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# Colonic metabolism of ranitidine: implications for its delivery and absorption

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**Abstract**

The aim of this study was to assess the in vitro stability of ranitidine to colonic bacteria by utilising a batch culture fermentation system to simulate the conditions of the colon. Three quantities of ranitidine, 100, 200 and 500 mg, in the form of the hydrochloride salt, were introduced into individual 100 ml fermenters consisting of buffer medium inoculated with freshly voided human faeces (10% w/v). Control experiments were also run in parallel using equivalent drug quantities in buffer medium without the presence of faeces. Samples were removed at pre-determined time intervals over a 24 h period and were subsequently analysed by high-performance liquid chromatography (HPLC) for drug concentration. A selection of the samples removed from the fermenters was also analysed by conventional UV spectroscopy and mass spectrometry. Subsequent to an initial dissolution phase in the fermentation system, a marked decline in ranitidine concentration was noted over time, thereby suggesting degradation and metabolism of the drug by colonic bacteria. No such decline in concentration was noted in the control buffer systems. The rate and extent of metabolism was rapid and complete within 12 and 24 h for the 100 mg and 200 mg samples, respectively, although the largest sample size, 500 mg, was only partly metabolised over the course of the experiment. UV and mass spectrometry analysis indicated that metabolism occurred via cleavage of an N–oxide bond within the molecule with the resultant loss of an oxygen atom, although further metabolic reactions are possible. Such metabolism may in part be responsible for the poor bioavailability of ranitidine from the colon. 

*Keywords:* Ranitidine; Metabolism; Colon; Bacteria; Fermentation system; Drug delivery

## 1. Introduction

The microflora of the gastrointestinal tract constitutes a highly complex ecosystem containing more than 400 bacterial species (Finegold et al., 1983). Although these bacteria are distributed throughout the gastrointestinal tract, the vast majority are found in the large intestine where they mediate hydrolytic digestive functions using carbohydrates and proteins, which escape degradation in the stomach and small intestine, as

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substrates. The end products of such fermentation reactions are diverse, but quantitatively, ammonia, the short chain fatty acids, acetate, propionate and butyrate, and the gases hydrogen, carbon dioxide and methane are the major metabolites that are formed (Cummings et al., 1989). In addition to the fermentation of carbohydrates and proteins, the microflora have the potential to metabolise drugs and other foreign compounds (Scheline, 1973; Ilett et al., 1990; Shamat, 1993). Moreover, it has been suggested that the gastrointestinal microflora have the ability to act as an organ with a metabolic potential equal to, or sometimes greater than, that of the liver (Scheline, 1973). While the majority of liver metabolic reactions involve oxidation and conjugation, the microflora tend to mainly catalyse hydrolytic and reductive reactions, by virtue of the anaerobic environment within the lower regions of the gastrointestinal tract. It follows that while the liver will be exposed to all drugs absorbed from the gut, only those drugs reaching the lower confines of the gastrointestinal tract will be susceptible to the metabolic effect of the microflora. Apart from direct rectal administration, such a situation is likely to arise via the oral route when the drug is incompletely absorbed from the upper regions of the gut or is administered in the form of an extended-release or colon-specific formulation. In addition, drugs that are rapidly and completely absorbed from the stomach and small intestine, or those that are given by the parenteral route, will also come in contact with the microflora if they undergo diffusion or secretion from the systemic circulation into the lumen of the gut. These drugs may also be subject to enterohepatic recycling, whereby they are excreted in the bile possibly as conjugates that the gut flora can metabolise to regenerate the parent compounds (Shamat, 1993).

After exposure to the gastrointestinal microflora, drugs may become pharmacologically active, inactive or even toxic. Sulphasalazine, for example, is a well-known prodrug that undergoes pharmacological activation by the gastrointestinal microflora. Sulphasalazine, which consists of sulphapyridine and 5-aminosalicylic acid linked by an azo bond, has been used in the treatment of inflammatory bowel disease. The drug is metabolised by reduction of the azo bond by the gastrointestinal microflora, thereby releasing both compounds (Peppercorn and Goldman, 1972), of which 5-aminosalicylic acid is the pharmacologically active agent (Azad Khan et al., 1977; van Hees et al., 1978). On the other hand, the action of the gastrointestinal microflora has rendered other drugs, such as digoxin, levodopa, isosorbide dinitrate and metronidazole, less pharmacologically active and in some instances toxic (Scheline, 1980; Ilett et al., 1990; Shamat, 1993).

