Chinese Journal of Digestive Diseases 2003; **4**; 140–146

Acid neutralization and bile acid binding capacity of hydrotalcite compared with other antacids: An *in vitro* study

S-E MIEDERER,* M WIRTZ† & B FLADUNG‡

*University of Bonn, Department of Internal Medicine, Gastroenterology and Metabolism, University of Bielefeld, †Bayer Health Care, Pharma Operations International and ‡Bayer Health Care, Consumer Care Europe R&D Medical Affairs, Leverkusen, Germany

OBJECTIVE: Antacids are used worldwide for the treatment of acid-related conditions such as heartburn and gastro-esophageal reflux disease. The present study investigated the *in vitro* acid neutralization and bile acid binding capacities of hydrotalcite compared with other antacids that are available in Germany and China. It has been reported that hydrotalcite is effective in the treatment of bile reflux gastritis because of its binding capacity to bile acids.

METHODS: Hydrotalcite and other preparations of antacids were tested in a comparative *in vitro* study. The neutralization properties were determined with static and dynamic tests (preliminary antacid test, acid neutralization capacity (ANC) test, Rossett-Rice (RR) test) and the bile acid binding test with a standardized commercial test kit (Merckotest bile acids).

RESULTS: In the static and dynamic tests hydrotalcite 500 mg and some other antacids, such as magaldrate, almasilate, algedrate Mg hydroxide, Ca/Mg carbonate and calcium carbonate, demonstrated favorable ANC reflected by rapid onset and long duration of action, and high buffering capacity. In the RR test, hydrotalcite dose of 1000 mg was able to keep the pH level above 3 for 76.9 min. The bile acid binding capacity test yielded that hydrotalcite had the highest binding potential to taurodeoxycholic acid, a lipophilic bile acid associated with cell and mucosa toxicity.

CONCLUSIONS: Hydrotalcite has a rapid onset of action, a high buffering capacity and a long duration of action. In particular, hydrotalcite binds cytotoxic bile acids. These pharmacochemical properties make hydrotalcite a most suitable antacid.

KEY WORDS: acid neutralization capacity, bile acid binding, duration of antacid action, hydrotalcite, *in vitro* comparison

INTRODUCTION

Antacids, which are used worldwide for the treatment of acid-related conditions such as heartburn and gastro-esophageal reflux disease (GERD), are the products of choice for self-medication of heartburn, stomach discomfort, bloating or acid eructation and other acid-related symptoms.[1] In the treatment of more serious disorders such as chronic gastritis, peptic ulcer or reflux esophagitis, antacids are an adjunct therapy.[2,3] The causes of acid-related conditions in the stomach are many, but there is a common pathomechanism: the critical imbalance between aggressive and protective factors. To a varying extent, antacids are able to neutralize the gastric acid or inactivate the bile acids and pepsin, which are the endogenous noxious agents.[1,2,4–6]

Because hyperacidity is not the only pathogenic factor in the development of acid-related gastrointestinal complaints, numerous important pharmacological properties have been identified as necessary for the

Correspondence to: Professor Siegfried-Ernst MIEDERER, Department of Internal Medicine, Gastroenterology and Metabolism, Ev. Krankenhaus University of Bielefeld, Germany.

Table 1. Antacid preparations tested including combinations available in Germany and China

Antacid preparation	Active ingredient(s)	Single dose
Kompensan tablets	300 mg Al-Na-CO_3-$(OH)_2$	1–2 tablets
Riopan stomach tablets	800 mg magaldrate	1 tablet
Simagel chewable tablets	430 mg almasilate	2 tablets
Talcid chewable tablets	500 mg hydrotalcite	1–2 tablets
Maalox 25mVal	400 mg algeldrate, 400 mg $Mg(OH)_2$	1–2 tablets
Rennie tablets	680 mg $CaCO_3$, 80 mg $MgCO_3$	1–2 tablets
Sidashu capsules	140 mg $Al(OH)_3$, 10 mg belladonna dry extract 50 mg iodine-methyl-methion	1 capsule
Pepcid dual chewable tablets	10 mg famotidine, 165 mg $Mg(OH)_2$, 800 mg $CaCO_3$	1 tablet

