ELSEVIER

International Journal of Pharmaceutics 237 (2002) 23–33

international journal of pharmaceutics

www.elsevier.com/locate/ijpharm

Susceptibility of the H_2-receptor antagonists cimetidine, famotidine and nizatidine, to metabolism by the gastrointestinal microflora

Abdul W. Basit [a,*], J. Michael Newton [a], Larry F. Lacey [b]

[a] *Department of Pharmaceutics, The School of Pharmacy, University of London, 29-39 Brunswick Square, London WC1N 1AX, UK*

[b] *GlaxoSmithKline, Greenford Road, Greenford, Middlesex UB6 0HE, UK*

Received 1 August 2001; received in revised form 20 December 2001; accepted 9 January 2002

Abstract

The H_2-receptor antagonist ranitidine has previously been shown to be a substrate for colonic bacterial metabolism. The objective of the present study was to assess the in vitro stability of the other H_2-receptor antagonists, cimetidine, famotidine and nizatidine, to colonic bacteria. One hundred milligrams of each drug were introduced into individual batch culture fermenters (100 ml) consisting of buffer medium inoculated with freshly voided human faeces (10% w/v). Control experiments, equivalent drug quantities in buffer medium without the presence of faeces, were also run in parallel. Samples were removed at set time intervals over a 24 h period and were subsequently analysed by HPLC. A selection of the samples removed from the fermenters was also subjected to analysis by UV spectroscopy and mass spectrometry. Following an initial dissolution phase in the fermentation system, a marked decline in nizatidine concentration was noted over time with virtually no drug remaining after 12 h, thereby suggesting degradation and metabolism of the drug by colonic bacteria. No such decline in concentration was noted for cimetidine or famotidine or for any of the drugs in the control buffer systems. The metabolic reaction pathway for nizatidine was complex, although UV and mass spectrometry analysis indicated that metabolism was initiated via cleavage of an N-oxide bond within the molecule. These results in combination with those obtained from a previous study indicate that of the four commercially available H_2-receptor antagonists, nizatidine and ranitidine are susceptible to metabolism by colonic bacteria, which in turn has ramifications for drug delivery and absorption.

Keywords: H_2-receptor antagonists; Metabolism; Colon; Bacteria; Drug delivery

1. Introduction

Prior to reaching the systemic circulation, an orally administered drug must firstly dissolve in the fluids of the gastrointestinal tract and then

* Corresponding author. Fax: +44-20-7753-5865.
E-mail address: abdul.basit@ulsop.ac.uk (A.W. Basit).

0378-5173/02/$ - see front matter
PII: S0378-5173(02)00018-2

traverse the gastrointestinal mucosa. Apart from these solubility and permeability issues, the stability of the drug within the lumen of the gut is a further factor that will have an impact on oral absorption. For instance, the presence of gastric acid, pancreatic enzymes and other intestinal secretions can lead to a gastrointestinal environment that is potentially conducive to drug degradation and/or metabolism, which in turn will limit oral bioavailability. Moreover, the resident gastrointestinal microflora may also have a role to play in this respect. The microflora, although present throughout the gut, predominantly colonize the distal regions of the gastrointestinal tract where the bacterial count in the large intestine is 10^{11} per g as compared to 10^4 per g in the upper small intestine (Finegold et al., 1983). The bacteria of the large intestine are essentially anaerobic in nature and are involved in the digestion of endogenous and exogenous materials such as carbohydrates and proteins that have escaped degradation in the stomach and small intestine (Cummings et al., 1989). The metabolic reactions performed by these bacteria and their respective enzymes are diverse and include hydrolysis, reduction, dehydroxylation, decarboxylation, dealkylation, dehalogenation, deamination, heterocyclic ring fission, aromatisation, nitrosamine formation, acetylation, esterification, and isomerisation among others (Scheline, 1973; Mikov, 1994). It is therefore not unduly surprising that these bacteria also have the ability to metabolise drugs and other xenobiotics (Scheline, 1973; Shamat, 1993). In practice, most orally administered drugs, by virtue of rapid and complete absorption from the proximal regions of the gastrointestinal tract, will not come in contact with the heavily colonised lower gut. However, those drugs that display poor solubility and/or permeation characteristics, or that are secreted from the systemic circulation into the lumen of the gut, or that are formulated as modified-release systems may be exposed to the microflora and its metabolic effect.

The H_2-receptor antagonist ranitidine (Fig. 1) has recently been shown in vitro to be susceptible to metabolism by colonic bacteria (Basit and Lacey, 2001). It was postulated that this finding might in part explain the poor bioavailability of ranitidine from the colon (Basit and Lacey, 2001),

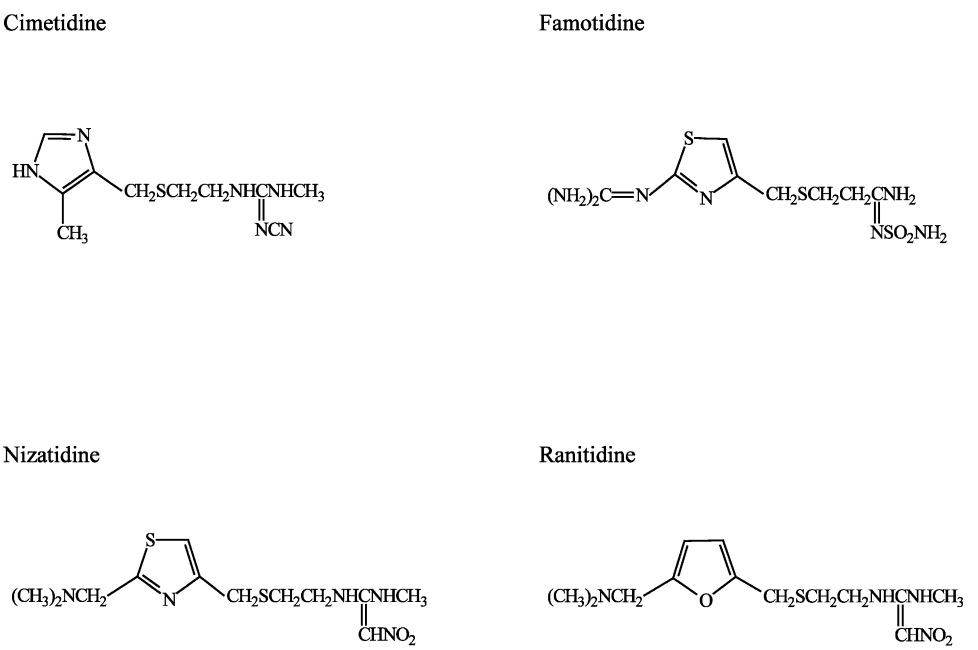

Fig. 1. Structures of the H_2-receptor antagonists.

