Adjuvant antibiotic therapy in duodenal ulcers treated with colloidal bismuth subcitrate

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Abstract

Persistence of *Helicobacter pylori* after duodenal ulcer healing is associated with high rates of ulcer relapse. We compared colloidal bismuth subcitrate alone with CBS combined with one of four antibiotic regimens in the treatment of duodenal ulcers. Endoscopy and antral biopsies were performed before treatment and four weeks afterwards. Biopsy specimens were examined for histological evidence of gastritis and by Gram stain and culture for *H. pylori* infection. Altogether 141 patients were allocated to one of five treatment groups. Giving CBS and metronidazole (400 mg tid for 7 days) with and without amoxycillin (500 mg tid) achieved higher clearance rates of *H. pylori* than treatment with CBS alone ($p < 0.01$). These two combinations also achieved higher rates of antral gastritis healing than CBS alone ($p < 0.01$ and $p < 0.05$ respectively). Susceptibility to metronidazole was tested in 29 isolates before and in seven isolates after treatment with metronidazole by disc diffusion test and minimum inhibitory concentration assay. Twenty seven (93%) of the isolates were sensitive before treatment while six of seven (86%) were resistant afterwards. Four of the six resistant strains had acquired resistance during treatment and one of these had acquired metronidazole resistance despite concomitant treatment with amoxycillin, to which it remained sensitive.

CBS with adjuvant metronidazole at a dose of 400 mg tid for seven days significantly improves the eradication of *H. pylori* compared with CBS alone. Acquired metronidazole resistance, however, seems to be an important cause of failure to eradicate *H. pylori*.

There is mounting evidence that *Helicobacter pylori* is the main cause of antral gastritis.¹ Up to 95% of patients with duodenal ulcer have an associated *H. pylori* positive antral gastritis.² There is a lower incidence of ulcer relapse at one year in patients in whom antral *H. pylori* is eradicated than in those in whom it persists.^{3,4} Although colloidal bismuth subcitrate (CBS) and cimetidine have equivalent ulcer healing rates, patients treated with CBS have a lower ulcer relapse rate at one year, and it is postulated that this is due to the well documented activity of CBS against *H. pylori*.^{3,4} Nevertheless, CBS alone eradicates *H. pylori* in fewer than 50% of cases.^{5,6} In this study patients treated with CBS alone and with CBS in combination with one of four different antibiotic regimens were compared in terms of ulcer healing and antral gastritis and eradication of *H. pylori*.

Methods

Altogether 141 patients with duodenal ulcer diagnosed at endoscopy, histological evidence of antral gastritis, and microbiological evidence of *H. pylori* infection were included in the study. Patients who had been taking H_2 antagonists, bismuth preparations, antibiotics, corticosteroids, and non-steroidal anti-inflammatory drugs during the previous month were excluded. The patients were consecutive and there were no other exclusions.

The patients were allocated to one of five treatment groups. All patients received CBS 120 mg four times a day for four weeks (DeNol swallow tablets-Gist Brocades). The five treatment groups were group 1, CBS alone ($n=43$); group 2, CBS with amoxycillin (500 mg three times a day for the first week) ($n=18$); group 3, CBS with metronidazole (200 mg three times a day for the first week) ($n=23$); group 4, CBS with metronidazole (400 mg three times a day for the first week) ($n=26$); and group 5, CBS with adjuvant metronidazole (400 mg three times a day) and amoxycillin (500 mg three times a day) both for the first week ($n=31$). As this is a combination of two studies the first 87 patients were allocated by sequential numbers to groups (1), (2), and (3). The remaining 64 patients were allocated by sequential numbers to groups (4) and (5). The endoscopist and histopathologist did not know to which treatment groups the patients belonged. The microbiologist was aware of the treatment groups but this was necessary because of the testing for resistance before and after treatment.

Patients were advised to take CBS half an hour before meals and to take the antibiotics with food. Compliance was monitored using tablet counts. The effectiveness of treatment was assessed by endoscopic healing of duodenal ulcers, healing of histological gastritis, and eradication of *H. pylori*.

Endoscopic examination and antral biopsy from two sites within 2 cm of the pylorus were performed before treatment and four weeks after the completion of treatment.

One of each pair of biopsy specimens was fixed in 10% buffered formalin, embedded in paraffin wax, and sectioned. Sections were stained with haematoxylin and eosin and examined for evidence of gastritis. The Warren Marshall classification for gastritis was used:

Grade 0 – normal;

Grade 1 – mild round cell infiltrate;

Grade 2 – massive round cell infiltrate with or without polymorph infiltrate but without epithelial migration or destruction;

Grade 3 – polymorph infiltration of the epithelial layers with or without glandular destruction.

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Grades 2 and 3 were regarded as positive for gastritis.

