Journal of Pharmaceutical & Biomedical Analysis
Vol. 7, No. 8, pp. 981–985, 1989
Printed in Great Britain

0731–7085/89 $3.00 + 0.00

Short Communication

Comparative pharmacokinetics of two crystalline forms of famotidine in dogs

M. KOZMA,* M. VAJDA and L. VERECZKEY

Department of Pharmacokinetics and Drug Metabolism, Chemical Works of Gedeon Richter Ltd, Budapest, Hungary

Keywords: *Famotidine, forms A and B; ion-pair extraction; HPLC; pharmacokinetics; dog.*

Introduction

Famotidine is a H_2-receptor blocker that inhibits gastric acid secretion in man and animals [1–4]. In the course of the synthesis of famotidine it was found that famotidine has two crystalline forms: the recently discovered, thermodynamically more stable form A (Eur, EP.Appl. 256747) which has a higher melting point and the less stable form B with a lower melting point which is widely used at present. Full details of the physicochemical properties of the two modifications have been published [5]. Since famotidine is dissolved under the acidic conditions in the stomach, it is very reasonable to suppose that the two modifications have identical pharmacokinetic properties. Nevertheless the dissolution and absorption of the two forms must be investigated *in vivo*. For the same reason bioequivalence studies are required, as well as *in vitro* dissolution tests. Therefore, a comparative pharmacokinetic study of the two crystalline forms was carried out. The bioequivalence of the two crystalline forms was studied in dogs.

Materials and Methods

Chemicals

Famotidine (both crystalline forms) and metiamid were synthesized at the Chemical Works of Gedeon Richter Ltd (Hungary). All solvents were HPLC grade. Sodium octanesulphonate was obtained from Merck (FRG). Sep-Pak cartridges were purchased from Waters Associates (USA).

Experiments in dogs

Six beagles (7.2–9.9 kg) received 20 mg kg^{-1} of famotidine crystals (in capsules) as a single oral dose in "cross-over" experiments at 2-week intervals. The weights of the dogs

*To whom correspondence should be addressed.

Table 1
Data of the beagles

Number of dogs	Weight (kg)	Sex	Dose (20 mg kg^{-1} p.o. in capsules)
I	8.40	F	168
II	8.80	F	176
III	8.20	F	164
IV	9.90	M	198
V	9.40	M	188
VI	9.40	M	188
Mean ± SD	9.02 ± 0.60		180.3 ± 12.02

and the doses of drug are shown in Table 1. The dogs were fasted overnight and no food was allowed until 10 h after administration of the drug.

Blood samples were taken at the following time intervals after drug administration: 0.25, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10 and 24 h. Sodium citrate solution (3.8%, m/v) was added to the blood samples (1:9, v/v) to prevent coagulation.

Extraction procedure

Sep-Pak C-18 cartridges were activated by washing with methanol and water according to the manufacturer's instructions. To 0.8 ml of plasma, 0.1 ml of sodium octanesulphonate solution (2%, m/v) in 0.017 M acetic acid) and 10 μl of metiamid solution (0.02 m/v in methanol) were added. The samples were applied to the cartridges and washed with 5 ml of water. The cartridges were then dried under vacuum. Two millilitres of acetonitrile saturated with ammonia [acetonitrile/14.68 M ammonia (50:0.5)] was applied to the column to elute the famotidine and metiamid. The eluate was evaporated in a stream of nitrogen at 40°C. The residue was reconstituted in 150 μl of 0.017 M acetic acid; 25 μl of sample was injected on the chromatograph.

The HPLC instrumentation comprised a Hewlett–Packard 1090 M system equipped with a ternary gradient SDS (DR5) and a diode-array UV detector and LC Workstation. The 200 × 4.6 mm, i.d., analytical column was filled with 10-μm LiChrosorb RP-8.

The mobile phase was 0.01 M KH_2PO_4-phosphoric acid buffer (pH 2.8)/acetonitrile (80:20, v/v). Famotidine and metiamid were detected at 267 and 230 nm, respectively. The analyses were performed at 28°C; the flow-rate was 1 ml min^{-1}.

Results and Discussion

The chemical structures and UV absorption spectra of famotidine (**I**) and metiamid (**II**) are shown in Figs 1 and 2.

Famotidine (**I**) is highly bound to plasma proteins so that any attempt to clean up plasma by protein precipitation resulted in low drug recovery. The pK_a value of the drug is 6.7 and its solubility in organic solvents is low.

Vincek *et al.* [6] reported an extraction method using a Bond-Elut 2.8 silica cartridge but they did not use any internal standard. The present authors could not reproduce this method. A possible reason was that Baker Bond-Elut silica cartridges were not available so that Waters Sep-Pak silica cartridges were tried with discouraging results.