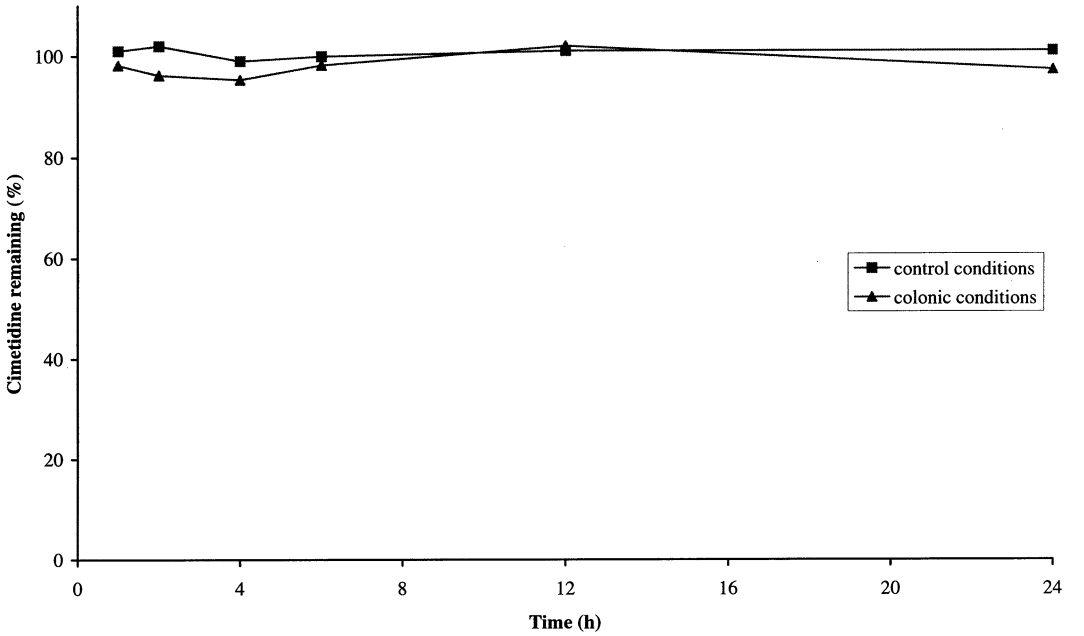

Fig. 2. Stability of cimetidine under both control and simulated colonic conditions of the fermentation system. (Each point represents the mean of three experiments and a coefficient of variation of less than 5%).

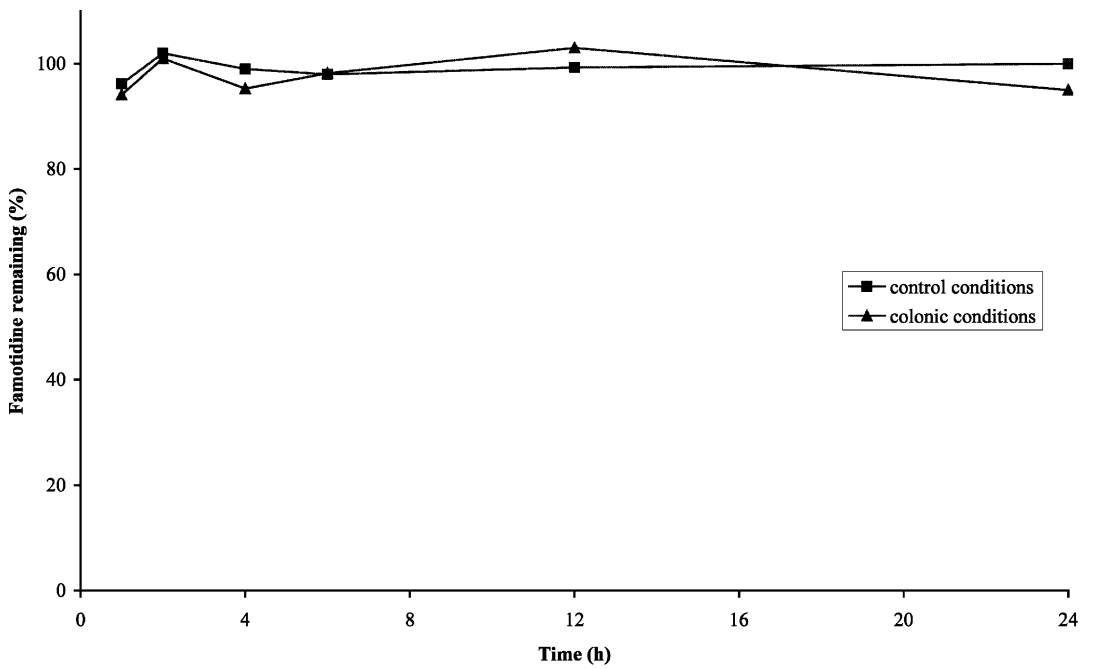

Fig. 3. Stability of famotidine under both control and simulated colonic conditions of the fermentation system. (Each point represents the mean of three experiments and a coefficient of variation of less than 5%).

and in turn precludes the development of a modified-release formulation for this drug. The aim of this study was to assess whether the other commercially available H_2-receptor antagonists, cimetidine, famotidine and nizatidine (Fig. 1), are also substrates for colonic bacterial metabolism.

