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PROSPECTIVE DOUBLE-BLIND TRIAL OF DUODENAL ULCER RELAPSE AFTER ERADICATION OF CAMPYLOBACTER PYLORI

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Summary 100 consecutive patients with both duodenal ulcer and *Campylobacter pylori* infection were followed up to see whether eradication of *C pylori* affected ulcer healing or relapse. Patients were randomly assigned to 8 weeks of treatment with cimetidine or colloidal bismuth subcitrate (CBS), with tinidazole or placebo being given concurrently from days 1 to 10, inclusive. Endoscopy, biopsy, and culture were done at entry, in weeks 10, 22, 34, and 62, and whenever symptoms recurred. There was no maintenance therapy. *C pylori* persisted in all of the cimetidine-treated patients and in 95% of those treated with cimetidine/tinidazole, but was eradicated in 27% of the CBS/placebo group and 70% of the CBS/tinidazole group. When *C pylori* persisted, 61% of duodenal ulcers healed and 84% relapsed. When *C pylori* was cleared 92% of ulcers healed ($p < 0.001$) and only 21% relapsed during the 12 month follow-up period ($p < 0.0001$).

Introduction

THE association between peptic ulcer disease and antral gastritis has been well described and is especially strong for duodenal ulcers (DU).[1] When we observed that over 90% of our DU patients were colonised with *Campylobacter pylori*,[2,3] we suspected that the bacterium caused the disease. This view was strengthened by observations that colloidal bismuth subcitrate (CBS, ['De-Nol', Gist Brocades, Delft, Holland]), which inhibits the growth of *C pylori*, led to healing of duodenal ulcers as effectively as did the H_2-receptor antagonists and prevented duodenal ulcer relapse.[4,5]

In a pilot study, we observed long-term eradication of *C pylori* in some patients treated with CBS-antibiotic combinations.[6] Here we describe how we tested the hypothesis that persistence of *C pylori* after ulcer healing is related both to active chronic gastritis and ulcer relapse.

Methods

Patient Selection

Patients had to have a duodenal or pyloric canal ulcer at endoscopy of at least 3 mm diameter, aged 18 to 75 years and, apart from their DU, be in good health. Those known to have taken bismuth-containing drugs, antibiotics, non-steroidal anti-inflammatory drugs, or corticosteroids in the month before diagnosis were excluded, and these drugs were forbidden for the duration of the study, except as required for the treatment regimen. Patients who had undergone gastric surgery more extensive than a selective vagotomy and pyloroplasty, had gastric ulcers greater than 5 mm diameter, or had a contraindication to gastric biopsy were also excluded. Female patients were required to practise contraception during the treatment phase of the study. The study was approved by the Royal Perth Hospital Human Rights Committee in June, 1984, and conducted at the hospital between April, 1985, and August, 1987.

Endoscopy and Biopsy

Four biopsy specimens were taken from antral mucosa 5 cm proximal to the pylorus. The first specimen was for a rapid urease test ('CLOtest', Delta West, Perth),[7] the second specimen was for microbiological examination, and the other two were for histological examination. Two biopsy specimens were also taken from the duodenal cap for histological examination—one from intact duodenal mucosa away from the ulcer, the other from the distal border of the ulcer.

If the CLOtest on the first antral biopsy specimen indicated the presence of urease (*C pylori* present) the patient was randomly assigned to one of the trial therapies. If the CLOtest was negative, the patient was given a consecutive study number but no therapy was assigned until proof of *C pylori* infection was obtained on histological examination or culture. If *C pylori* was not detected by histology or culture (7 patients), the patient was removed from the study and treated conventionally.

Doctors who managed the patients were blinded to the histology and microbiology findings except for those required for

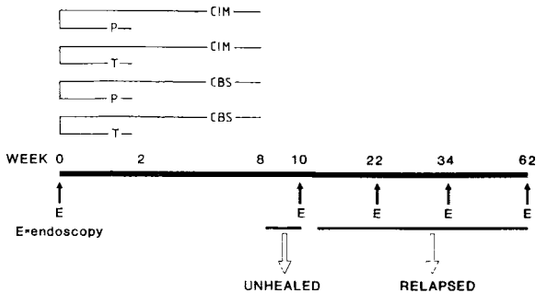

Fig 1—Study design.

randomisation. Endoscopic and laboratory findings were not revealed to patients unless they had completed, or were being removed from, the study.