The second biopsy specimen was transported in nutrient broth. A smear of the specimen was Gram stained and the remainder was inoculated onto chocolate agar and incubated at 37°C in a humidified aerobic jar flushed with CO₂ for up to five days. The growth of small translucent colonies positive for urease (Christensen's urea medium) was taken as positive for *H. pylori*.

Histological and microbiological assessments were carried out without knowledge of clinical details or *H. pylori* status.

The susceptibility to metronidazole of 29 isolates obtained from the 57 patients in groups 4 and 5 was assessed by disc diffusion technique using 5 µg discs and by minimum inhibitory concentration (MIC) determination. The MIC was done on brain-heart infusion agar with 10% horse blood using a multipoint inoculator. A faintly turbid suspension of isolates in nutrient broth (approximately 10⁶–10⁷ cfu/ml) was used for inoculation. The plates were read after 48 and 72 hours of incubation at 37°C in an atmosphere of CO₂ and humidity. Amoxycillin sensitivity was tested using amoxycillin discs (10 µg) and a zone of inhibition of 5 cm diameter and above was taken as sensitive. In the disc diffusion test for metronidazole a zone size of 2 cm diameter and above was considered sensitive.

The results were analysed by Z test.

Results

The ulcer healing rates with different regimens are shown in Table I. Although ulcer healing was better in groups 4 and 5, there was no statistically significant difference between the five groups. There was no significant correlation between ulcer healing and *H. pylori* eradication.

Table II summarises the results of *H. pylori* eradication. Group 4 (metronidazole 400 mg) and group 5 (metronidazole 400 mg and amoxycillin 500 mg) had significantly higher rates of *H. pylori* eradication than group 1 (on CBS alone) ($p < 0.01$). Group 2 (amoxycillin 500 mg) and group 3 (metronidazole 200 mg) had intermediate eradication rates but did not differ significantly from groups 1, 4, and 5.

Healing of histological gastritis (Table III) was also significantly better in groups 4 and 5 compared with group 1 ($p < 0.05$, $p < 0.05$, and $p < 0.01$ respectively).

The results of sensitivity testing of paired isolates before and after treatment with metronidazole are shown in Table IV. Ninety three per cent of isolates were sensitive to metronidazole before treatment. Six of seven (86%) isolates obtained after treatment with metronidazole

TABLE II Eradication of *H. pylori* from the gastric antrum after treatment

Group	No of patients	<i>H. pylori</i>	
		Eradicated (%)	Persistent (%)
1 CBS	43	14 (32)	29 (68)
2 CBS/Amox	18	9 (50)	9 (50)
3 CBS/M200	23	13 (57)	10 (43)
4 CBS/M400	26	19 (73)	7 (27)
5 CBS/M400/Amox	31	23 (74)	8 (26)

* $p < 0.05$ (confidence intervals at 1% (69.8–12.2)).

** $p < 0.01$ (confidence intervals at 1% (69.2–14.8)).

CBS=colloidal bismuth subcitrate; Amox=amoxycillin 500 mg; M200=metronidazole 200 mg; M400=metronidazole 400 mg.

were resistant to this drug. Four of these six resistant isolates had acquired resistance during treatment. All isolates were sensitive to amoxycillin before treatment. One isolate which persisted after treatment with metronidazole and amoxycillin had acquired metronidazole resistance but was still sensitive to amoxycillin.

Discussion

Since the establishment of the link between *H. pylori* eradication and improved ulcer relapse rates efforts have been made to devise ulcer healing treatment regimens that completely eradicate *H. pylori*. Most regimens, such as that used by Coghlan *et al* in the study of *H. pylori* and ulcer relapse, have included CBS.³ The duration of treatment with CBS in the present study is four weeks as no significant improvement in *H. pylori* clearance seems to be achieved by prolonging treatment.⁷ While CBS 120 mg four times a day is as effective as 240 mg twice a day in healing duodenal ulcer, *H. pylori* eradication is significantly better with the former.^{8,9} Therefore all our patients received CBS 120 mg four times a day.

Although *H. pylori* is sensitive in vitro to a wide range of antibiotics including penicillin, metronidazole, tinidazole, cephalosporins, some quinolones, gentamicin, tetracycline, erythromycin, and rifampicin, the clinical efficacy of most of these is poor.^{10–12} Erythromycin, for example, is not effective in the low pH environment of the stomach.

In this study the best clearance rates for *H. pylori* were achieved by the two groups that received metronidazole at a dose of 400 mg three times a day for seven days. These groups also had the highest rates of gastritis healing.