Therefore a new ion-pair method has been developed for the extraction of famotidine. Metiamid (**II**) served as the internal standard. The extraction was performed on C-18

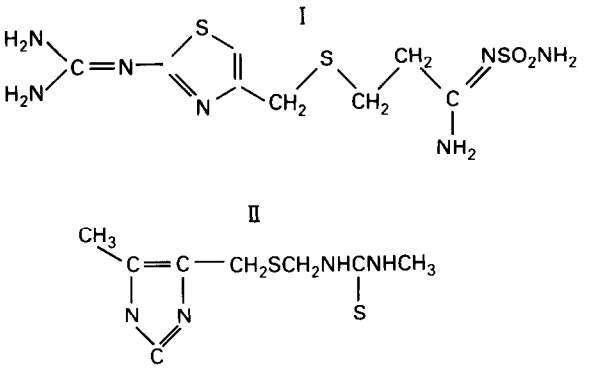

Figure 1
The structures of famotidine (**I**) and metiamid (**II**).

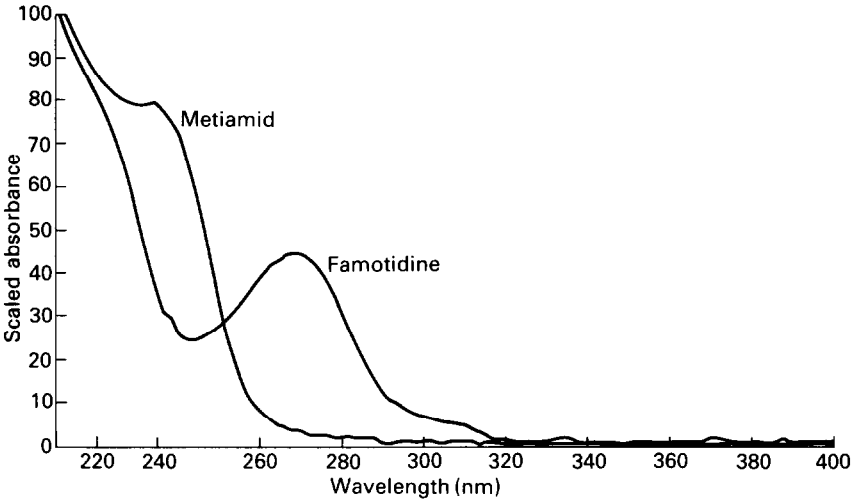

Figure 2
The UV spectra of famotidine and metiamid.

Sep-Pak cartridges with octanesulphonate as the counter-ion in an acid medium. Famotidine and metiamid were eluted from cartridges with acetonitrile saturated with ammonia.

Famotidine and metiamid could be extracted from blood plasma by the new method with a yield of 51.5% (±7.91 SD) and 63% (±5 SD), respectively. The results are shown in Table 2. The sensitivity of the method was 100 ng ml^{-1} for famotidine.

Famotidine is retained on the reversed-phase cartridge only as an ion-pair. The ion-pair equilibrium is partially disturbed by the protein binding of famotidine; the extraction losses are probably due to loss of the compound by elution in the washing cycle in the protein-bound form. It is also possible that protein denaturation occurs during elution from the cartridges and that famotidine is absorbed in this precipitate.

Table 2
Analytical recovery of famotidine and metiamid from human plasma

Concentration (μg ml^{-1})	Extraction recovery % SD	*n*
Famotidine		
0.25	61.6 ± 16.8	5
0.5	60.8 ± 10.47	4
1.0	35.2 ± 7.50	4
2.5	45.0 ± 11.4	4
5.0	45.0 ± 8.46	4
Mean %	51.5 ± 7.91	5
Metiamid		
1.0	58.0 ± 5.35	3
2.0	68.0 ± 5.35	6
Mean %	63.0 ± 5.0	

The calibration curves, obtained by adding known amounts of famotidine to blank plasma, were linear in the concentration range of interest (0.1–5 μg ml^{-1}). The linear regression curves could be described by the equation:

$$y = 0.3454x + 0.10376.$$

The correlation coefficient was 0.99897.

Famotidine and metiamid were well resolved from endogenous sources of interference as shown in Fig. 3. The method is suitable for the determination of famotidine and metiamid in specimens for clinical study. The detection of famotidine and metiamid was carried out at their UV absorption maxima at 267 and 230 nm, respectively.