Histology Methods

Pathology specimens were placed in buffered formol-saline, mounted in paraffin, and stained with haematoxylin and eosin (H&E) for general cytology and histology, and with Giemsa and also Warthin Starry (WS) stains to show the bacteria.

The H&E section was graded under low and medium power for gastritis, as previously described.[6] Polymorphonuclear neutrophil leucocytes (PMNs) and mononuclear leucocytes (monos) were scored 0 to 3. A gastritis "grade" of 0–6 was obtained by adding these scores.

All specimens were then examined for the presence of *Campylobacter*-like organisms (CLO) in the H&E, Giemsa, and WS stained sections.

For microbiological examination, a corner of the specimen was cut off with a sterile scalpel blade and a gram stain of that material was examined for CLO. The remainder was ground and cultured on selective and non-selective media for 6 days.[8] *C pylori* organisms were identified as gram-negative curved rods which produced catalase, urease, and oxidase. The susceptibility of each isolate to tinidazole was determined as reported elsewhere.[9]

Randomisation and Therapy

Patients were stratified for gender, age, smoking, and duration of ulcer disease. Within each group, the four therapies were assigned in random order. Patients received either cimetidine (CIM), 400 mg twice a day, or colloidal bismuth subcitrate (CBS,'De-Nol') one tablet four times a day (480 mg bismuth per day calculated as Bi_2O_3). CBS was given on an empty stomach, 30 min to 1 h before meals and at bedtime. Patients also received either tinidazole (T) 500 mg twice a day ('Fasigyn', Pfizer, Sydney), or an identical placebo (p) from the first to the tenth day of therapy. The four treatment groups were cimetidine/placebo (CIM/p), cimetidine/tinidazole (CIM/T), CBS/placebo (CBS/p), and CBS/tinidazole (CBS/T).

To blind the endoscopist to the temporary staining of the mouth and stools seen in some patients who take CBS, all therapy was withdrawn for 14 days before the second endoscopic examination at week 10. Patients with probable ulcer symptoms and/or ulcer craters were withdrawn from the study. Patients who were well and whose ulcers had healed were examined by endoscopy and biopsy 22, 34, and 62 weeks after entry (fig 1). There was no maintenance therapy, and patients were cautioned not to take antibiotics, antacids, or bismuth-containing drugs.

During therapy, problems with medication (eg, severe pain or possible side-effects) were managed over the telephone by the pharmacist who had usually not seen the patient but who did have access to the treatment codes. After therapy, if symptoms recurred, they also called the pharmacist (if available) or the investigators in an emergency. As far as possible there was no communication between patients and the chief investigator, so that the ulcer drug given remained investigator-blind.

Before each endoscopic examination the patient, with the aid of a research assistant, completed a symptom questionnaire and Zung depression scale.[10] Vital signs were then recorded, a brief physical examination was done, and a blood sample was drawn for *C pylori* serology.[11]

Completion Criteria

Patients completed the study if they had an ulcer (unhealed ulcer or relapse documented endoscopically) at any time after completing therapy; if their symptoms persisted during and after therapy, or recurred (symptomatic relapse); or if they remained ulcer-free and symptom-free and completed the follow-up period of 12 months (fig 1).

Study Design and Analysis

At each evaluation, *C pylori*-positive (CP+) patients were defined as those in whom the bacteria were detected by culture or histology. *C pylori*-negative (CP−) patients were those in whom *C pylori* was not detected by culture or histology.

For the statistical analysis and tables, patients were grouped as healed/unhealed at 10 weeks (second endoscopy), and relapsed/not relapsed at 62 weeks (1 year post-treatment). Patients with healed ulcers but persistent ulcer symptoms at the second endoscopy were therefore grouped as "healed DU" and "symptom relapse 0 weeks after healing".

For comparison of the treatment groups at baseline, a one-way analysis of variance was used for continuous variables and a χ^2 test was used for nominal or ordinal scale variables. Life-table analysis was done using the survival analysis available on the SAS package. The results obtained were consistent with significance values calculated with a Fisher's exact test, so for simplicity only the latter values are cited in the text. Histology grades were compared with Wilcoxon's test.

