The Relationship between Helicobacter Pylori and Oxygen-Derived Free Radicals in the Mechanism of Duodenal Ulceration

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This prospective randomized study investigated the possibility that duodenal ulcer relapse associated with Helicobacter Pylori infection is mediated by oxygen-derived free radicals. To this end, the radical scavengers allopurinol (50 mg 4 times daily) and dimethyl sulphoxide (DMSO, 500 mg 4 times daily) were administered orally. One hundred and forty-six consecutive patients with previous symptomatic endoscopy proven duodenal ulceration, which had been shown endoscopically to have healed in the presence of gastric mucosal infection with Helicobacter Pylori, were randomized to receive for the period of one year either placebo, or cimetidine 400 mg at bedtime, or allopurinol, or DMSO. In one hundred and twenty-six patients evaluable for efficacy, the cumulative relapse at one year was: placebo 47%, cimetidine 24%, allopurinol 6% and DMSO 6%. Cimetidine was significantly effective in preventing the relapse ($p<0.01$), however allopurinol and DMSO were superior to cimetidine in this respect ($p<0.05$). In the patients who relapsed, ulcer recurrence tended to occur early in those on placebo and cimetidine and to be evenly distributed over the year in those on free radical scavenging therapy. In all groups, ulcer recurrence throughout the maintenance year was more frequently symptomatic than silent. The incidence of infection with Helicobacter Pylori was not influenced by any of the regimens employed and the bacterium was detected with every relapse noted in this study and during the follow-up endoscopy which was carried out at 6 months and at 12 months during the maintenance year. The results suggest that oxygen-derived free radicals are involved in the relapse of duodenal ulceration in patients infected with Helicobacter Pylori.
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Key words: ulcer, relapse, infection, bacterium

Introduction

Oxygen is required to transform various substrates for the release of energy, to oxidize endogenous compounds and to detoxify xenobiotics. During these processes 95% of molecular oxygen in biological systems undergoes controlled reduction through the addition of four electrons in the mitochondrial cytochrome oxidase system to form water (1–5). The remaining molecular oxygen, however, undergoes sequential, univalent reduction to produce a series of reactive chemical intermediates (1–5). Two of these intermediates, the superoxide anion and hydroxyl species, are known as oxygen-derived free radicals. These are highly reactive species capable of widespread, indiscriminate oxidation and peroxidation of proteins, lipids and nucleic acids, which can lead to significant cellular damage and even tissue and/or organ failure (4, 5). Of particular importance in this respect is the autocatalytic free radical mediated destructive process of lipid peroxidation whereby polyunsaturated fatty acids in cell membranes undergo degradation to form lipid hydroperoxides (4, 5). Accumulation of these peroxides in a membrane disrupts its function and can cause it to collapse (4, 5). In addition, lipid hydroperoxides can decompose to yield a range of highly cytotoxic products such as the aldehydes (4).

Among the oxyradicals the hydroxyl radicals are particularly capable of causing degradation of hyaluronic acid, the principle component of the epithelial basement membrane thereby promoting mucosal injury (4, 5).

Consequently, in order to survive, aerobic organisms are equipped with an antioxidant multilayer defensive system to protect them from the effects of free radicals (1–5). The primary

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defense is provided by the enzymes, superoxide dismutase, catalase and glutathione peroxidase. Further defense against oxidant induced cellular injury is afforded by nonenzymatic scavengers such as thiols, especially reduced glutathione, α-tocopherol, carotenoids, ascorbate, uric acid and methionine (1–5).

Recent experimental and clinical evidence suggests that oxygen-derived free radicals are directly responsible for the development of duodenal ulceration and that scavenging these radicals stimulates healing and prevents recurrence of ulceration (6–10). On the other hand, the pathogen Helicobacter Pylori has been implicated in the mechanism of peptic ulceration (11–15) and infection with this bacterium triggers polymorphonuclear leukocyte and macrophage infiltration into its vicinity (16). These cells play a role in the pathogenesis of tissue damage at inflammatory sites (4, 17). To kill some of the bacterial strains that they can engulf, the phagocytes undergo oxidative bursts that yield oxygen-derived free radicals (4, 17). The production of these radicals outside the cell may cause tissue injury as stated above (17). Consequently, the possibility exists that oxygen-derived free radicals mediate the role of Helicobacter Pylori in the mechanism of peptic ulceration, a possibility which this study was designed to examine.

Patients and Methods

Drugs

A 1% solution of allopurinol (Burroughs Wellcome Co., Research Triangle Park, N.C., U.S.A.) was prepared by dissolving the powder in double distilled water containing the molar equivalent of 0.1 M NaOH. A 10% solution of dimethyl sulphoxide – pharmaceutical grade, B.P. (DMSO, Sigma, St. Louis, MO., U.S.A.) was prepared by diluting the stock solution with double distilled water. Cimetidine (Tagamet, 400 mg tablets) was purchased from SK & F (Hertfordshire, England). The placebo tablets were identical in appearance to the cimetidine tablets. Solutions were placed in dark coloured glass bottles of similar appearance containing 300 ml. Patients were issued a fresh supply of solutions every 15 days.