2. Materials and methods

2.1. Materials

Cimetidine was obtained from GlaxoSmithKline (formerly SmithKline Beecham), Harlow, UK. Famotidine was received from Merck Sharpe and Dohme, Hoddesdon, UK, and nizatidine was obtained from Eli Lilly, Basingstoke, UK. All other chemicals were of AnalaR or HPLC grade and were purchased from Merck, Poole, UK.

2.2. Fermentation studies

A batch culture fermentation system was used to simulate the conditions of the colon (Basit and Lacey, 2001). Such fermentation systems have traditionally been used in studies concerned with food metabolism in the distal gut (Englyst and Macfarlane, 1986; Macfarlane et al., 1993; Silvester et al., 1995), and more recently as a means to investigate the in vitro performance of bacterial enzyme degradable film coating formulations for colonic delivery (Milojevic et al., 1996a,b; Siew et al., 2000a,b). One hundred milligrams of each drug were introduced into individual 100 ml fermenters inoculated with human faeces (10% w/v). The fermenters were prepared by homogenising the faeces, which were freshly obtained and pooled from three healthy female volunteers, in a phosphate-based buffer of pH 6.8. The fermenters were sealed under positive nitrogen pressure to establish an anaerobic environment and then incubated at 37 °C in an orbital incubator. Control experiments were also run in parallel using equivalent drug quantities in the same buffer without the presence of faeces. Each drug was assessed in triplicate. Two millilitre samples were removed at 0, 1, 2, 4, 6, 12 and 24 h from both the fermentation and control systems. These samples were

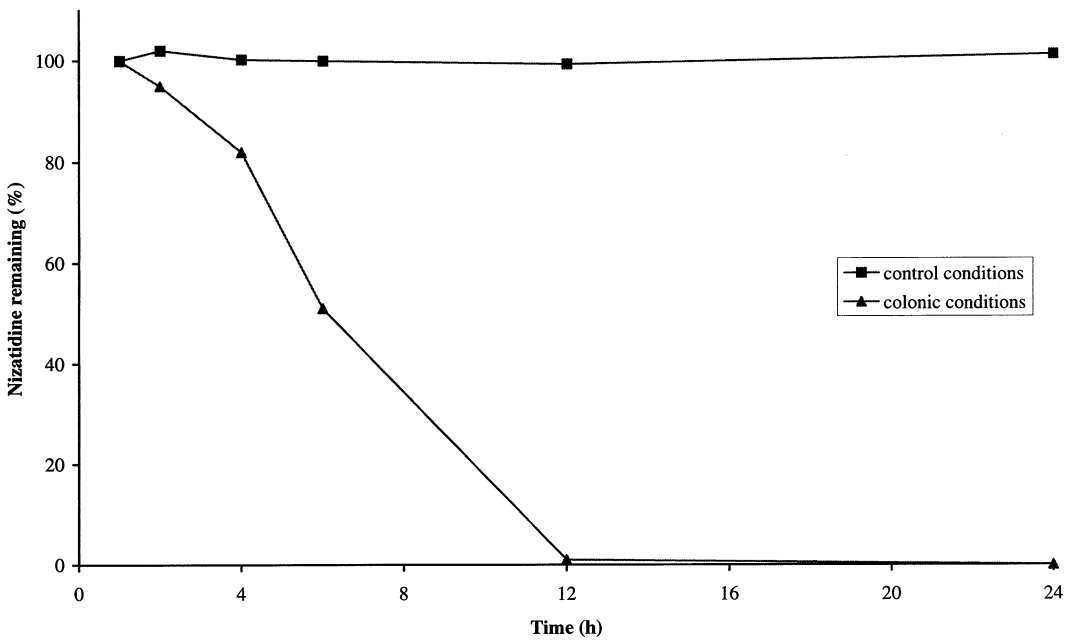

Fig. 4. Stability of nizatidine under both control and simulated colonic conditions of the fermentation system. (Each point represents the mean of three experiments and a coefficient of variation of less than 5%).

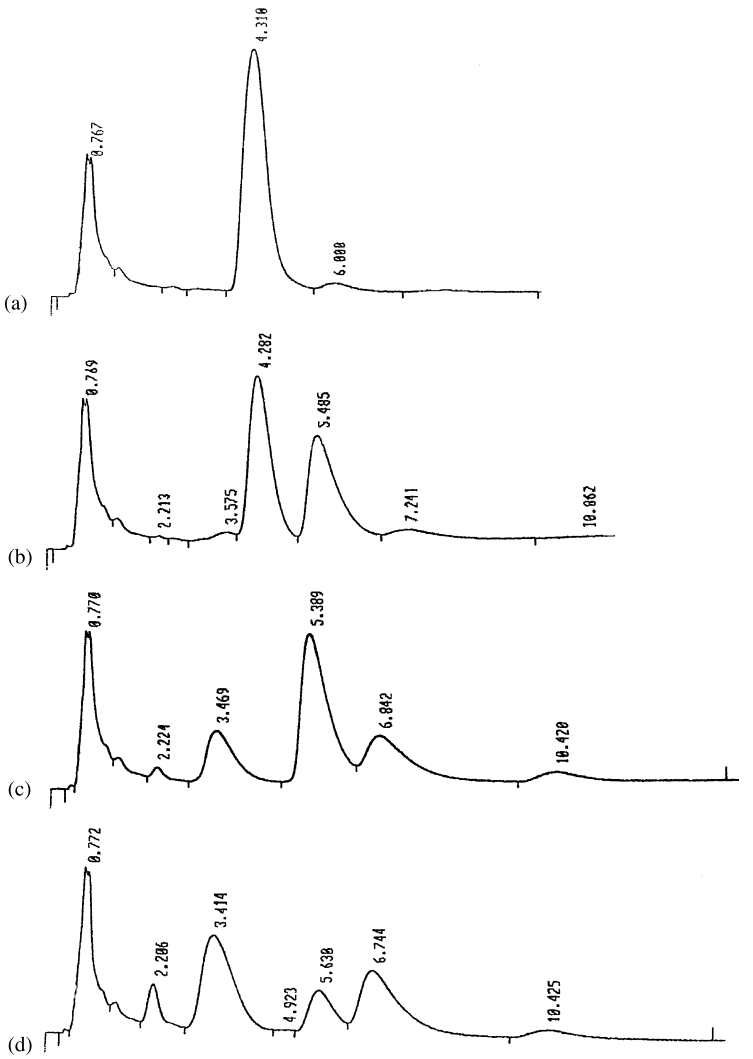

Fig. 5. HPLC chromatograms for nizatidine after exposure to the simulated colonic conditions of the fermentation system, (a) 2 h, (b) 6 h, (c) 12 h, (d) 24 h.