Results

Of 107 consecutive eligible patients with duodenal ulcer, 7 were withdrawn from the study because *C pylori* could not be detected on histological examination or culture. Thus 100 patients were randomised to therapy. There were no major differences between the four treatment groups (table I).

All except 2 patients completed all aspects of the study. 1 man did not attend for his second (week 10) endoscopy because he felt well and had taken a job in the outback. 3 weeks later, during an apparent ulcer relapse with vomiting episodes, he had a myocardial infarction. Barium meal revealed a duodenal ulcer crater 1·5 cm in diameter and *C pylori* was found at endoscopy in week 23. For the analysis he was classed as CP+, healed, with subsequent relapse in week 15. A second man left Australia, but before his departure in week 29 he was endoscopically and clinically normal with a CP− biopsy.

Effect of Therapy on C pylori *and Gastritis*

CIM/p had little effect on *C pylori*. All 22 patients taking only cimetidine had the organism at the second and subsequent evaluations (table II). CIM/T eradicated

TABLE I—BASELINE COMPARISON OF PATIENTS

Variable	CBS/p (n=22)	CBS/T (n=27)	CIM/p (n=22)	CIM/T (n=29)	All groups (n=100)
Male sex (%)	16 (72%)	19 (70%)	17 (77%)	19 (66%)	..
Smokers (%)	12 (55%)	15 (56%)	13 (59%)	15 (52%)	..
Previous ulcer (%)	20 (90%)	25 (93%)	19 (86%)	24 (83%)	..
Mean age (yr)	46·6	46·3	42·2	47	45 4
Mean duration of disease (yr)	8·5	14·0	10 8	12·6	11·5
Duration of current episode (wk)	3·6	4·6	6·5	2·9	4 53
Pain score (0–10) at entry	5·8	5·5	7 1	5 1	5·8
Ulcer diameter (mm)	11·8	13·2	12 0	9 2	11 5

Significance of difference between groups for pain score at entry 0·1; p > 0·2 for all other variables.

TABLE II—HEALING AND RELAPSE DATA BY THERAPY AND BY C PYLORI STATUS AFTER THERAPY

Group (n)	Unhealed/ healed at 10 wk	Relapsed			Well (%)
		3 mo	6 mo	12 mo	
CIM/p					
CP+ (22)	9/13	9	2	1	1 } 5%
CP− (0)	0	0	0	0	0 }
CIM/T					
CP+ (28)	7/21	13	2	3	3 } 14%
CP− (1)	0/1	0	0	0	1 }
CBS/p					
CP+ (15)	7/8	5	2	0	1 } 32%
CP− (5)	0/5	1	0	0	4 }
Recrudescent (1)	0/1	0	0	0	1
Reinfected (1)	0/1	0	0	0	1
CBS/T					
CP+ (7)	5/2	0	0	0	2 } 56%
CP− (19)	2/17	2	1	1	13 }
Recrudescent (1)	0/1	0	1	0	0
All CP+ cases (72)	28/44	27	6	4	*7–10%*
All CP− cases (25)	2/23	3	1	1	*18–72%*
Recrudescences (2)	0/2	0	1	0	1
Reinfection (1)	0/1	0	0	0	1
Column totals (100)	30	30	8	5	27

C pylori in only 1 of 29 patients (4%). The antibiotic failed because a tinidazole-resistant isolate emerged in nearly all cases in which CBS was not being taken concurrently[8] (see below).