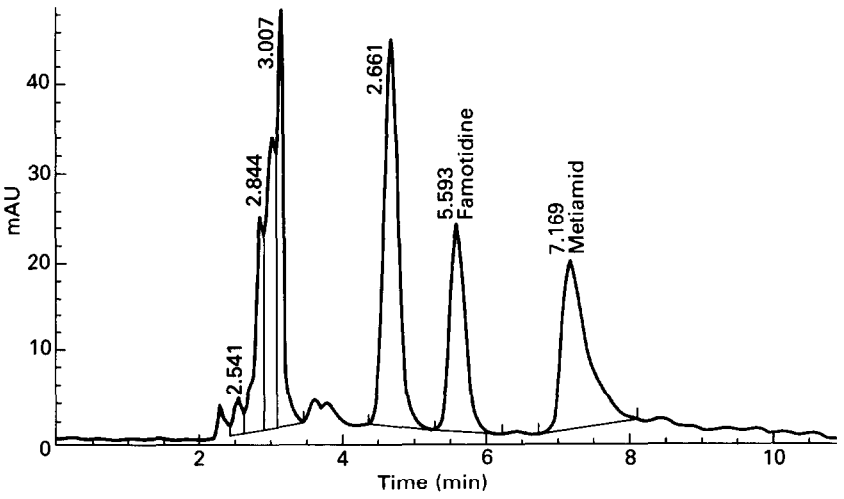

Figure 3
Chromatogram of a plasma sample taken 2 h after drug administration.

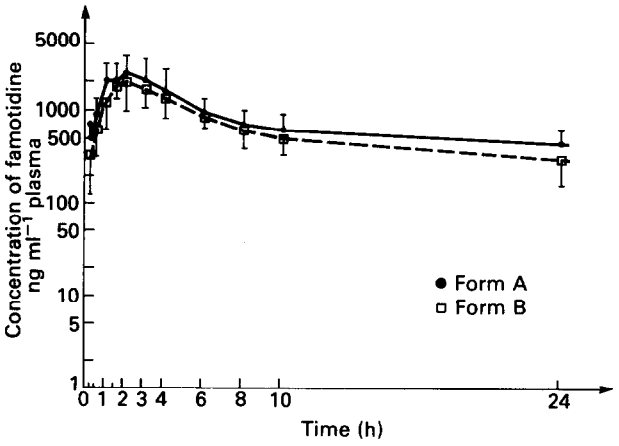

Figure 4
Log concentration–time curves of famotidine in forms A and B in dog plasma.

The log concentration–time curves of the two crystalline forms are presented in Fig. 4.

It was found that the rate of absorption was the same for the two preparations since the T_{max} values as well as the C_{max} values did not differ significantly:

$$C_{max} \text{ form A} = 2.44 \ \mu g \ ml^{-1} \ (\pm 1.02 \text{ SD}) \text{ at a } T_{max} \text{ of 2 h},$$
$$C_{max} \text{ form B} = 1.91 \ \mu g \ ml^{-1} \ (\pm 0.85 \text{ SD}) \text{ also at a 2-h } T_{max}.$$

The extent of absorption can be characterized by the area under the log concentration–time curve (AUC). To avoid the errors arising from extrapolation to infinity the AUC_{0-24h} values were calculated to compare the bioavailability of the two forms:

$$AUC_{0-24h} \text{ form A} = 15.95 \pm 8.08 \text{ SD } (n = 6),$$
$$AUC_{0-24h} \text{ form B} = 15.78 \pm 5.31 \text{ SD } (n = 6).$$

If it is assumed that absorption ended 24 h after administration of the drug, on the bases of the non-significant differences between AUC_{0-24h} values, it can be concluded that the two crystal forms are bioequivalent.

References

[1] C. Staiger, B. Korodnay, J. X. DeVries, E. Weber, P. Muller, B. Simon and H. G. Dammann, *Br. J. Clin. Pharmac.* **18**, 105–106 (1984).
[2] C. Staiger, B. Korodnay, J. X. DeVries, E. Weber, P. Muller, B. Simon and H. G. Dammann, *Arzneim.-Forsch.* **34**, 1041–1042 (1984).
[3] R. Kawai, S. Yamada and S. Kawamura, *Pharmacokinetics* **27**, 73–77 (1984).
[4] R. Kawai, H. Imasaki and S. Kawamura, *Pharmacokinetics* **26**, 927–933 (1983).
[5] B. Hegedüs, P. Bod, K. Harsányi, P. Imre, A. Kálmán and L. Párkányi, *J. Pharm. Biomed. Anal.* **7**, 563–569 (1989).

[*Received for review 28 January 1988; revised manuscript received 7 April 1989*]