The purpose of this study was to assess whether ranitidine (Fig. 1), an $H_2$ receptor antagonist widely used in the treatment of conditions associated with excess gastric acidity, is a substrate for metabolism by colonic bacteria. Studies have shown that the bioavailability of ranitidine is markedly lower from the human colon than the upper regions of the gut (Williams et al., 1992; Pithavala et al., 1998). Potential reasons for this may include the small surface area available for absorption within the colon or that ranitidine is thought to be absorbed across the gastrointestinal epithelium in part via the paracellular route (Gan et al., 1993), a route that operates to a lesser extent within the colon (Hayton, 1980). However, it is not inconceivable that the colonic microflora may also have a role to play in this respect by way of metabolism, thereby reducing the amount of unchanged drug reaching the systemic circulation. This study was therefore concerned with assessing the in vitro stability of ranitidine under simulated conditions of the colon.

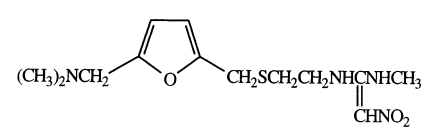


Fig. 1. Structure of ranitidine.

## 2. Materials and methods

### 2.1. Materials

Ranitidine, in the form of the hydrochloride salt, certified as 99% pure, was a gift from GlaxoSmithKline, Ware, UK. All other chemicals were of AnalaR or HPLC grade and were obtained form Merck, Poole, UK.

### 2.2. Fermentation studies

A batch culture fermentation system was utilised to simulate the conditions of the colon (Silvester et al., 1995; Milojevic et al., 1996; Siew et al., 2000a,b). Three quantities of ranitidine hydrochloride, equivalent to 100, 200 and 500 mg of the free base, were introduced into individual 100 ml fermenters inoculated with freshly voided human faeces (10% w/v). The faeces were obtained, and pooled, from three healthy female subjects. The fermenters were prepared by homogenising the faeces in a buffer medium comprising 0.15% potassium dihydrogen orthophosphate, 0.15% dipotassium hydrogen orthophosphate, 0.45% sodium chloride, 0.05% magnesium chloride hexahydrate, 0.005% ferrous sulphate heptahydrate, 0.015% calcium chloride dihydrate and sufficient sodium hydroxide to obtain a pH of 6.8. Prior to this, the buffer was boiled for at least 15 min to aid dissolution of the salts as well as removal of oxygen. The buffer solution was then cooled in a water bath to approximately 37 °C under nitrogen gas. The required quantity of faeces was weighed, added to the buffer solution and homogenised using a stomacher (model Stomacher 3500, Colworth, UK). The faecal slurry was passed through a 500 μm sieve to remove any unhomogenised fibrous material. The fermenters were sealed under positive nitrogen pressure to establish an anaerobic environment and then incubated at 37 °C in an orbital incubator. Each sample weight of ranitidine was assessed in triplicate. Control experiments were also run in parallel using equivalent drug quantities in buffer edium without the presence of faeces. Two millilitre samples were removed at 0, 1, 2, 4, 6, 12 and 24 h. These samples were centrifuged at 13 000 rpm for 5 min and filtered through 0.2 μm filters prior to ranitidine analysis using a validated HPLC assay (British Pharmacopoeia, 1998). Briefly, the assay method involved pumping the mobile phase, comprising 85% methanol and 15% 0.1 M ammonium acetate buffer, at a flow rate of 2 ml/min through a 200 mm × 4.6 mm column packed with 10 μm particle size Partisil (model 200ODS, Hichrom, Reading, UK). The detection wavelength was set at 322 nm. However, in order to determine the presence of metabolites, the fermentation samples were re-assayed using an HPLC assay procedure slightly modified from the aforementioned method. In this case, the mobile phase, consisting of 30% methanol and 70% 0.1 M ammonium acetate buffer, was pumped at a flow rate of 2 ml/min through a 100 mm × 4.6 mm column packed with 10 μm particle size Partisil. The wavelength of detection was set at 228 nm.