CBS/p led to clearance of *C pylori* in 7 of 22 patients (32%). Recrudescence of infection occurred in 1 patient, so the eradication rate for CBS/p was 27% (6/22), which was significantly better than that obtained with CIM/T ($p = 0.02$, or CIM/p ($p = 0.01$). CBS/T cleared the infection in 20 of 27 patients (74%), and 19 of these patients remained CP− during follow-up—these findings were significantly better than the result obtained with cimetidine ($p < 0.0001$) or CBS alone ($p < 0.01$). When the initial *C pylori* isolate was sensitive to tinidazole in vitro, the bacterium was eradicated by the CBS/T combination in 85% of cases.[8]

Of the 28 patients cleared of *C pylori* at the 10-week biopsy, 3 were later found to have *C pylori*. 1 man given CBS/p was CP+ at the 22-week study. He had oesophagitis with mild symptoms but did not have a relapse. A woman given CBS/T became CP+ at 22 weeks and began to have symptoms at 25 weeks. We believe that these 2 patients had recrudescent infections and that the 10-week biopsy specimens, immediately after treatment, gave false-negative results. They were included in the healing analysis as CP− after therapy but were excluded from the relapse analysis. One reinfection occurred. A man given CBS/p was CP− at the 10- and 22-week endoscopies, but histological evidence of gastritis and *C pylori* were noted at the 34-week study. He remained well and completed the study. He was included in both the healing and relapse analyses and grouped with the CP− patients (ie, patients in whom *C pylori* was eradicated by therapy).

Clearance of *C pylori* organisms resulted in improvement in the histology. PMN scores decreased from 1·79 to zero in patients cleared of *C pylori* ($p < 0.0001$). In contrast, PMN scores were unchanged in patients with persistent infection (average grade after therapy 1·74 [SD 0·62]). Mononuclear cell scores also improved significantly, falling from a mean of 2·5 (0·5) to 1·6 (0·5) in patients cleared of *C pylori*, but not changing in patients with persistent infection ($p < 0.001$) (fig 2).

Ulcer Healing

Healing occurred in 92% of patients in whom *C pylori* was not detected at the 10-week endoscopy, whereas only 61% of patients with persistent *C pylori* healed (43/71) ($p = 0.001$).

Ulcer and Symptom Relapse

There were 70 patients with healed ulcers at the 10-week endoscopy, 35 of whom had received cimetidine. During the 12-month follow-up period, relapse occurred in 12 of 13 CIM/p patients (92%) and 18 of 22 CIM/T patients (82%) (fig 3). This difference was not significant. The relapse rate for all cimetidine-treated patients was thus 86% in 12 months.

In the CBS/p-treated group, 8 of 15 had a relapse (53%). The relapse rate was less than that in the combined cimetidine groups ($p = 0.027$). In this group, all 7 patients whose ulcers did not heal were found to be still CP+ ($p = 0.037$). In those whose ulcers healed but were still CP+, relapse then occurred in 87% (7/8), whereas only 17% (1/6) of CP− cases relapsed ($p < 0.026$) (table II).

Of 20 patients whose ulcers healed with CBS/T only 5 went into relapse (25%). This was clearly better than the result in the combined cimetidine groups ($p < 0.0001$). Although maintenance of remission was commoner with CBS/T than with CBS/p, the difference was not significant ($p = 0.15$). However, if with unhealed ulcers and relapses patients were combined for analysis as "treatment failures", CBS/T therapy was superior to CBS/p (12/27 failed *vs* 17/22, $p = 0.04$).

The observed differences in relapse between the four treatment groups could be accounted for by *C pylori*. Excluding the 2 patients with recrudescent infection, relapse

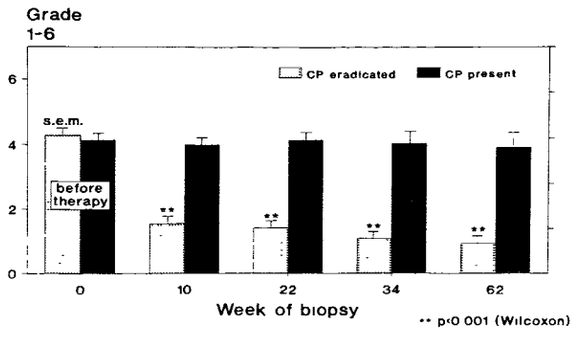

Fig 2—Effect of eradication of *C pylori* on gastritis grade.