The study design

This was a prospective randomized controlled trial conducted on consecutive non-smokers immediately after healing of symptomatic duodenal ulceration in the presence of gastric mucosal infection with Helicobacter Pylori. The study was carried out at the Medical City in Baghdad, Iraq, which is the largest medical establishment in the country and its main referring centre. It serves a population well in excess of one million. The patients seen in this city do not represent a homogenous group extracted from one region of the country.

Patients were judged suitable for the study only when the following conditions existed:

1. Previous symptomatic endoscopy proven duodenal ulceration (sharply demarcated circular or oval punched-out breaches in the duodenal mucosa regardless of their depth) occurring for the first time and shown endoscopically to be solitary, confined to the first part of duodenum, and larger than 0.5 cm in diameter (but not giant ulcers, i.e. larger than 2.5 cm in diameter).
2. Cimetidine was the only agent used to treat this ulceration.
3. The ulceration had been shown endoscopically to have healed (completely intact duodenal mucosa without any breaches) within twelve weeks of treatment (not refractory) and the patient had achieved complete symptomatic relief.
4. Biopsy specimens taken from the gastric antrum during this endoscopy were positive by modified Giemsa staining and tissue culture for Helicobacter Pylori. Four biopsy specimens were obtained from the gastric antrum within 5 cm from the pyloric channel. Specimens were stained with haematoxylin and eosin to detect neutrophil and macrophage infiltrates in the lamina propria as an index of a reaction to the presence of bacterial infection, and with modified Giemsa stain to identify Helicobacter Pylori. This examination was only regarded as H. Pylori-positive if many bacteria were seen sporadically and also lying in clusters (the observation of sporadic or many bacteria without clusters was considered negative H. Pylori presence for the purposes of this study). One specimen was cultured by standard laboratory procedures for H. Pylori (in unselective horse blood agar and Columbia agar supplemented with 10% horse blood and vancomycin-trimethoprim-amphotericin-cefsulodin selective supplement, incubated at 37°C under microaerobic conditions).
5. The patient was recruited into the study within seven days of the latter endoscopy.

Recruiting criteria

Patients were not recruited into the study if one or more of the following were identified: previous anti-ulcer surgery; erosive/ulcerated oesophagitis; co-existent or previously noted gastric ulcers; a history of taking antibiotics, bismuth-containing compounds, aspirin or other NSAIDs within the previous seven days; the presence of significant symptoms from other gastrointestinal disorders which would make it difficult to evaluate efficacy and safety of the trial drugs – for example, severe irritable bowel syndrome; Zollinger–Ellison syndrome; cardiorespiratory problems; hepatic or renal disorders; pregnancy; alcoholism.

The patients recruited into the study had their history recorded and were physically examined. They were then randomized to one of the study groups; randomization was carried out by drawing sealed envelopes. Standard haematology and biochemistry measurements and urine examination were performed. Patients were instituted on maintenance treatment or placebo for one year and were seen at monthly intervals. At each visit a detailed assessment of symptoms and possible adverse events was made. The standard laboratory investigations were repeated every three months. Follow-up endoscopy with biopsies and histologic examination (as detailed above) were undertaken at six months and at twelve months (± one week) if asymptomatic, or earlier whenever symptoms recurred (one or more days of typical ulcer pain). The compliance of

patients with their therapeutic regimen was carefully monitored using special charts specifically supplied for this reason. In addition, patients were required to return empty bottles of drugs on follow-up visits and pill counts were made at each of these visits to determine the amount of cimetidine taken.

Endoscopy was performed under sedation where the oesophagus, stomach, and duodenum to the ampulla were closely examined for ulcers, scars or inflammation. Patients were withdrawn from the study whenever recurrence was detected (re-appearance in the duodenal mucosa of circular or oval breaches larger than 0.5 cm in diameter, which are sharply demarcated and punched-out regardless of their degree of penetration). This definition was adopted to avoid confusing erosions with ulceration.

The end point for this study was at the end of the maintenance therapy year.

Ethical considerations

This investigation was approved by the Ethical Committee on Human Experimentation and every patient gave written informed consent.

The study groups

One hundred and forty-six consecutive patients with healed duodenal ulceration were randomized into the following four groups and treated by mouth; 1) placebo 400 mg at bedtime and 5 ml of the vehicle solution of allopurinol every six hours; 2) cimetidine 400 mg at bedtime and 5 ml of the vehicle solution of allopurinol every six hours; 3) placebo 400 mg at bedtime and 5 ml of 1% allopurinol (50 mg) every six hours; 4) placebo (as in group one) and 5 ml of 10% DMSO (500 mg) every six hours. All patients were given the same number of tablets and volumes of solutions and were treated for one year (maintenance).