### 2.3. UV spectroscopy

The samples obtained from the fermentation experiments with 100 mg ranitidine were further evaluated using conventional UV spectroscopy. The samples were initially diluted 1 in 20 with water and then analysed using an UV-Vis spectrophotometer (model 554, Perkin Elmer, Ueberlingen, Germany) between the wavelengths of 190 and 400 nm. These samples were analysed against an identically diluted blank fermentation sample ($t = 0$ h) to counteract the influence of the faecal components on the UV absorption profile.

### 2.4. 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

The relative inaccessibility of the healthy human colon for routine experiments means that most studies concerned with fermentation or metabolism are conducted in animals or in vitro. In vitro investigations usually take the form of incubating the substrate with gut contents of an animal or man in a suitable medium (Ilett et al., 1990; Shamat, 1993). In terms of cost, availability and relevance, human faeces would appear to be the culture source of choice. Macfarlane et al. (1992) have shown that cultures taken from the human sigmoidal/rectal region and the more proximal regions of the colon, while displaying differences in their fermentation reactions, produced similar digestion products. In vitro studies using human faeces can therefore provide an indication of in vivo events in the colon. In addition to the choice of culture source, consideration must be given to the type of fermentation system to be employed (Macfarlane et al., 1993). These range from the relatively simple batch culture system to the more complex continuous system. In the continuous system, the source of nutrients is constantly replenished and the waste products selectively removed. This system mimics the constant change within the colon and may be set up as a multi-stage system to simulate the different regions of the colon. However, continuous culture systems involve a very complex culturing procedure and are only useful for experiments lasting over 24 h. Batch culture systems, on the other hand, are closed systems whereby the nutrients are not replenished and the waste products not removed. For experiments lasting 24 h or less, batch culture systems are cheaper, easier to set up and hence more practical. Therefore, batch cultures using freshly voided human faeces were used as means of simulating colonic conditions in vitro for the purpose of assessing ranitidine stability. Moreover, such a system has previously been used to assess the in vitro performance of bacterial enzyme-degradable film coatings based on amylose for colonic drug delivery (Milojevic et al., 1996; Siew et al., 2000a,b).

All three quantities of ranitidine dissolved rapidly in the fermentation systems with approximately 100% present in solution within 1 h (Fig. 2). After the first hour, however, a marked decline