modern antacid of choice:[1,2,7] pH interval of 3–5, but no acid rebound provocation; fast onset of action; long-lasting efficacy; high neutralization capacity; reversible inactivation of pepsin; binding of cytotoxic bile acids; stimulation of gastric prostaglandin synthesis; minimal absorption and stimulation of heat shock proteins (chaperones).[8]

Antacids can be classified into carbonates and hydrogen carbonates such as $MgCO_3$, $CaCO_3$ and $NaHCO_3$, hydroxides such as $Mg(OH)_2$, $Ca(OH)_2$ and $Al(OH)_3$, layer–lattice structures such as hydrotalcite, magaldrate and almasilate, and combinations of these. Although carbonates and hydrogen carbonates have a strong antacid action, the immediate release of the acid neutralization capacity (ANC) may lead to a steep increase in gastric pH (pH >6), which may provoke acid rebound and result in adverse effects such as bloating, meteorism and eructation[2,3] because of the release of a large volume of carbon dioxide (CO_2). Furthermore, immediate release of the entire ANC may result in early exhaustion of the neutralization capacity, which is then reflected in a shorter duration of action. The variable duration of antacid action was investigated in the present *in vitro* study.

Bile acids are breakdown products of cholesterol and because of their bipolar structure they act as natural detergents in the emulsification of alimentary fats. They consist mainly of cholic acid, deoxycholic acid and chenodeoxycholic acid, and are largely present as conjugates of the amino acids glycine and taurine. In the event of a duodenogastric reflux, the bile fluid can reach the stomach and if there is existing GERD, bile acids may enter the esophagus and act as endogenous irritative agents, which with long-term exposure result in ulceration and considerably increase the likelihood of a malignant development.[5,9,10] It has been reported that hydrotalcite is effective in the treatment of bile reflux gastritis[11] and for this reason, the observed binding of harmful bile acids is regarded as an additional benefit of the antacids with a layer-lattice structure, and was therefore determined in the present *in vitro* study as an additional parameter.[2,6,7]

MATERIALS AND METHODS

The preparations and doses compared in this study are shown in Table 1.

Acid neutralization

The preliminary antacid test (PAT) is a static test derived from the United States Pharmacopeia 23 and it examines the antacid efficacy of a product in accordance with the FDA standard (Code of Federal Regulations). We used a Mettler DL77 Titrator, Mettler Toledo balance AX 205 and the following reagents: purified water, 0.5 N HCl (Merck) and buffer solutions (pH 2–7) (Merck, Darmstadt, Germany; for calibration of the glass electrode). The tested preparations were crushed and mixed with water and 0.5 N HCl was added. Finally the pH value was determined.

The acid neutralization capacity test (ANC test) is also a static test derived from USP 24 and it determines the acid binding capacity. We used a Mettler DL77 Titrator, Mettler Toledo balance AX 205 and the following reagents: purified water, 1.0 N HCl (Merck), 0.5 N NaOH (Merck), buffer solutions (pH 2–7) (Merck; for calibration of the glass electrode).

The tested preparations were crushed and suspended in water, and 1.0 N HCl was added. After 5 min, the excess acid was back-titrated with 0.5 N NaOH up to pH 3.5. The entire process was carried out at 37°C. The ANC is determined in milli-equivalents per gram (mEq/g) substance. The test was carried out three times with each preparation; the arithmetic mean was calculated and the neutralization capacity per gram preparation and per tested dose was determined.