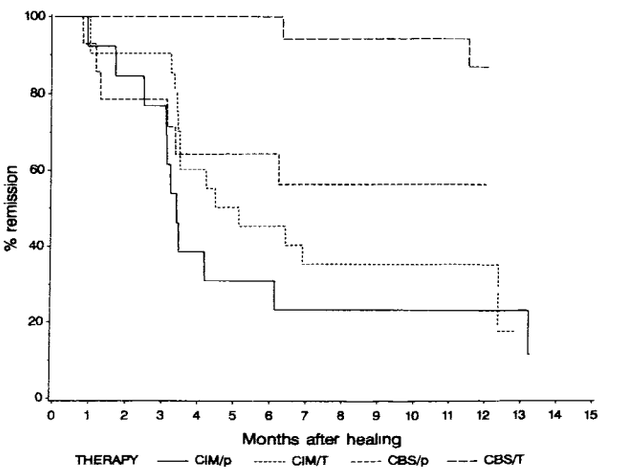

Fig 3—Effect of treatments on relapse.

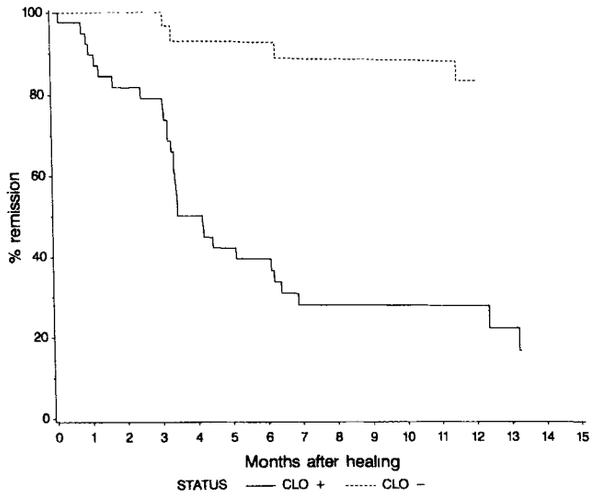

Fig 4—Differences between CP + and CP − groups on relapse.

occurred in 84% of CP + patients (37/44) but in only 21% (5/24) of the CP − patients ($p < 0.0001$) (fig 4).

The clinical picture at relapse was more acute and severe in CP + than in CP − patients. 5 CP − patients with relapses completed the study at the appointed follow-up endoscopy times; none of them had symptoms severe enough to warrant endoscopy. In contrast, 8 of the 38 CP + patients who went into relapse required an additional endoscopic examination because of severe ulcer symptoms ($p = 0.033$).

Of the 2 patients with recrudescent infection, 1 went into relapse. The second, and also the reinfected patient, completed the full 12-month follow-up period.

Sex, age, smoking, and history of a previous ulcer had no significant effect on relapse provided *C pylori* had been eradicated (table III). 28 patients with three or more of these risk factors did not relapse more often when *C pylori* had been eradicated. In contrast, of 16 CP + patients with three or more risk factors the relapse rate was 100% ($p = 0.04$). Relapse was also more common in CP + patients if they smoked ($p = 0.03$), but in this study male sex or increasing age was not a disadvantage in CP + patients.

Gastric Metaplasia and C pylori *in the Duodenum*

At the first endoscopic examination, adequate ulcer border and duodenal bulb biopsy specimens were obtained from 88 of the 100 CP + patients. Gastric metaplasia was more common in the ulcer border than in the adjacent bulb

TABLE III—SUCCESS OF THERAPY BY SEX, AGE, SMOKING, AND ULCER HISTORY

Variable	All healed patients (n = 70)		CP eradicated healed (n = 24)	
	Success	Relapse	Success	Relapse
Sex				
Female	8	12 (60%)	5	1 (16%)
Male	19	31 (62%)	14	4 (14%)
Age				
< 35	7	7 (54%)	5	0 (0%)
35–49	15	22 (59%)	10	2 (17%)
> 49	5	14 (75%)	4	3 (43%)
Smoking				
No	13	20 (61%)	6	3 (33%)
Yes	14	23 (62%)	13	2 (13%)
Previous ulcer				
No	2	6 (75%)	1	0 (0%)
Yes	25	37 (60%)	18	5 (22%)
No of risk factors				
0, 1, or 2	18	24 (57%)	10	2 (17%)
3 or 4	9	19 (67%)	9	3 (25%)

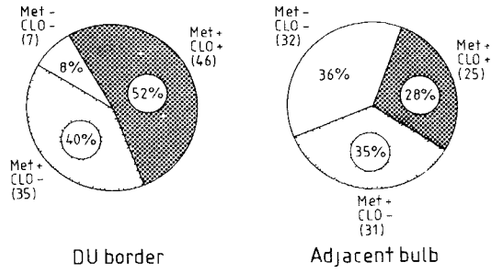

Fig 5—Gastric metaplasia in duodenal ulcer border and adjacent bulb.