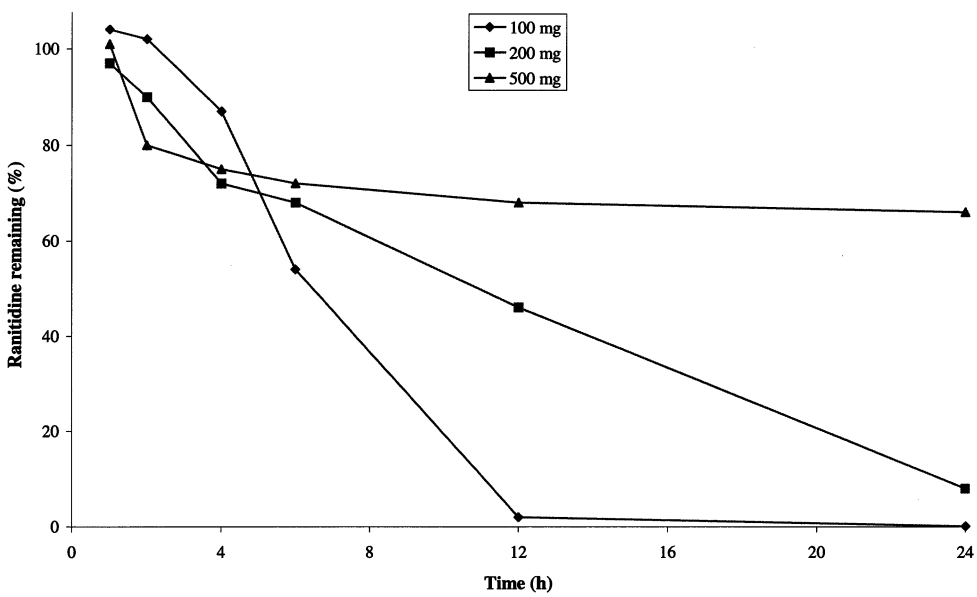


Fig. 2. Stability of different quantities of ranitidine within the simulated colonic conditions of the fermentation system. (Each point represents the mean of three experiments with a coefficient of variation of less than 5%).

in ranitidine concentration was seen, thereby suggesting instability of the drug. In contrast, no such decline in concentration was noted in the control phosphate buffer systems (data not shown). These results would imply that ranitidine was being metabolised or degraded within the fermentation system. More specifically, it would appear that the presence of bacteria within the homogenised faeces of the fermentation system was responsible for this effect. The rate and extent of ranitidine metabolism, although rapid and complete for the two smallest sample weights, terminates and reaches a plateau after a finite time in the case of the largest sample weight. The reason for this may be related to the high sample weight of drug added, which may have led to the drug and/or the resultant metabolite(s) having a bacteristatic or bactericidal action on the system. Overall though, the rate of metabolism was proportional to the sample weight of ranitidine.

As a means of elucidating the structure of the resultant ranitidine metabolite(s), the fermentation samples were subjected to analytical techniques such as UV spectroscopy and mass spectrometry. UV spectroscopy, despite being a primitive tool in structure determination, provides a simple means of identifying chromophores within organic molecules. Chromophores are essentially electron containing regions that are responsible for UV absorption. Hence, any structural changes on conversion from the parent drug to the metabolite(s) may also manifest in changes to the chromophores, thereby revealing different UV spectra. The UV spectra obtained for the early samples (1 and 2 h) displayed two discrete UV absorption maxima at 228 and 313 nm (data not shown). In the later samples, however, the peak at 313 nm became less pronounced and was completely absent from the 24 h sample. The rate of disappearance of this peak appeared to correlate with the ranitidine degradation rate data obtained by HPLC (Fig. 2). The UV results would indicate that the bacteria within the fermentation system were altering the chromophore