The Rossett-Rice (RR) test is a dynamic test in which the antacid is exposed to a defined quantity of acid, and in addition, a constant flow of acid is added. The objective of this procedure is to match the *in vitro* test with physiological conditions as far as possible. We used a Mettler DL77 Titrator (the method was programmed using dynamic titration), Mettler Toledo balance AX 205, circulation thermostat D8-G Haake, 665 Dosimat Metrohm (Filderstadt, Germany) and the following reagents: purified water, 0.1 N HCl (Merck), buffer solutions (pH 2–7) (Merck; for calibration of the glass electrode) and ethanol (96% v/v).

The RR test has been described previously.[12–14] Briefly, a crushed single dose of antacid is suspended in 96% ethanol, mixed with water and 0.1 N HCl. Subsequently, a constant flow of 0.1 N HCl with a flow rate of 3 mL/min is begun. The pH value is automatically recorded every 30 s with the cut-off at pH <2.

Bile acid binding

The static analytical model used in the present study was adopted from other studies.[5,15,16] The bile acid concentrations applied should reflect the concentration range of refluxate from *in vivo* measurements.[10] The method of quantifying the bile acids was also adopted from previous studies[15,16] and carried out using a standardized, commercial test kit (Merckotest bile acids). The analytical procedure was calibrated first.

We used an Ikamag Reo IKA Labortechnik (magnetic stirrer), pH meter 763 Knick, Omnifuge 2.ORS Heraeus Sepatech (centrifuge), HP 8452 Diode Array Spectral Photometer, Mettler Toledo balance AX 205 and a water bath 1023 GLF (Giessen, Germany). The following reagents were used: purified water, 0.1 N HCl (Merck), bile acids (Sigma-Aldrich; bile acids, conjugated 1 kit), Merckotest bile acids (Merck) and buffer solutions (pH 2–7) (Merck; for calibration of the glass electrodes).

A crushed single dose of antacid was suspended in 0.1 N HCl and then 5 mmol/L concentrated solution of the corresponding bile acid (e.g. glycocholic acid, taurocholic acid, taurodeoxycholic acid as the sodium salts) was added. The concentration of the added artificial bile corresponded to *in vivo* conditions[14] and, as mentioned before, yielded an end concentration corresponding to that of the refluxate (i.e. ≈250 μmol/L).[10] A sample was then taken every 10 min from the solution, which continued to be stirred continuously without replacing the removed volume. The pH value was noted at the time every sample was taken. The removed sample was immediately centrifuged and processed. Finally a triple photometric measurement was made against a blank sample at λ = 500 nm.

The principle of the analytical procedure is based on the oxidation of the 3α-hydroxy bile acids by 3α-steroid dehydrogenase to their ketone derivatives. The resulting NADH (nicotinamide adenine dinucleotide) reduces nitrotetrazolium blue to a formazan derivative, which absorbs at λ = 500 nm.

Table 2. pH values obtained in the preliminary antacid test (PAT)

Drug	pH value after PAT
Al/Na carbonate	3.61
Magaldrate	5.02
Almasilate	3.92
Hydrotalcite	4.31
Algedrate/Mg hydroxide	4.29
Ca/Mg carbonate	5.46
Belladonna dry extract/ iodine-methyl-methion	1.10
Ca/Mg carbonate/famotidine	6.23

RESULTS

Acid neutralization

The PAT, which only measures the pH after acid exposure, yielded large differences between the different preparations tested. Belladonna dry extract/iodine-methyl-methion achieved a pH of only 1.1, and Al/Na carbonate and almasilate elevated the pH only slightly above 3.5. Magaldrate, hydrotalcite 500 mg and algedrate/Mg hydroxide yielded pH values above 4. Ca/Mg carbonate and Ca/Mg carbonate/famotidine produced pH values greater than 5 (Table 2).