(92% *vs* 63%, $p = 0.0001$), but *C pylori* was present in about half of the areas of metaplasia seen, irrespective of its location (fig 5).

Effect of Therapy on Symptoms

For patients whose ulcers healed and who remained in the study after the second endoscopic examination, there was no difference among the four treatment groups in response of symptoms to therapy. The Zung scale was significantly lower for patients cleared of *C pylori* (mean Zung score = 0·32) than for patients in whom *C pylori* was detected at the second endoscopic examination (mean Zung score = 0·38). The difference was significant even though patients whose ulcers remained unhealed or relapsed at 10 weeks were excluded from the analysis ($p = 0.014$, analysis of variance). The difference increased slightly during the follow-up period, with mean Zung values of 0·30 and 0·37 for the CP − and CP + patients, respectively, at the third endoscopic examination ($p = 0.003$, analysis of variance).

Side-Effects

There were more side-effects in patients who received tinidazole compared with those who did not, but the difference was not significant. 2 patients taking CBS/T had severe diarrhoea (but were able to continue therapy) and 2 others noticed more frequent stools. 2 patients complained of a temporary "burning anal irritation" while 3 others complained of eructation, flatulence, or bloating. Constipation was uncommon. 1 case of oral candidosis occurred in each of the cimetidine groups.

Discussion

In the early 1980s, interest in bismuth compounds was revived when it was noted that the relapse rate for duodenal ulcers fell when ulcers were healed with CBS.[4,5] The ability of CBS not only to heal but also to cure some people of the disease suggested to us that it had another action besides mucosal protection, an action directed at the underlying duodenal ulcer diathesis.

The isolation and culture of *C pylori* and its association with type B gastritis led to the hypothesis that this new bacterium was the cause of the gastroduodenal inflammation in patients with duodenal ulcer. The findings that *C pylori* was inhibited by some bismuth salts,[6] and that suppression of infection led to healing of gastritis[7] lent further support to the thesis that the ulcer diathesis was closely related to *Campylobacter pylori* gastritis.

How bacterial infection in the antrum can lead to ulceration in the duodenal bulb is at present unknown. However, we found gastric metaplasia in over 90% of the duodenal ulcer borders; and adherent *C pylori* were also commonly found in this location, more so than elsewhere in the bulb. The known association between *C pylori* and

active duodenitis with gastric metaplasia[12] suggests that the bacterium causes "gastritis" in the bulb, just as it does in the antrum. Other reported abnormalities such as deficient mucosal bicarbonate secretion[13] could be direct or indirect consequences of this action.

When the present ulcer trial was planned in 1984, the aim was permanent eradication of *C pylori*, in a controlled fashion, from patients with duodenal ulcer disease. We had no clinical experience with tinidazole in combination with cimetidine, but in-vitro studies suggested that tinidazole had high activity against *C pylori*. In this study, the development of tinidazole resistance by *C pylori* enabled the bacterium to survive in 28/29 patients treated with CIM/T. CIM/p had no effect on the presence of *C pylori*, as was expected.

The two cimetidine therapies produced very similar healing and relapse rates, and *C pylori* was not cleared in either group. CIM/p and CIM/T patients were therefore combined in the analysis to give one CP+ cimetidine-treated control group.

C pylori was cleared from 30% of those treated with CBS/p and 74% of those treated with CBS/T.