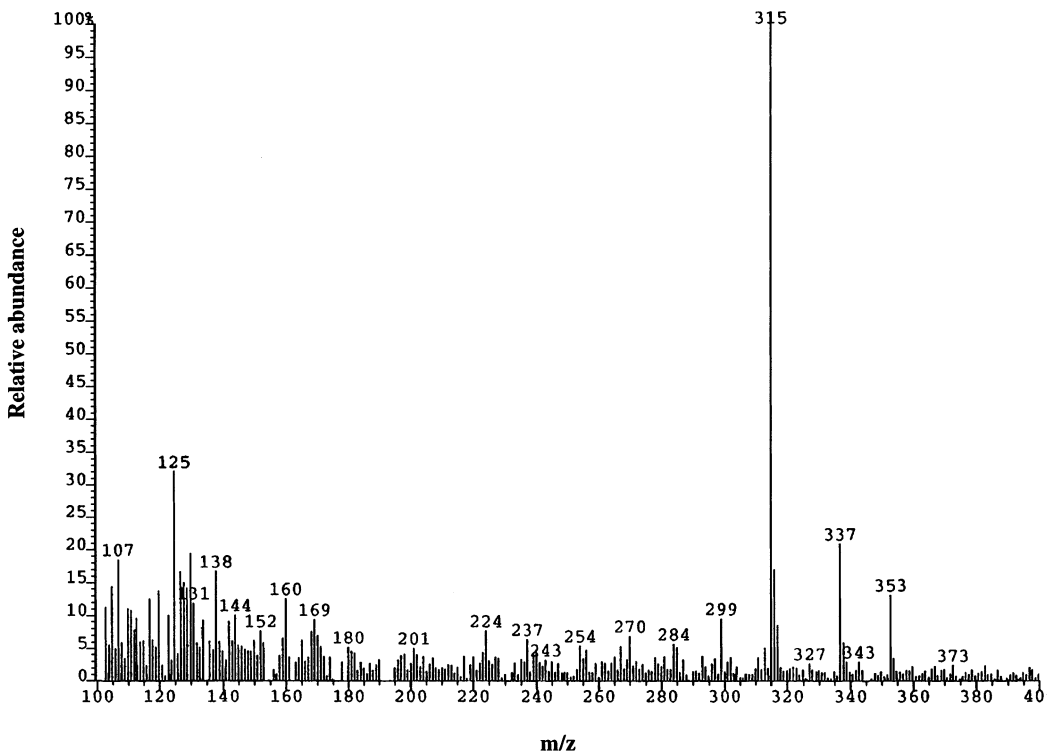


Fig. 3. Mass spectrum for ranitidine after exposure to the simulated colonic conditions of the fermentation system (2 h).

responsible for ranitidine absorption at 313 nm. From inspection of the structure of ranitidine (Fig. 1), two distinct chromophores are present within the molecule. The furan ring is responsible for the absorption maximum at 228 nm, whereas the diaminonitroalkene portion on the right of the structure is responsible for the peak at 313 nm. The bacteria were therefore altering the diaminonitroalkene region of the structure.

Since the resultant metabolite(s) absorb at 228 nm, it was therefore deemed possible to detect their presence using an HPLC assay, slightly modified from the original method, at a detection wavelength of 228 nm. On re-assaying the early ranitidine samples, a major peak appeared on the chromatograms after the ranitidine peak. With time this metabolite peak became more intense, whereas the main drug peak became smaller in size, and in fact completely disappeared from the later samples. In addition to the primary metabolite peak, three further peaks were observed in the later samples, thereby leading to the suggestion that ranitidine was being metabolised to at least four different metabolites.

The ranitidine fermentation samples were further evaluated using mass spectrometry in order to determine the molecular weights of the metabolites. The mass spectra obtained for the ranitidine samples at times 2, 6 and 24 h are shown in Figs. 3–5, respectively. In the 2 h sample, the abundant peak at 315 is the $(M + H)^+$ peak for ranitidine (molecular mass = 314). At later times, the 315 peak is less prominent and the peak at 299 begins to overshadow all others. The appearance of this peak coincides with the disappearance of the parent peak and implies that ranitidine is being metabolised to a metabolite of a molecular weight lower than the parent drug by 16 mass units. This is in contrast to the HPLC results, which indicate the presence of more than one metabolite. Nevertheless, since reduction is a primary mechanism of metabolism by colonic bacteria, the metabolite of