In the ANC test, the neutralization capacity of the antacids tested differed considerably. Belladonna dry extract/iodine-methyl-methion did not demonstrate significant acid neutralizing capacity, whereas Al/Na carbonate had an ANC of slightly less than 10, hydrotalcite 500 mg, and Ca/Mg carbonate showed a moderately strong ANC of 10–17 mEq, and magaldrate, almasilate, algedrate/Mg hydroxide, and Ca/Mg carbonate/famotidine all had an ANC greater than 20 mEq per tested dose (Table 3).

The highest ANC per gram substance was achieved by almasilate followed by algedrate/Mg hydroxide and hydrotalcite (Figure 1).

Table 3. Overview of measured acid neutralization capacity (ANC)

Drug	ANC_1 (mEq/g)	ANC_2 (mEq/g)	ANC_3 (mEq/g)	Mean ANC (mEq/g)	Average mass/ unit (g)	Average ANC/ tested dose (mEq)	Single dose tested
Al/Na carbonate	10.36	8.39	10.35	9.70	1.02	9.89	1 tablet
Magaldrate	14.49	11.23	12.19	12.64	2.00	25.28	1 tablet
Almasilate	21.98	19.71	21.21	20.97	0.51	21.39	2 tablets
Hydrotalcite	17.78	15.26	17.19	16.77	1.01	16.94	1 tablet
Algedrate/Mg hydroxide	17.38	16.94	17.13	17.15	1.21	20.75	1 tablet
Ca/Mg carbonate	11.75	11.78	11.74	11.76	1.33	15.64	1 tablet
Belladonna dry extract/ iodine-methyl-methion	9.66	9.99	9.59	9.75	0.25	2.44	1 Kaps
Ca/Mg carbonate/famotidine	12.25	11.79	12.28	12.11	1.78	21.56	1 tablet

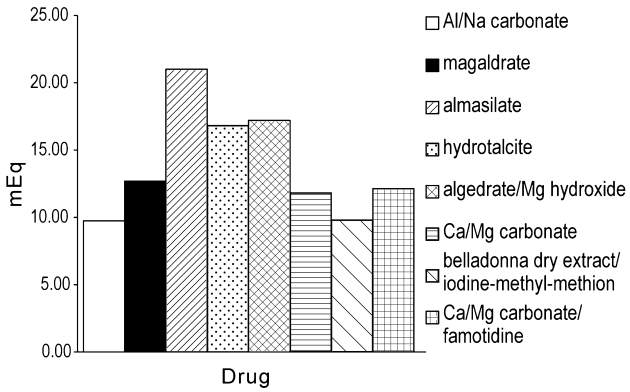

Figure 1. Mean acid neutralizing capacity (ANC) per gram of preparation.

The RR test as a dynamic test is closest to the conditions in the human gastric luminal environment because hydrochloric acid is constantly added over time. With regard to the ANC per dose, 1000 mg of hydrotalcite compares to 800 mg magaldrate.

The pH curve over time for the antacid preparations tested in the RR test and the different effects of antacids on the pH of the test solution are shown in Table 4 and Figure 2. Several antacids elevate the pH above 6, but maintain it above 3 for less than 50 min. Only hydrotalcite in the dose of 1000 mg maintained the pH above 3 for 76.9 min.

Bile acid binding

Taurodeoxycholic acid is a very lipophilic bile acid and has the highest damage potential to the gastric mucosal epithelium. The study of the bile acid binding capacity demonstrated that hydrotalcite, in contrast to all the other products examined, had by far the highest binding potential to taurodeoxycholic acid, which is associated with the highest cytotoxic potential[16] (Figure 3). The other antacids products tested showed comparable binding potential with the more polar bile acids.

DISCUSSION

Antacids are heavily used worldwide for the treatment of symptoms of acid-related conditions (i.e. heartburn, functional dyspepsia and GERD). The clinical endpoint, 'relief', however, depends on the performance of antacids within certain pharmaceutical parameters, which can be measured using well-established test methods. The elimination of excess acid by antacids is critical for the relief of the resultant symptoms (i.e. heartburn, stomach pain). The outcome of such acid neutralization tests can therefore be predictive of the clinical effectiveness of the antacid preparation to relieve gastrointestinal symptoms.