centrifuged at 13 000 rpm for 5 min, filtered through 0.2 μm filters and then immediately frozen prior to drug analysis.

2.3. HPLC assay

The samples were assayed by reversed-phase HPLC using a system comprising a pump (model Spectroflow 400, Kratos Analytical, Ramsey, NJ), a variable wavelength detector (model LDC Spectromonitor III, Milton Roy Co. FL) and an integrator (model 3393A, Hewlett Packard, Bedford, UK). The column, 100 × 4.6 mm Partisil (10 μm particle size) (model 100ODS, Hichrom, Reading, UK), was eluted with a mobile phase consisting of a mixture of methanol (30%) and 0.1 M ammonium acetate buffer (70%) at a flow rate of 2 ml/min. The detection wavelength was 220, 278 and 255 nm for cimetidine, famotidine and nizatidine, respectively.

2.4. UV spectroscopy

A selection of the samples obtained from the fermentation experiments were further assessed using conventional UV spectroscopy. The samples were initially diluted 1 in 20 with water and then analysed, against a blank (diluted reference fermentation sample obtained at 0 h), using an UV–Vis spectrophotometer (model 554, Perkin Elmer, Ueberlingen, Germany) between the wavelengths of 190 and 400 nm.

2.5. Mass spectrometry

The fermentation samples subjected to UV spectroscopy were also presented for analysis by mass spectrometry. Analysis was performed using a mass spectrometer (model ZAB-SE, VG Analytical, Manchester, UK) utilising fast atom bombardment mode.

3. Results and discussion

All three drugs dissolved rapidly in both the fermentation and control systems with peak concentrations reached within 1 h (Figs. 2–4). For the remainder of the experiment little or no change in drug concentration was noted for cimetidine and famotidine in the fermentation or control systems (Figs. 2 and 3). In contrast, a striking decrease in the concentration of nizatidine within the fermentation system was observed, with virtually no drug remaining after 12 h (Fig. 4). No such change in nizatidine concentration was noted in the control system (Fig. 4). Nizatidine, unlike

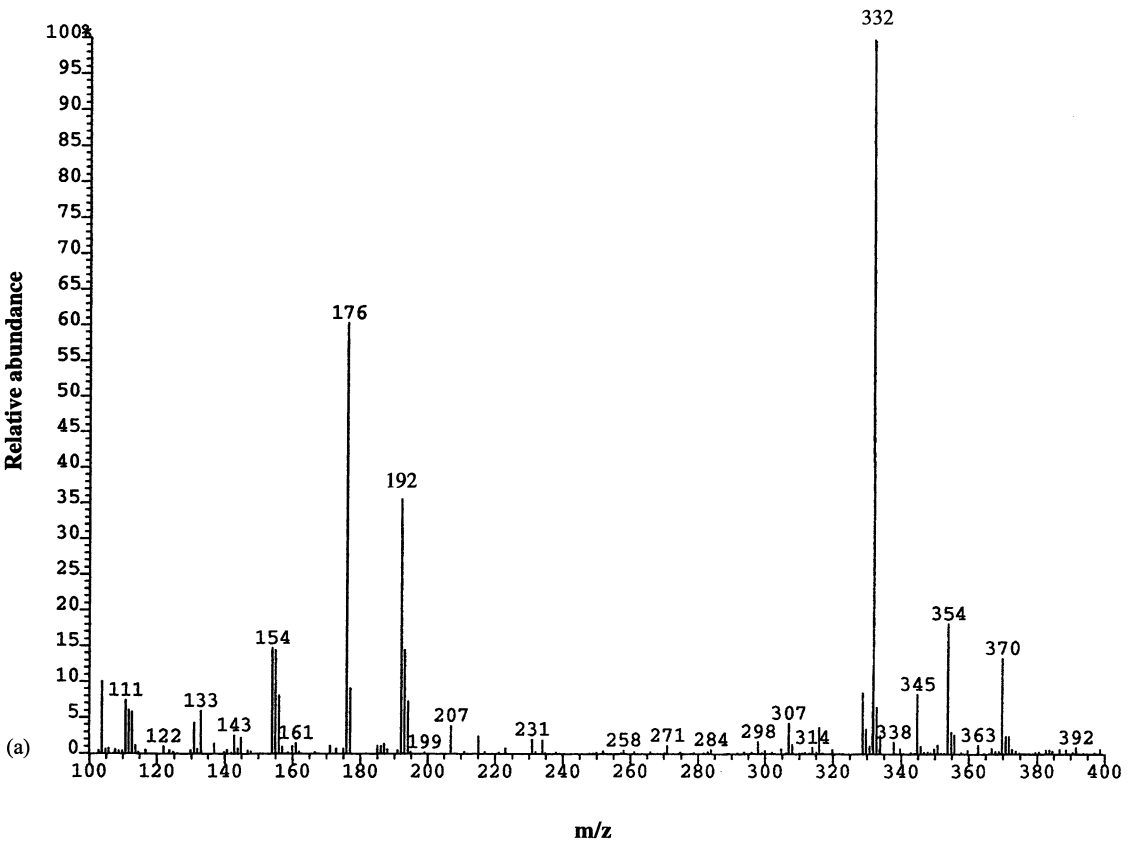

Fig. 6. Mass spectra for nizatidine after exposure to the simulated colonic conditions of the fermentation system, (a) 2 h, (b) 6 h, (c) 12 h, (d) 24 h.