Statistical analysis

Results are expressed as percentages. The X^2 test with Yates' correction was employed to determine the statistical significance ($p<0.05$) of observed differences in ulcer relapse.

Life table analyses with Mantel-Cox (log rank) and Breslow generalized Wilcoxon's statistics were used to evaluate the statistical differences among treatments. Pairwise comparisons were made between groups with or without active therapy. Cox proportional hazards models were then applied to investigate the effect of the radical scavengers on maintenance by cimetidine when account was taken of the other patient factors as covariates.

Exclusion of patients from efficacy analysis

This exclusion was based on the following rules which were strictly applied:

1. treatment with antacids, anticholinergics, salicylates, non-steroidal anti-inflammatory drugs, corticosteroids, phenothiazines, antibiotics, or bismuth-containing compounds.
2. intolerance to treatment.
3. failure to accurately comply with the therapeutic regimen.
4. failure to be endoscoped at six months and at twelve months (± one week) if asymptomatic or earlier whenever symptoms recurred.

Additional intention-to-treat analyses were carried out re-including such patients and using various theoretically possible outcomes to examine what influence their exclusion might have had on the conclusions reached.

In preliminary studies on ten healthy volunteers, the vehicle solution of allopurinol, 5 ml every six hours for seven days, had no adverse macroscopical or microscopical effects on the integrity of the gastroduodenal mucosa when studied 6 hours and seven days after start of treatment and was well tolerated.

Results

Patient characteristics

All the patients recruited in this study were social drinkers (having no more than 14 units per week – a unit equals half a pint of beer, a glass of wine, or a standard measure of spirits) who did not indulge heavily or have a history of alcoholism, and who drank coffee every day. The study groups were comparable with regard to the amounts of coffee and alcohol consumed every day.

Thirty-five patients (27 men and 8 women, age range 19–71 years; mean 44) were randomized to the placebo group. Thirty-seven patients (28 men and 9 women, age range 20–68 years; mean 48) were randomized to the cimetidine group. Thirty-six patients (29 men and 7 women, aged range 20–70 years; mean 49) were randomized to the allopurinol group. Thirty-eight patients (28 men and 10 women, aged range 21–67 years; mean 46) were randomized to the DMSO group. These patients were recruited from a total of 191 patients seen during the study period. Five patients in the placebo group, 4 patients in the cimetidine group, 4 patients in the allopurinol group and 7 patients in the DMSO group were excluded from evaluability for maintenance treatment. The characteristics of the remaining patients are presented in Table 1 and they appear similar in terms of number, age and sex ratio among the groups.

Comparison between the groups

Approximately 10% of the patients in each of the active therapy groups experienced adverse events. Most were gastrointestinal complaints of a type to be expected in patients with a history of peptic ulceration. These events were mainly headache, nausea, vomiting, dyspepsia and abdominal pain. They were sufficiently troublesome, however, to lead to withdrawal in one patient from each of the cimetidine (headache) and DMSO (abdominal pain) groups and in 2 patients from the allopurinol group (one case of dyspepsia and nausea and another of nausea and vomiting) (Table 2). There were no obvious treatment related changes in haematology or biochemistry values.

Relapse rates

In the cases that relapsed, ulcer recurrence tended to occur early on placebo and cimetidine, whereas on radical scavengers it was similar in the first and second six months of the year. At

Table 1. Patient Characteristics

	Placebo 400 mg	Cimetidine 400 mg	Allopurinol 50 mg × 4	DMSO 500 mg × 4
n	30	33	32	31
Age (years)				
range	19–69	21–68	20–67	23–66
mean	47	46	48	45
Males	24	25	26	24
Females	6	8	6	7
Mean length of ulcer history (months)	2.4	2.3	3.1	2.7

DMSO: dimethyl sulphoxide.

Table 2. Evaluability of Patients

	Placebo 400 mg	Cimetidine 400 mg	Allopurinol 50 mg × 4	DMSO 500 mg × 4
Total entered	35	37	36	38
Fully evaluable	30	33	32	31
Not evaluable because:				
withdrawn for adverse events	–	1	2	1
failed to attend for endoscopy	1	2	–	2
prohibited drugs used	3	1	1	2
non-compliant with the therapeutic regimen	1	–	1	2
Total not evaluable	5	4	4	7

DMSO: dimethyl sulphoxide.

six months, the relapse rate on cimetidine (15%) was lower than that on placebo (30%), but the radical scavengers allopurinol and DMSO were more potent in this respect (Table 3). At twelve months, cimetidine was significantly ($p<0.01$) more effective than placebo in preventing ulcer relapse (24% vs 47%), but allopurinol and DMSO were equally superior to placebo ($p<0.001$) and cimetidine ($p<0.05$) in protecting the duodenum against ulceration. The recurrence rate was 6% on allopurinol and DMSO (Table 3). In all the groups, ulcer recurrence throughout the maintenance year was more frequently symptomatic than silent (Table 3).