In the acid neutralization tests performed in the present *in vitro* study the antacid properties of hydrotalcite and other relevant antacids available in Germany and China were investigated. The preliminary antacid test, which only measures the pH after acid exposure, yielded large differences between the different preparations tested: belladonna dry extract/ iodine-methyl-methion (Sidashu) achieved a pH of only 1.1 and Al/Na carbonate and almasilate elevated the pH slightly above 3.5, whereas the other preparations (i.e. magaldrate, hydrotalcite 500 mg, algedrate/ Mg hydroxide, Ca/Mg carbonate and Ca/Mg carbonate/ famotidine) clearly raised the pH above 3.5. Only two preparations (Ca/Mg carbonate and Ca/Mg carbonate/ famotidine) raised the pH above 5. Although this strong effect on the pH appears desirable at first

Table 4. Comparison of antacid preparations in Rossett-Rice (RR) test

Drug	Time to pH >3 (s)	RR time (min) pH = 3–5	Duration (min) pH >2	pH >3	pH >5
Al/Na carbonate	57	3.9	11.1	3.9	0.0
Magaldrate	18	48.5	62.8	48.5	0.0
Almasilate	35	45.3	70.2	45.3	0.0
Hydrotalcite (1000 mg)	21	76.6	91.6	76.9	0.0
Algedrate/Mg hydroxide	61	43.8	67.2	43.8	0.0
Ca and Mg carbonate	24	22.2	36.5	30.5	7.9
Belladonna dry extract/iodine-methyl-methion	No effect	0	0	0	0
Ca/Mg carbonate/famotidine	16	21.9	56.9	46.1	23.9

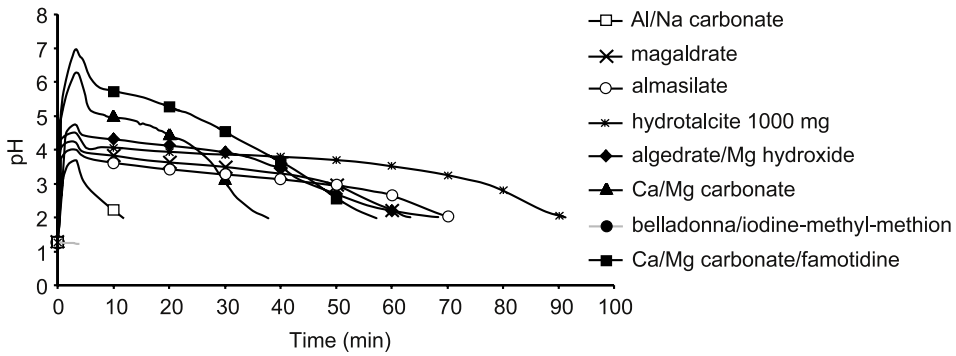

Figure 2. pH curve over time for the antacid preparations tested in the Rossett-Rice test.

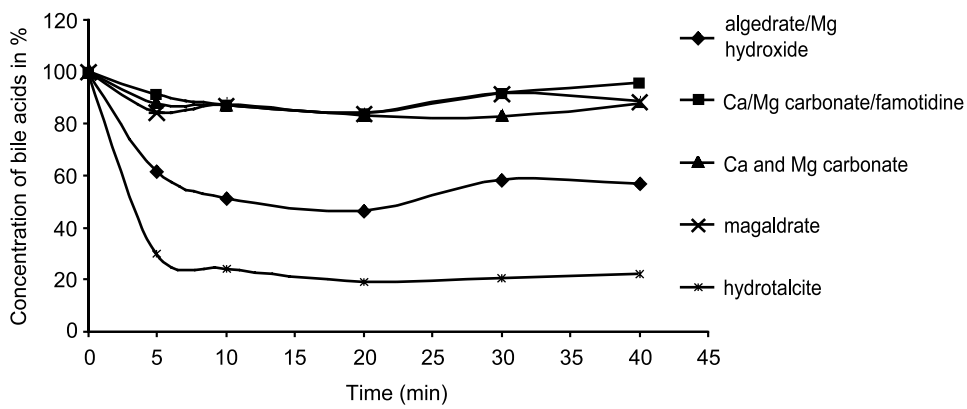

Figure 3. Binding of taurodeoxycholic acid by the tested antacid preparations.