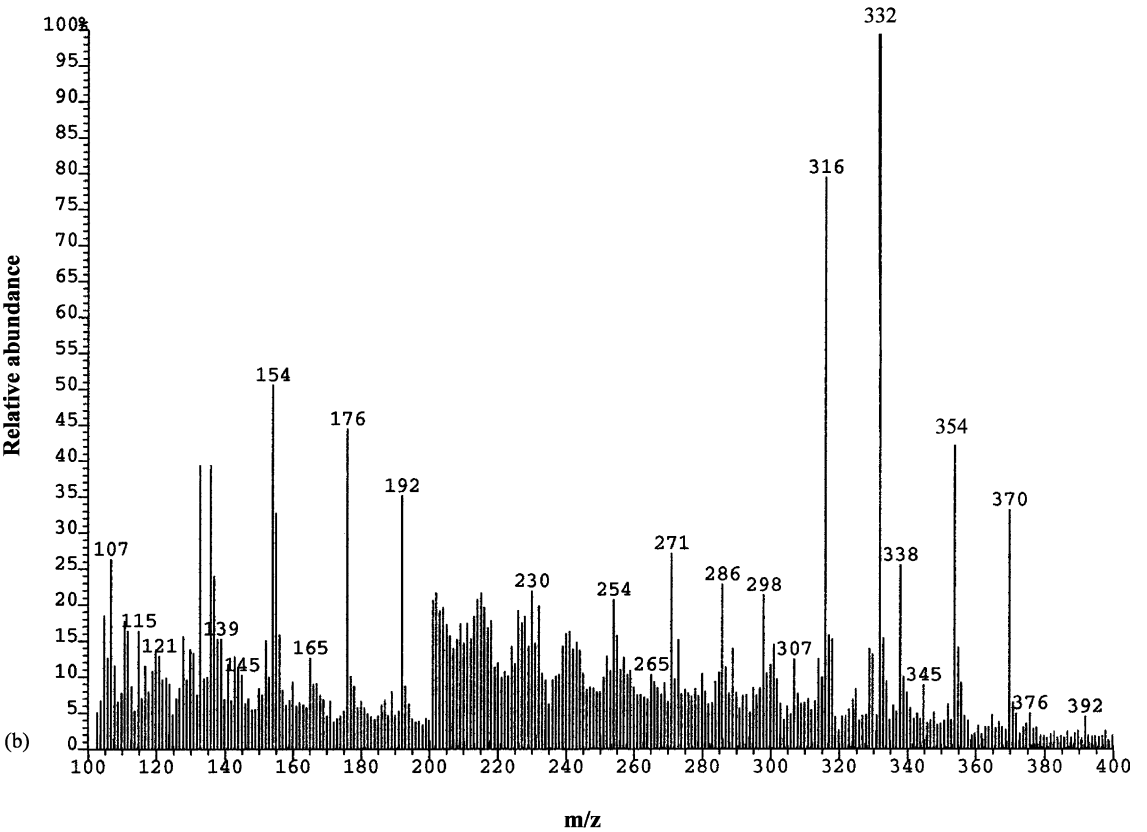

Fig. 6. (*Continued*)

cimetidine and famotidine, is therefore inherently unstable within the in vitro simulated colonic conditions of the fermentation system. This instability is likely to be due to metabolism of the drug by bacterial enzymes within the homogenised faeces of the fermentation system. The rate and extent of nizatidine metabolism was very similar to that observed previously with an equivalent quantity of ranitidine under identical testing conditions (Basit and Lacey, 2001). It was also found in this previous study with ranitidine that the rate and extent of metabolism was proportional to the quantity of drug ($100 > 200 > 500$ mg). Although it would have been of interest, for comparison purposes, to assess the stability of 200 and 500 mg sample sizes of cimetidine, famotidine and nizatidine in the present study, this was not deemed possible as these drugs are less soluble than ranitidine and, in some cases, would not have fully dissolved in the fermentation or control systems.

The HPLC chromatograms for the nizatidine samples obtained at selected times (2, 6, 12 and 24 h) from the fermentation experiments are shown in Fig. 5. In the early sample, one peak (retention time = 4.3 min), which can be assigned to the drug itself, is present on the chromatogram. Over time (6 and 12 h) this nizatidine peak becomes less prominent and is superseded by a major metabolite and a number of secondary metabolite peaks. The major metabolite also appears to undergo metabolism, as indicated by a reduction in its concentration in the final sample (24 h). These results suggest that the metabolic reaction pathway is complex, but nevertheless it would appear that, as was the case with ranitidine, nizatidine is metabolised to at least four different metabolites.

In order to identify the region of the nizatidine molecule that was being modified by the bacteria, UV spectroscopy was performed on the fermentation samples. Two distinct maxima at 258 and 314 nm were present on the UV spectra of the early samples (data not shown). The thiazole ring of the nizatidine molecule is responsible for the absorption maximum at 258 nm, while the diaminonitroalkene functional group on the right of the structure is responsible for the maximum at 314 nm. With time no significant change in the maximum at 258 nm was noted, whereas the maximum at 314 nm was less pronounced and was conspicuous by its absence from the 24 h sample, thereby inferring that the bacteria were metabolising the diaminonitroalkene region of the nizatidine structure.