The *H. pylori* eradication rates in this study (74%) are comparable with the results achieved by Borsch *et al*,¹³ who, in a pilot study of peptic ulcer patients with *H. pylori* positive gastritis, used a two week combination of bismuth sub-salicylate (600 mg three times a day), metro-

TABLE I Duodenal ulcer healing four weeks after completion of treatment in five treatment groups

Group	No of patients	Healed (%)	Not healed (%)
1 CBS	43	28 (65)	15 (35)
2 CBS/Amox	18	14 (78)	4 (22)
3 CBS/M200	23	14 (61)	9 (39)
4 CBS/M400	26	22 (85)	4 (15)
5 CBS/M400/Amox	31	26 (84)	5 (16)

CBS=colloidal bismuth subcitrate; M200=metronidazole 200 mg; M400=metronidazole 400 mg; Amox=amoxycillin 500 mg.

TABLE III Effect of treatment on healing of antral gastritis

Group	No of patients	Healed (%)	Persistent (%)
1 CBS	43	17 (40)	26 (60)
2 CBS/Amox	18	11 (61)	7 (39)
3 CBS/M200	23	12 (52)	11 (48)
4 CBS/M400	26	18 (69)	8 (31)
5 CBS/M400/Amox	31	22 (71)	9 (29)

* $p < 0.05$ (confidence intervals at 5% (31.4–26.6)).

** $p < 0.05$ (confidence intervals at 1% (34.1–27.8)).

CBS=colloidal bismuth subcitrate; Amox=amoxycillin; M200=metronidazole 200 mg; M400=metronidazole 400 mg.

TABLE IV Development of metronidazole resistance in *H pylori* with treatment

Treatment	No tested	Sensitive (%)	Resistant (%)
Before	29	27 (93)	2 (7)
After	7	1 (14)	6 (86)

nidazole (500 mg three times a day), and amoxycillin (500 mg three times a day) and achieved 90% eradication rate four weeks after completion of treatment. Some of the patients were given H_2 antagonists with the above regimen. No antibiotic toxicity was reported.

Marshall *et al*⁴ improved the *H pylori* eradication rate in duodenal ulcer patients from 27% to 70% by adding tinidazole (500 mg twice a day for 10 days) to an eight week course of CBS (120 mg four times a day). Minor toxic effects were more common in patients treated with tinidazole.

A study by Borody *et al*¹⁴ (including both duodenal ulcer and non-ulcer dyspeptic patients) used a four week combination of CBS (120 mg four times a day), tetracycline (500 mg four times a day), and metronidazole (200 mg four times a day) for two weeks. Toxic effects seem to have been more common with this regimen than with the other above mentioned regimens. The eradication rate of *H pylori* eight weeks after treatment was 94%. A follow up of 21 duodenal ulcer patients who were *H pylori* negative at the eight week stage showed that only three (14%) had become *H pylori* positive and all of these positive patients had an ulcer relapse. Interpretations of the findings of this study are complicated by the fact that some of the patients received amoxycillin rather than tetracycline.

Antibiotics alone or in combination with H_2 antagonists have a poor record in eradicating *H pylori* gastritis despite impressive in vitro sensitivity.^{14,15} This failure seems to be due to the development of antibiotic resistance during treatment. Bayerdorffer *et al* found that virtually all strains of *H pylori* treated with a combination of ofloxacin and bismuth developed resistance to ofloxacin during treatment.¹⁶ Goodwin *et al* found that patients treated with a combination of cimetidine and tinidazole had very high rates of acquired tinidazole resistance. In contrast the development of tinidazole resistance was almost abolished by concomitant treatment with CBS.¹⁷ In the present study of seven patients with persistent *H pylori* infection, two were infected with resistant strains and four acquired resistance to metronidazole during treatment. Acquisition of resistance may be a major factor in determining *H pylori* eradication and seems to be more common than pretreatment resistance. The addition of CBS to metronidazole did not prevent the development of resistance. We assume, but cannot prove, that the persistence of *H pylori* in these patients is due to resistance rather than early infection, as this concurs with experience of other studies.^{4,16}

All isolates in our study were sensitive to amoxycillin before treatment. Amoxycillin resistance did not develop in the one strain which was tested after treatment with combined amoxycillin and metronidazole and in which metronidazole resistance had developed. A possible explanation for this occurrence is suggested

by Hollingsworth *et al*,¹⁸ who found that metronidazole was secreted in gastric mucus whereas amoxycillin was not and who postulated that some antibiotics may be active only topically resulting in high but transient clearance of *H pylori* with the organism persisting in the gastric pits. It seems that the best clinical results for eradication of *H pylori* are obtained by regimens that include imidazoles, despite in vitro studies showing that amoxycillin and other agents have higher sensitivity rates.^{11,12} It is also worth noting that metronidazole was found to have equivalent ulcer healing to cimetidine in a study that did not assess *H pylori* status.¹⁹