In 92% of patients cleared of *C pylori* the ulcers healed (26/28), but for those with detectable *C pylori* after therapy the healing rate was only 61% (44/72). These data support findings reported by Bayerdorffer et al,[14] who noted enhanced DU healing when the quinolone antibiotic ofloxacin was added to standard ranitidine therapy. In our study no individual therapy had superior healing properties. In the CBS groups, any advantage conferred by rendering patients CP− may have been diluted by slightly worse healing in patients who remained CP+. For example, in 50 patients who remained CP+ after cimetidine therapy, 34 had ulcers that healed (68%), but in 21 patients who remained CP+ after receiving CBS, only 9 had ulcers that healed (42%) ($p = 0{\cdot}06$). The trend suggests that healing of ulcers with CBS is related more to its antibacterial action than to its "mucosal protective" action.[15]

The poor healing seen in our two cimetidine-treated groups may have been an artifact produced by our study design. In studies in which higher healing rates have been reported with H_2-receptor antagonists,[16,17] patients were assessed while still taking the drug. In our study some patients whose ulcers healed at 8 weeks could well have relapsed by the second endoscopic examination at 10 weeks. As a consequence, these patients completed the study as "unhealed" rather than as "relapsed".

Of the 28 patients who were CP− at the 10-week endoscopy, 2 had ulcers that had not healed and 2 had rapid recrudescence of the infection, leaving only 24 CP− patients who could be observed for ulcer relapse. Sequential biopsy of these patients over a 12-month period demonstrated that *C pylori* could be permanently eradicated and that reinfection is unusual in adults. Only 1 patient had reinfection as we defined it. Our data support those of Rauws et al,[18] who reported a similar incidence of *C pylori* reinfection—about 5% per annum.

Of the patients from whom *C pylori* was eradicated and whose ulcers healed, only 21% went into relapse. The statistical significance of the result in such a small sample means that eradication of *C pylori* had a considerable clinically useful benefit. In accord with this, when CBS/p or CBS/T did not eradicate *C pylori*, the relapse rate was no different from that in the cimetidine group. Thus, presence of the bacterium, not type of therapy, was the factor which determined relapse. The findings support those of Coghlan,[19] who noted that the benefit of bismuth therapy over H_2-blocker therapy was confined to those patients in whom *C pylori* had been eradicated.

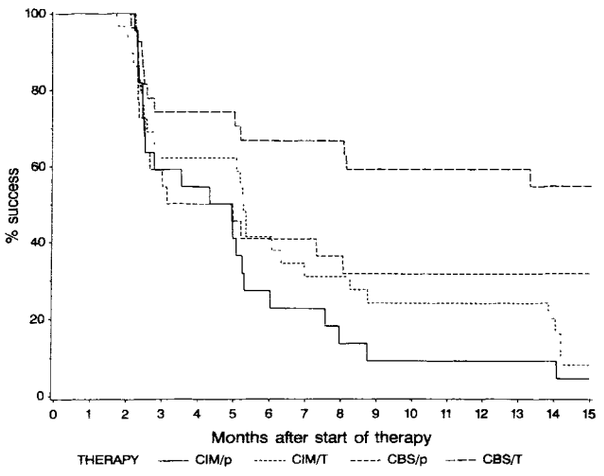

Fig 6—Differences between treatments by % success of therapy.

In other studies of ulcer relapse, the importance of age, sex, and smoking have been emphasised,[20] perhaps because they were the only factors other than continuing ulcer therapy that seemed to make any prognostic difference. How important are they compared with *C pylori*? Apparently they are of secondary importance. Once *C pylori* had been eradicated the patient did well, even if he (or she) smoked, or had a previous history of severe relapsing disease, or had multiple adverse factors. On the other hand, patients with persistent *C pylori* infection had an adverse prognosis if they had multiple risk factors, or if they smoked.

A one-time therapy which both heals duodenal ulcers and stops relapse is, by definition, curative. In future studies the distinction between unhealed ulcers and ulcers which relapse within 12 months of therapy may be unnecessary since both outcomes are really treatment failures. Conversely, by defining treatment success as a patient whose ulcer heals and who remains well for 12 months without therapy, a striking difference is evident between conventional H_2-receptor antagonist therapy and our anti-*C pylori* therapy. Treatment success in patients treated with CIM/p was 5%, with CIM/T 14%, with CBS/p 32%, and with CBS/T 56% (fig 6).

Our results imply that *C pylori* is the most important aetiological factor so far described for duodenal ulcer. We propose that detection of *C pylori* should be part of the routine management of patients with acid peptic disease and eradication of the bacterium a major therapeutic goal.