As a means of elucidating the molecular weights of the nizatidine metabolites, the nizatidine fermentation samples were further evaluated using mass spectrometry. The mass spectra obtained for the nizatidine samples at times 2, 6, 12 and 24 h are presented in Fig. 6. In the 2 h sample, a major peak at 332 can be seen on the spectrum. This represents the molecular ion + 1 $(M + H)^+$ for nizatidine (molecular mass = 331). In the later samples, this peak is less prominent and a peak at 316 begins to dominate over all others. The emergence of this peak coincides with the disappearance of the parent peak and implies that nizatidine is being metabolised to a metabolite of 16 mass units lower than the parent drug. In combination with the UV spectroscopy data, these results would indicate that the loss of an oxygen atom, presumably by an enzymatic reductive process, from the diaminonitroalkene region of the nizatidine molecule is responsible for the generation of the metabolite. An identical mecha-

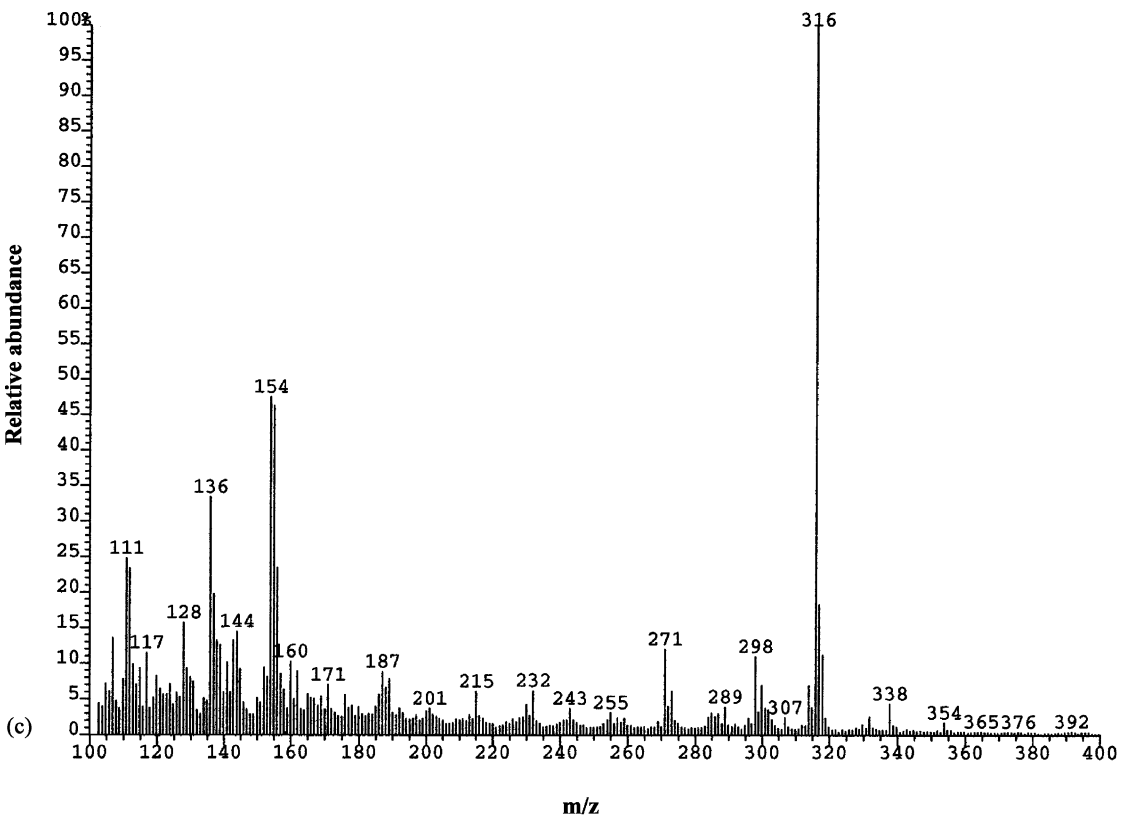

Fig. 6. (*Continued*)

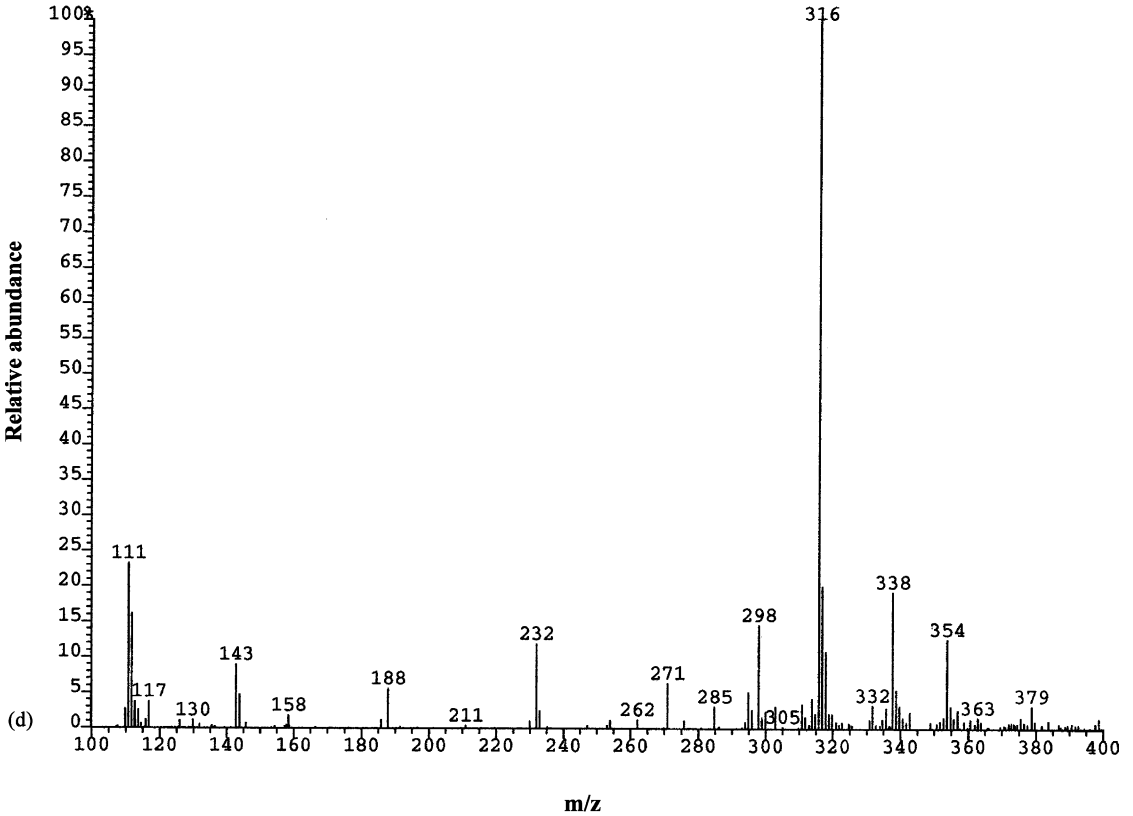

Fig. 6. (*Continued*)