glance, there are two potential disadvantages of a pH greater than 5.

1 Does the high pH achieved by an antacid preparation reflect immediate depletion of the entire antacid capacity?

2 Would it not be more useful to preserve some antacid capacity in order to keep a balanced pH over an extended period of time?

The second static test, the ANC test, compared the neutralization capacity of the antacid, providing

information on the potency per gram and per tested dose. In this test belladonna dry extract/iodine-methyl-methion did not demonstrate a satisfactory antacid effect given that it showed almost no ANC. Although Al/Na carbonate demonstrated a mean ANC of just below 10, hydrotalcite 500 mg, and Ca/Mg carbonate exceeded a mean ANC of 15, and magaldrate, almasilate, algedrate/Mg hydroxide and Ca/Mg carbonate/famotidine all had a mean ANC above 20. As belladonna dry extract/iodine-methyl-methion is obviously not a suitable antacid preparation, smaller differences in ANC may be balanced by an increase of the recommended dose. Antacids are drugs that have only local effect and little or no toxicity, which explains the high variability of recommended doses. For this reason hydrotalcite, which was tested in the 500 mg dose, is also available in 1000 mg preparations.

More important than the ANC values is the issue of how long a pH above 3 can be sustained. The RR test, which imitates the physiological process of constant addition of hydrochloric acid, provided important results in this respect. Although all the antacids started working within 1 min (with the exception of belladonna dry extract/iodine-methyl-methion, which in confirmation of the ANC test result also did not show neutralization properties in this test according to USP 23), the antacid preparations differed considerably with regard to the RR time. Hydrotalcite 1000 mg demonstrated by far the longest time during which the pH is kept between 3 and 5 (76.9 min), followed by magaldrate (48.5 min), almasilate (45.3 min) algedrate/Mg hydroxide (43.8 min) and Ca/Mg carbonate/famotidine (21.9). Because Ca/Mg carbonate/famotidine elevated the pH above 5 for another 23.9 min, the overall duration for pH above 3 was 46.1 min The RR time of Ca/Mg carbonate was 22.2 min (30.5 min >pH 3) and of Al/Na carbonate 3.9 min These results indicate that for those preparations in which high acid neutralization capacity is not depleted immediately by an immediate rise of the pH beyond 5 or even 6, there is a longer duration of the pH being held above 3. A pH >5 achieved by certain antacid preparations may be the result of immediate release of the entire antacid capacity, but it may be an advantage to preserve some antacid capacity in order to keep a balanced pH over an extended period of time, as confirmed by the results of the RR test.

In terms of the binding of taurodeoxycholic acid, hydrotalcite 500 mg was the most potent antacid preparation tested, which is important to note because taurodeoxycholic acid is a very lipophilic bile acid with the highest cytotoxic potential.[5,16] The investigation of the pH dependence of the binding of taurodeoxycholic acid with hydrotalcite confirmed a binding maximum at pH >4, as determined in other publications.[15,16]

Ca/Mg carbonate and Ca/Mg carbonate/famotidine exhibited a markedly lower binding capacity to taurodeoxycholic acid than hydrotalcite 500 mg, which was not surprising considering that the binding surface is rapidly reduced by the antacid carbonates. However, this is contradicted by the results on the binding of taurocholic acid because Ca/Mg carbonate and Ca carbonate/famotidine seemed to partly bind better than the layer–lattice structures.[16] Belladonna dry extract/iodine-methyl-methion could not be adequately compared in the present study because its poor neutralization properties resulted in a low pH value and precipitation of bile acids. The exact mechanism of bile acid binding by antacids has not yet been thoroughly investigated, but is likely to be based on adsorption and/or ionic bonding between the bile acid anions and the positive charge on the surface of the antacids.[16]

ACKNOWLEDGMENT

The authors express their gratitude to Mr Stefan Oswald who performed the tests at Bayer AG, Consumer Care laboratories.