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VARIATION OF APOLIPOPROTEIN-B GENE IS ASSOCIATED WITH OBESITY, HIGH BLOOD CHOLESTEROL LEVELS, AND INCREASED RISK OF CORONARY HEART DISEASE

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Summary A random sample of 290 white men was examined for association between restriction fragment length polymorphism (RFLP) haplotypes (closely linked RFLPs on a single chromosome) of the apolipoprotein-B gene and serum levels of cholesterol, triglyceride, and high-density lipoprotein, obesity, smoking, alcohol consumption, and coronary heart disease. Haplotype or single RFLP frequencies differed significantly for obesity ($p < 0.005$), serum cholesterol ($p < 0.005$), and coronary heart disease ($p < 0.05$), but for no other variable. Obesity was associated with haplotypes involving minimum *Pvu*II and *Xba*I RFLPs that do not change the aminoacid sequence. These RFLPs are likely to be in linkage disequilibrium with nearby functional variation predisposing to obesity. Significant variation in serum cholesterol levels was associated with three functional alleles defined by *Msp*I and *Eco*RI RFLP pairs ($p < 0.03$). These RFLPs correspond to charged aminoacid variants at positions 3611 (arginine to glutamine) and 4154 (glutamic acid to lysine), which lie near the low-density-lipoprotein (LDL) receptor binding region of apolipoprotein-B. The three alleles showed stratification of serum cholesterol between low, normal, and high levels. Coronary heart disease was associated with minimum haplotypes involving *Xba*I and *Msp*I RFLPs. Together these results suggest that inherited variations of the apolipoprotein-B gene, probably in the form of charged aminoacid substitutions, influence circulating cholesterol concentration, and that these and other functional variants of the apolipoprotein-B gene affect susceptibility to coronary heart disease and obesity.

Introduction

IN times of nutritional surplus the liver synthesises triglyceride and cholesterol from excess dietary carbohydrate. This lipid is incorporated into very-low-density lipoprotein (VLDL) and secreted into the circulation.[1-4] VLDL contains apolipoprotein-B100 as its major apolipoprotein. The VLDL is transported to peripheral capillaries, where the triglyceride is hydrolysed by lipoprotein lipase. The fatty acids released are taken up by fat cells, re-esterified, and stored as triglyceride. After removal of core-triglycerides, the VLDL is enriched in cholesteryl-ester, and is designated low-density lipoprotein (LDL). Apolipoprotein-B100 is the sole protein component of LDL, and is the ligand that delivers cholesterol to all tissues of the body by the LDL receptor pathway. The circulating levels of LDL-cholesterol and apolipoprotein-B are strongly correlated with the risk of coronary heart disease.[4]

Dietary triglyceride and cholesterol are packaged within the intestinal absorptive cell into lipoproteins called chylomicrons. The major structural component of chylomicrons is apolipoprotein-B48. Chylomicrons are secreted into intestinal lymph and enter the blood for transport to the periphery, where core-triglycerides are hydrolysed by the lipoprotein lipase that also catabolises VLDL triglyceride. In times of dietary excess the fatty acids released are stored in the form of triglyceride as an energy reserve in fat cells; during carbohydrate depletion fatty acid is used by skeletal and cardiac muscle as an energy source. The chylomicron remnant is rapidly cleared from the blood by the interaction of apolipoprotein-E, another apolipoprotein, with hepatic lipoprotein receptors. Apolipoprotein-B48 lacks the carboxyl terminal domain, present in apoprotein-B100, that interacts with the LDL receptor, and thus has no active role in clearance of chylomicron remnants.[4]

Thus, apolipoprotein-B is the principal lipid transport protein in the blood. Several studies have shown that genetic variation at the apolipoprotein-B locus is associated with altered circulating cholesterol, triglyceride, and apolipoprotein-B concentrations.[5-14] However, in population association studies of restriction fragment length polymorphisms (RFLPs) of the apolipoprotein-B gene, findings on lipid, lipoprotein, or apolipoprotein levels have been contradictory.[6-8,10-14] Moreover, some workers have found no association at all between RFLPs of the apolipoprotein-B gene and lipid features, but have detected

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