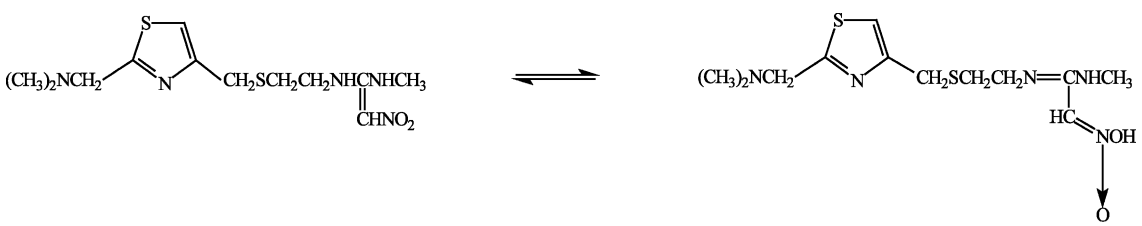

Fig. 7. Possible tautomeric structures of nizatidine.

nism was proposed for the metabolism of ranitidine (Basit and Lacey, 2001). Comparing the structure of nizatidine with that of ranitidine reveals very little difference between the two (Fig. 1). The only difference is the ring structure, which is of the thiazole variety for nizatidine and furan type for ranitidine. It is therefore perhaps not overly surprising that nizatidine also undergoes colonic bacterial metabolism. It has previously been proposed that the structure of ranitidine undergoes tautomerisation, and hence exists chemically in a different form to that usually represented in the literature (Hohnjec et al., 1986; Basit and Lacey, 2001). By virtue of the almost identical structure of nizatidine, it is feasible that it also undergoes tautomerisation in accord with the scheme shown in Fig. 7. In the tautomeric form of the structure, cleavage of the N-oxide bond would result in the loss of the oxygen atom and the formation of the metabolite shown in the

upper part of Fig. 8. This reaction pathway has not only been previously reported for ranitidine (Basit and Lacey, 2001) but also for nicotine N-oxide (Beckett et al., 1970) and loperamide N-oxide (Lavrijsen et al., 1995). In addition to this reaction, the nizatidine metabolite may also undergo further biotransformation. For instance, cleavage of the hydroxyl group attached to the nitrogen atom, a dehydroxylation reaction known to be initiated by the gastrointestinal microflora (Williams et al., 1970; Scheline, 1973), and loss of the hydrogen atom associated with the carbon atom on the other side of the double bond would generate the metabolite depicted at the bottom of Fig. 8. The loss of the hydroxyl group and hydrogen atom is essentially the removal of water and 18 mass units. This new metabolite would therefore be expected to have a molecular weight of 298. Interestingly, the mass spectra for the fermentation samples obtained at later time points display a peak, albeit one that is relatively small, at 298, thereby providing support for the proposed structure.

The HPLC results of the study suggest that nizatidine was metabolised to at least four different metabolites, which is in contrast to the mass spectrometry data that highlight the presence of only one or two metabolites. Potential reasons for this discrepancy are twofold. Firstly, the additional metabolite peaks may in fact be present on the mass spectra but are merely masked by the background components of the fermentation system, and hence difficult to identify. Alternatively, the metabolites may have simply fragmented during the ionisation process within the mass spectrometer. Despite the contradictory results obtained from these analytical techniques, it is possible that nizatidine and/or the metabolite will undergo further metabolism than that proposed in Fig. 8.

Overall, the results of this and the previous study (Basit and Lacey, 2001) clearly demonstrate that of the four commercially available H_2-receptor antagonists, nizatidine and ranitidine are susceptible to bacterial metabolism within the simulated colonic conditions of the fermentation system. On the assumption that a similar degree of metabolism also occurs in vivo, both drugs are liable to be poorly absorbed from the colon, as is the case with ranitidine (Williams et al., 1992). These findings not only have implications for the delivery and absorption of these drugs but also far more general consequences. The increasing interest in utilising extended- and colonic-release formulations will lead to a greater number of established and novel compounds being delivered

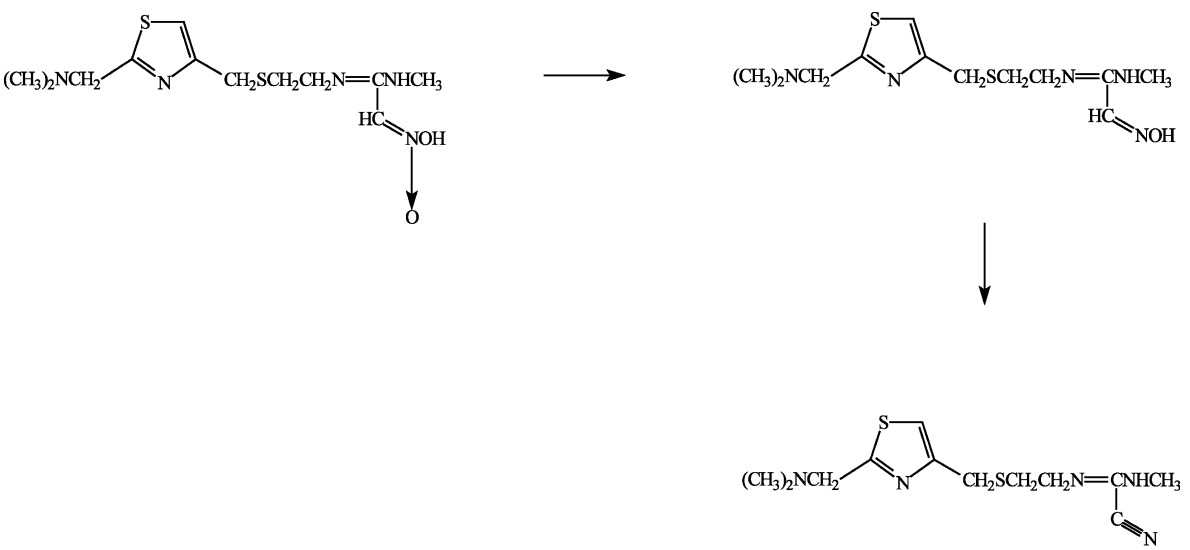

Fig. 8. Possible reaction pathway for the metabolism of nizatidine by colonic bacteria.