REFERENCES

1 Konturek SJ, Brzozowski T, Marks IN, Miederer S, Peskar BM. Antacids and mucosal protection. *Eur J Gastroenterol Hepatol* 1992; **4**: 954–65.
2 Brackmann HP. Physiological action principles in ulcer therapy. *Therapiewoche* 1985; **35**: 4007–23.
3 Kovar KA, ed. *Pharmazeutische Praxis*, 6th edn. Stuttgart: Wissenschaftliche Verlagsgesellschaft, 2001.
4 Holtermuller KH, Liszkay M, Bernard I, Haase W. Therapy of stomach ulcer: a comparison between the low dosage antacid hydrotalcite and ranitidine: results of a randomized multicenter double-blind study (Talcivent Study Group Z). *Gastroenterology* 1992; **30**: 717–21.
5 Llewellyn AF, Tomkin GH, Murphy GM. The binding of bile acids by hydrotalcite and other antacid preparations. *Pharm Acta Helv* 1977; **52**: 1–5.
6 Reimann HJ, Schmidt U, Bluemel G, Liszkay M. Mucosa protecting effect of Hydrotalcite against aspirin-induced lesions in men. *Dig Dis Sci* 1986; **31**(Suppl. 10): 202S.
7 Playle AC, Gunning SR, Llewellyn AF. The in vitro antacid and anti-pepsin activity of hydrotalcite. *Pharm Acta Helv* 1974; **49**: 298–302.
8 Tarnawski AS, Wang H, Tomikawa M, Pai R, Sarfeh IJ. Talcid triggers induction of heat shock proteins HSP-70 in gastric mucosa: a key to its mucosal protective action? *Gastroenterology* 1999; **116**: A331.

9 Nehra D, Howell P, Pye JK, Beynon J. Assessment of combined bile acid and pH profiles using an automated sampling device in gastro-oesophageal reflux disease. *Br J Surg* 1998; **85**: 134–7.
10 Ali SL. Comparative in vitro investigation of antacids. *Pharm Zeit* 1982; **127**: 1482–9.
11 Xu GM, Li ZS, Zou DW *et al.* Effect of hydrotalcid on bile reflux gastritis and 24-hour intragastric bile. *Chin J Intern Med* 1998; **37**: 598.
12 Prieto R, Martinez-Tobed A, Fabregas JL, Beneyto JE. In vitro comparison of the antacid potencies of almagate in tablets and suspension with those of other commercially available antacid preparations. *Arzneimittelforschung* 1984; **34**: 1360–4.
13 Vatier JL, Gao Z, Fu-Cheng XM *et al.* Evidence for the interaction between antacid and gastric mucosa using an 'artificial stomach' model including gastric mucosa. *J Pharmacol Exp Ther* 1992; **263**: 1206–11.
14 Metza K, Röesch W. Comparison of commercially-available antacids. *Z Gastroenterol* 1981; **19**: 212–21.
15 Mendelsohn D, Mendelsohn L. Hydrogen ion, pepsin and bile acid binding properties of hydrotalcite. *S Afr Med J* 1975; **49**: 1011–14.
16 Hänsel W, Herzog T. Antacids in gallic acid reflux: In vitro study on the binding of gallic acids by calcium and magnesium ions. *Deutsche Apotheker Zeit* 1998; **138**: 2536–9.