic Acid Solubilities (μg/ml) and Ranges for Each pH/Bile Salt Concentration Combination[a]

[NaTC], m*M*	pH 5.0	pH 5.5	pH 6.0	pH 6.5
0	0.47[b]	4.75 (4.63–5.13)	10.9 (9.91–11.9)	25.7 (22.6–27.5)
0.1	1.14[b]	2.51 (2.39–2.62)	7.17 (7.04–7.31)	25.6 (25.2–26.3)
1.0	1.12 (1.01–1.21)	2.70 (2.59–2.77)	7.18 (6.92–7.42)	25.7 (25.4–26.4)
4.0	1.17 (1.05–1.29)	3.01 (2.92–3.10)	7.63 (7.51–7.73)	21.3 (21.2–21.4)
10.0	2.33 (2.22–2.48)	5.23 (5.15–5.29)	11.9 (11.5–12.5)	37.8 (37.6–38.1)
20.0	9.75 (9.52–9.87)	14.3 (13.9–14.5)	31.3 (30.2–31.9)	76.2 (74.1–77.6)

[a] $n = 3$ at each pH/bile salt combination.
[b] Combined data.

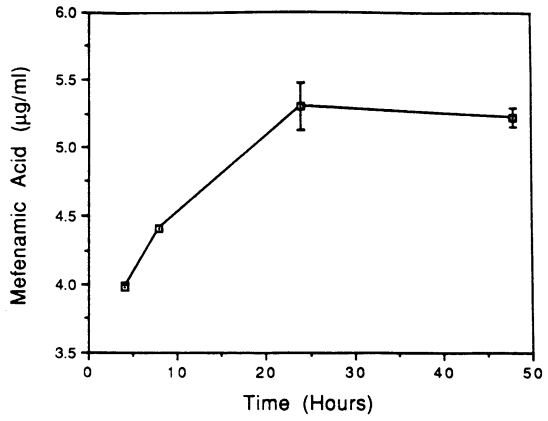

Fig. 1. Concentration of mefenamic acid in solution as a function of time at pH 5.5 and 10 m*M* NaTC.

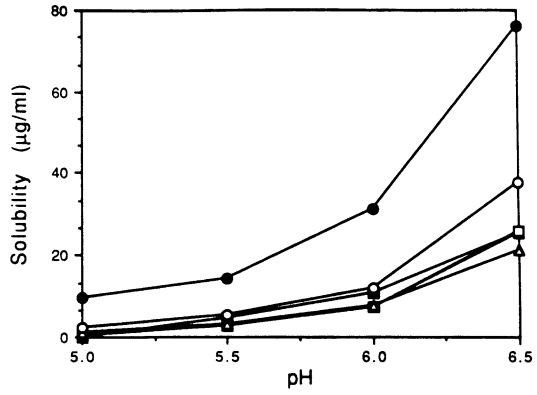

Fig. 2. Solubility of mefenamic acid as a function of pH, at six concentrations of sodium taurocholate and 37°C: (—■—) 0 m*M* NaTC; (—▲—) 0.1 m*M* NaTC; (—□—) 1.0 m*M* NaTC; (—△—) 4.0 m*M* NaTC; (—○—) 10 m*M* NaTC; (—●—) 20 m*M* NaTC.

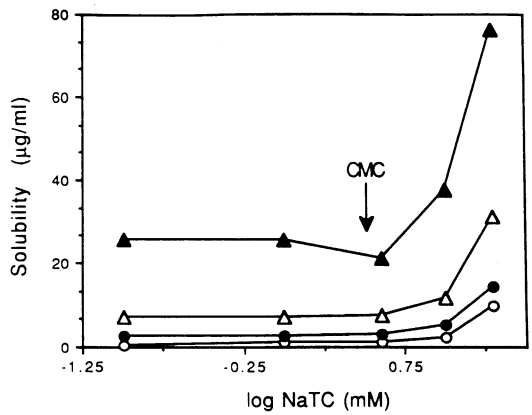

Fig. 3. Solubility of mefenamic acid measured at 37°C as a logarithmic function of sodium taurocholate, at four pHs: (—○—) pH 5.0; (—●—) pH 5.5; (—△—) pH 6.0; (—▲—) pH 6.5.

In both the fed and the fasted states, one would, however, expect to observe a wide interindividual variation in the *in vivo* dissolution of such drugs. Under fasted-state conditions, where the bile salt concentration usually falls within the 1–4 m*M* range and pH is between 5.5 and 6.5 in 80% of the observed data (7), the solubility of mefenamic acid ranges from <3 to 21 μg/ml. Under fed-state conditions, where bile salt concentrations typically range from 10 to 20 m*M* and the pH is between 5 and 6 in 70% of the observed data (7), there is an even wider range of solubilities, from 2.3 to 31.3 μg/ml. As all of these solubilities are very low and the recommended dose of mefenamic acid is 250 mg, the dose-to-solubility ratio (11) is expected to be a major limitation to the absorption of this drug under either fasted- or fed-state dosing conditions.

CONCLUSIONS

Within the range of values encountered in the intestinal tract, both the bile salt concentration and the pH can profoundly affect the solubility of mefenamic acid. Considerable variation in dissolution rate would be expected in both the fasted and the fed state, as a result of interindividual variation in pH and bile salt concentration. Despite the higher levels of bile salt in the fed state, dissolution is not expected to be greater than in the fasted state. The decrease in luminal pH associated with the fed state tends to result in a decreased solubility for mefenamic acid, offsetting the increased solubilizing effects of the bile salts.

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