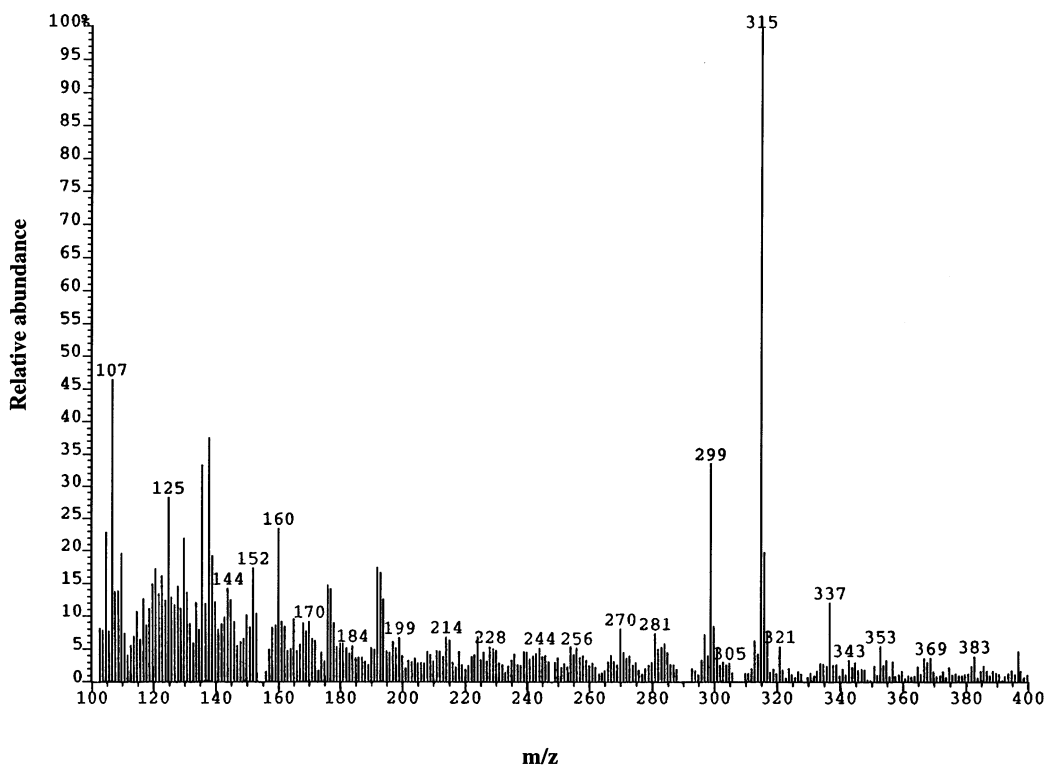


Fig. 4. Mass spectrum for ranitidine after exposure to the simulated colonic conditions of the fermentation system (6 h).

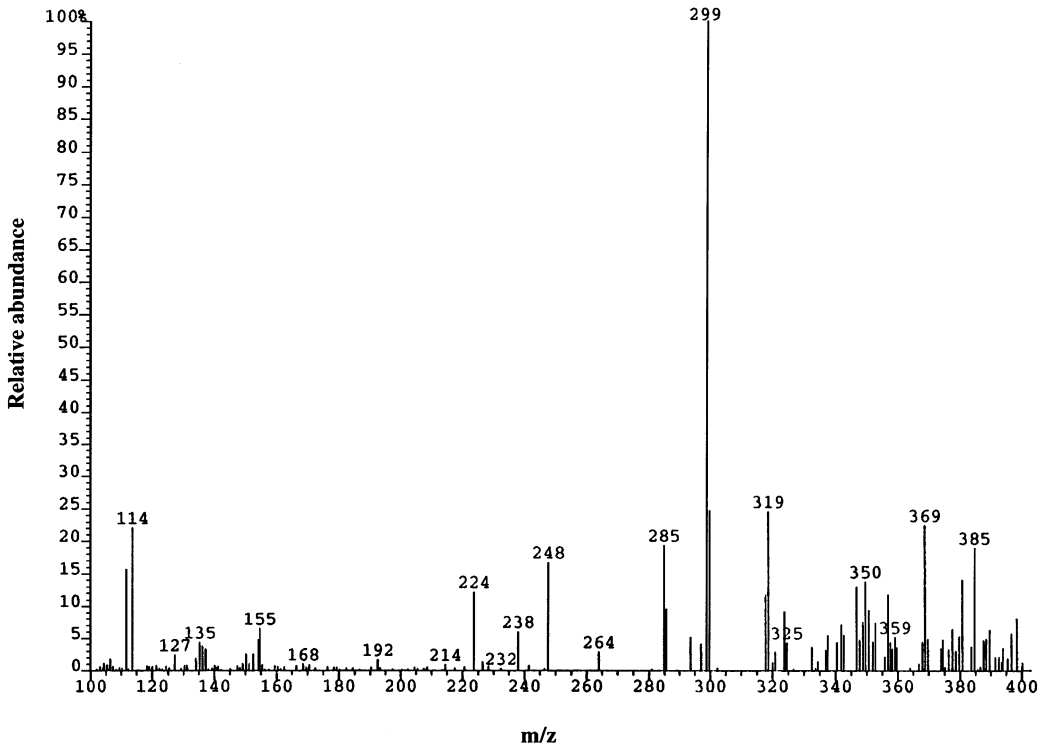


Fig. 5. Mass spectrum for ranitidine after exposure to the simulated colonic conditions of the fermentation system (24 h).