Eradication of *H pylori* is increasingly regarded as the single most important prognostic factor for duodenal ulcer relapse.⁵ Our results justify the use of adjuvant metronidazole at a dose of 400 mg three times a day for seven days in duodenal ulcer patients treated with CBS. It produced a 73% clearance with no appreciable toxicity. We were unable to show a significant benefit from the addition of amoxycillin to metronidazole at this dose. Because of the size of the groups, a small additional benefit from amoxycillin cannot be excluded on statistical grounds. Furthermore, we have shown the development of metronidazole resistance not only in the presence of CBS but also in the presence of amoxycillin to which the isolate was sensitive indicating that adjuvant amoxycillin may not prevent metronidazole resistance. The increasing awareness of *H pylori*'s ability to develop resistance to imidazoles suggests that this may be the main barrier to *H pylori* clearance. It is reasonable to look for another antibacterial agent to counter this resistance. To date the evidence from this and other studies has yet to show conclusively that amoxycillin should be used with CBS and metronidazole to achieve total clearance of *H pylori*.

- Marshall BJ. Unidentified curved bacilli on gastric epithelium in active chronic gastritis. *Lancet* 1983; i: 1273-5.
- Dooley CP, Cohan H. The clinical significance of *Campylobacter pylori*. *Ann Intern Med* 1988; 108: 70-9.
- Coghlan JG, Gilligan D, Humphreys H, *et al*. *Campylobacter pylori* and recurrence of duodenal ulcers - a 12 month follow up study. *Lancet* 1987; ii: 1109-11.
- Marshall B, Goodwin CS, Warren JR, *et al*. Prospective double blind trial of duodenal ulcer relapse after eradication of *Campylobacter pylori*. *Lancet* 1988; ii: 1437-42.
- Goodwin CS. Duodenal ulcer, *Campylobacter pylori* and the 'leaking roof' concept. *Lancet* 1988; ii: 1467-9.
- Humphreys H, Bourke S, Dooley C, *et al*. Effect of treatment on *Campylobacter pylori* in peptic disease. A randomised prospective trial. *Gut* 1988; 29: 279-83.
- Lambert JR, Borromeo M, Eaves ER, Hanskey J, Korman M. Efficacy of different dosage regimes of bismuth in eradicating *Campylobacter pylori*. *Gastroenterology* 1988; 94: A248.
- Coghlan JG, Gilligan D, Humphries H, *et al*. Efficacy of different dosage regimens in duodenal ulcer healing and eradication of *Campylobacter pylori* [Abstract]. *Gut* 1987; 28: A1409.
- Hollanders D. Twice daily tripotassium dicitrato bismuthate in the treatment of duodenal ulceration. *Postgrad Med J* 1986; 62: 19-21.
- Marshall BJ, McGeechie DB, Rogers PA, Glancy RJ. Pyloric *Campylobacter* infection and gastroduodenal disease. *Med J Aust* 1985; 142: 439-44.
- Van Caekenberg D, Breyssens J. In vitro synergistic activity between bismuth subcitrate and various antimicrobial agents against *Campylobacter pyloridis* (*C. pylori*). *Antimicrob Agents Chemother* 1987; 31: 1429-30.
- McNulty CAM, Dent JC, Ford GA, Wilkinson SP. Inhibitory antimicrobial concentrations against *C. pylori* in gastric mucosa. *J Antimicrob Chemother* 1988; 22: 729-38.
- Borsch A, Mai U, Opperkuch W. Oral triple therapy (OTT) may effectively eradicate *C. pylori* in man: A pilot study. *Gastroenterology* 1988; 94: A44.
- Borody T, Cole P, Noonan S, *et al*. Long term *Campylobacter pylori* recurrence post-eradication. *Gastroenterology* 1988; 94: A43.

- 15 Oderda G, Dellolio D, Morra I, Ansaldi N. *Campylobacter pylori* gastritis long term results of treatment with amoxycillin. *Arch Dis Child* 1989; **64**: 326-9.
- 16 Bayerdorffer E, Simon TH, Bastlein CH, Ottenjann R, Kasper G. Bismuth/ofloxacin combination for duodenal ulcer. *Lancet* 1987; **ii**: 1467-8.
- 17 Goodwin C, Marshall BJ, Blincow ED, Wilson DH, Blackburn S, Phillips M. Prevention of nitroimidazole resistance in *Campylobacter pylori* by co-administration of colloidal bismuth subcitrate: clinical and in vitro studies. *J Clin Pathol* 1987; **41**: 207-10.
- 18 Hollingsworth J, Goldie J, Silletti Li, Richardson H, Hunt RH. Gastric secretion of antibiotics used for *Campylobacter pyloridis* [Abstract]. *Gut* 1987; **28**: A1409.
- 19 Quintero Diaz M, Sotto Eschobar A. Metronidazole versus cimetidine in treatment of gastroduodenal disease. *Lancet* 1986; **i**: 907.