to the lower regions of the gastrointestinal tract. This will therefore increase the number of potential substrates for the microflora. The batch culture fermentation system described here, although not providing an exact replica of the human situation, can be used as a preliminary screen to assess the colonic stability of drugs that are likely to be exposed to the distal regions of the gut prior to embarking on a complex, time-consuming and expensive formulation development program.

Acknowledgements

The authors would like to thank Dr C.W. Leong, Dr L.F. Siew, Ms S.M. Man and Ms M.E. Shew for able technical assistance, Dr R.A. Watt for helpful discussions and GlaxoSmithKline (formerly GlaxoWellcome) for supporting this work.

References

Basit, A.W., Lacey, L.F., 2001. Colonic metabolism of ranitidine: implications for its delivery and absorption. Int. J. Pharm. 227, 157–165.

Beckett, A.H., Gorrod, J.W., Jenner, P., 1970. Absorption of (−)-nicotine-1′-N-oxide in man and its reduction in the gastrointestinal tract. J. Pharm. Pharmacol. 22, 722–723.

Cummings, J.H., Macfarlane, G.T., Draser, B.S., 1989. The gut microflora and its significance. In: Whitehead, R. (Ed.), Gastrointestinal and Oesophageal Pathology. Churchill-Livingstone, Edinburgh, pp. 201–219.

Englyst, H.N., Macfarlane, G.T., 1986. Breakdown of resistant and readily digestible starch by human gut bacteria. J. Sci. Food Agric. 37, 699–706.

Finegold, S.M., Sutter, V.L., Mathisen, G.E., 1983. Normal indigenous intestinal flora. In: Hentges, D.J. (Ed.), Human Intestinal Flora in Health and Disease. Academic Press Inc, London, pp. 3–13.

Hohnjec, M., Kuftinec, J., Malnar, M., Skreblin, M., Kajfez, F., Nagl, A., Blazevic, N., 1986. Ranitidine. In: Florey, K. (Ed.), Analytical Profiles of Drug Substances, vol. 15. Academic Press Inc, London, pp. 533–562.

Lavrijsen, K., Van Dyck, D., Van Houdt, J., Hendrickx, J., Monbaliu, J., Woestenborghs, R., Meuldermans, W., Heykants, J., 1995. Reduction of the prodrug loperamide oxide to its active drug loperamide in the gut of rats, dogs, and humans. Drug Metab. Dispos. 23, 354–362.

Macfarlane, G.T., Gibson, G.R., Macfarlane, S., 1993. Short chain fatty acid and lactate production by human intestinal bacteria grown in batch and continuous culture. In: Binder, H.J., Cummings, J.H., Soergel, K. (Eds.), Short Chain Fatty Acids. Kluwer Academic Publishers, London, pp. 44–60.

Mikov, M., 1994. The metabolism of drugs by the gut flora. Eur. J. Drug Metab. Ph. 19, 201–207.

Milojevic, S., Newton, J.M., Cummings, J.H., Gibson, G.R., Botham, R.L., Ring, S.G., Stockham, M., Allwood, M.C., 1996a. Amylose as a coating for drug delivery to the colon: preparation and in vitro evaluation using 5-aminosalicylic acid pellets. J. Control. Release 38, 75–84.

Milojevic, S., Newton, J.M., Cummings, J.H., Gibson, G.R., Botham, R.L., Ring, S.G., Stockham, M., Allwood, M.C., 1996b. Amylose as a coating for drug delivery to the colon: preparation and in vitro evaluation using glucose pellets. J. Control. Release 38, 85–94.

Scheline, R.R., 1973. Metabolism of foreign compounds by gastrointestinal microorganisms. Pharmacol. Rev. 25, 451–523.

Shamat, M.A., 1993. The role of the gastrointestinal microflora in the metabolism of drugs. Int. J. Pharm. 97, 1–13.

Siew, L.F., Basit, A.W., Newton, J.M., 2000a. The properties of amylose–ethylcellulose films cast from organic-based solvents as potential coatings for colonic drug delivery. Eur. J. Pharm. Sci. 11, 133–139.

Siew, L.F., Basit, A.W., Newton, J.M., 2000b. The potential of organic-based amylose–ethylcellulose film coatings as oral colon-specific drug delivery systems. AAPS PharmSciTech 1 (3) article 22 (http://www.pharmscitech.com).

Silvester, K.R., Englyst, H.N., Cummings, J.H., 1995. Ileal recovery of starch from whole diets containing resistant starch measured in vitro and fermentation of ileal effluent. Am. J. Clin. Nutr. 62, 403–411.

Williams, J.R., Grantham, P.H., Marsh, H.H., Weisburger, J.H., Weisburger, E.K., 1970. Participation of liver fractions and of the intestinal bacteria in the metabolism of *N*-hydroxy-*N*-2-fluorenylacetamide in the rat. Biochem. Pharmacol. 19, 173–188.

Williams, M.F., Dukes, G.E., Heizer, W., Han, Y.H., Hermann, D.J., Lampkin, T., Hak, L.J., 1992. Influence of gastrointestinal site of drug delivery on the absorption characteristics of ranitidine. Pharm. Res. 9, 1190–1194.