molecular weight 299 may be attributable to the loss of an oxygen atom from the parent compound. These results, in combination with those obtained by UV spectroscopy, would suggest that the oxygen atom was cleaved from the diaminonitroalkene region of the ranitidine molecule. In this region of the molecule, two oxygen atoms form part of a nitro functional group. However, infrared and X-ray diffraction studies have revealed that ranitidine undergoes tautomerisation and exists chemically, not in the form usually represented in the literature (Fig. 1 and on the left hand side of Fig. 6) but in the tautomeric form shown on the right of Fig. 6 (Hohnjec et al., 1986). In this form, an N–oxide grouping is clearly present. Cleavage of this N–oxide bond would result in the loss of the oxygen atom and the formation of the ranitidine metabolite depicted in Fig. 7. A similar mechanism of metabolism was shown to occur with nicotine N–oxide (Beckett et al., 1970) and loperamide N–oxide (Lavrijsen et al., 1995), in which cleavage of the oxygen atom by bacterial N–oxide reductase enzymes in the gut resulted in the formation of nicotine and loperamide, respectively.

In spite of the reaction pathway and metabolite structure proposed in Fig. 7, it is likely that the drug and/or metabolite will undergo further biotransformation. Regardless of the precise mechanisms involved and the final end product of the reaction, this study amply demonstrates that colonic bacteria play a significant role in the metabolism of ranitidine. Furthermore, the limited colonic bioavailability of ranitidine might in part be attributed to bacterial metabolism, assuming that a similar mechanism of metabolism occurs in vivo. In general terms, the consequences of such metabolism have important implications in the development of extended-release and colon-specific drug delivery systems. For drugs that are substrates for bacterial metabolism, extending or targeting release beyond the small intestine holds

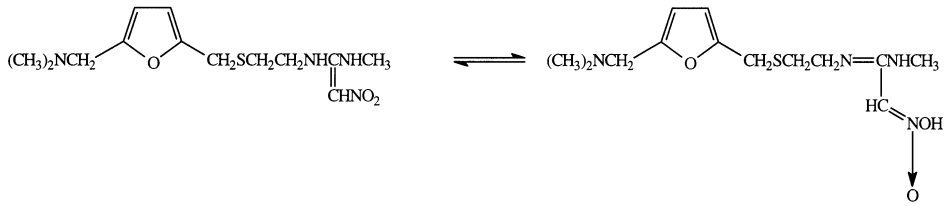

Fig. 6. Tautomeric structures of ranitidine.

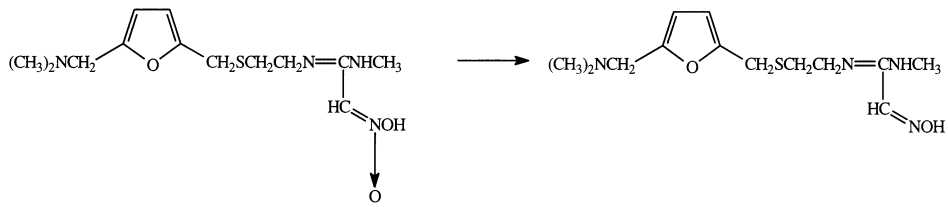

Fig. 7. Possible reaction pathway for the metabolism of ranitidine by colonic bacteria.

little promise in attaining therapeutic levels of drug in the blood or locally within the colon. Moreover, drugs that are incompletely absorbed during passage through the stomach and small intestine, either because of poor aqueous solubility or membrane permeability, are also potential candidates for metabolism — notwithstanding the fact that the small intestine may also be a site for drug metabolism as it contains similar bacterial species to those in the colon, albeit to a lesser extent. Therefore, studies on the gastrointestinal microflora as a potential site for drug metabolism should be considered an essential part of the development program for those compounds that are likely to be presented to the lower confines of the gut.

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