The incidence of infection with Helicobacter Pylori was not influenced by any of the study regimens and the bacterium was detected with every relapse noted throughout the investigation and at every follow-up endoscopy. Histologically there was no obvious effect on the bacterial population and the clusters of Helicobacter Pylori remained visible in every case. Moreover, these clusters were always associated with mononuclear and polymorphonuclear cells infiltrating both the lamina propria and epithelium.

A series of Cox proportional hazards models was fitted using, as covariates, all factors other than treatment with radical scavengers to obtain a group of patients and conditions which independently and significantly influence the rate of ulcer relapse. Treatment with allopurinol and DMSO was then added as separate covariates. Increasing age, duration of symptoms and duration of ulcer existence had a significant ($p<0.001$) detrimental effect on the recurrence rate of ulceration at the 5 percent level. When these and all the other non-significant variables were allowed for, treatment with allopurinol or DMSO

Table 3. Relapse Rates During Maintenance Therapy

	Placebo 400 mg			Cimetidine 400 mg			Allopurinol 50 mg × 4			DMSO 500 mg × 4		
Overall relapse rate	No.S	No.R	%	No.S	No.R	%	No.S	No.R	%	No.S	No.R	%
6 months	30	9	30%	33	5	15%	32	1	3%	31	1	3%
12 months	30	14	47%	33	8	24%	32	2	6%	31	2	6%
Type of relapse (n)												
6 months												
symptomatic	7			4			1			1		
silent	2			1			–			–		
12 months												
symptomatic	10			6			2			2		
silent	4			2			–			–		

No.S: Number of patients studied, No.R: Number relapsing, DMSO: dimethyl sulphoxide.

continued to exert a significant beneficial effect in the prevention of ulcer relapse ($p<0.001$).

The influence of the method of analysis on the recurrence rate of duodenal ulceration was studied. Intention-to-treat analyses were carried out to determine what might have happened if all the patients had been evaluable. This required postulating that some patients' ulceration would have healed and others' ulceration would have recurred at various time periods. When the ulcers of all the patients who were excluded from the study were assumed to have remained healed, cimetidine continued to afford significant ($p<0.05$) protection against ulcer recurrence, however both allopurinol and DMSO were equally and significantly ($p<0.05$) more effective in this respect. On the other hand, when the ulcers of all the patients who were excluded from the study were assumed to have recurred, cimetidine afforded significant ($p<0.01$) protection against ulcer relapse, an action practiced more effectively by both DMSO and allopurinol, but only significantly so ($p<0.05$) by the latter agent. If, however, the assumption was that while the ulcers of those patients excluded from the placebo and cimetidine groups remained healed, a recurrence occurred in all of the cases excluded from the other groups, the protection against ulcer relapse by cimetidine ($p<0.05$) was not significantly different from that afforded by allopurinol or DMSO. Under this circumstance, allopurinol ($p<0.01$) and DMSO ($p<0.05$) continued to practice significant protection against ulcer relapse.

Discussion

The results confirm those of a previous report showing that free radical scavengers are significantly more effective than H_2-receptor blockade in preventing duodenal ulcer relapse (8). It must be stressed that the similarity in efficacy between allopurinol and DMSO (Table 3) and the knowledge that the only action they share is scavenging oxyradicals, supports the impression that this scavenging was responsible for the results achieved.

Studies in peptic ulcer patients (18, 19) suggest that the integrity of the duodenal mucosa is the principal aetiological factor in the mechanism of its ulceration, i.e. forces detrimental to the duodenal mucosa will reduce its resistance to the already existing injurious agents thereby allowing them to breach the mucosa and then to sustain its ulceration. Recent experiments in rats demonstrated that peptic ulceration is produced by the adrenergic hypothalamovagal pathway (20–22), a neuroanatomical tract which controls gastroduodenal blood flow and the mechanism of acid secretion. Low magnitude stimulation of this pathway, such as certain stressful events like goal frustration, enhances gastric acid secretion. Diffusion of acid into the duodenal mucosa changes its pH value which can then convert the enzyme xanthine dehydrogenase into the superoxide radical generating agent xanthine oxidase, an action that may also be achieved by the acid-induced irritation of the deeper layers of the duodenal mucosa (4). Furthermore, such an irritation triggers inflammatory cell infiltration and these cells can then generate free radicals by their oxidative bursts (4). In addition, oxyradicals can be produced by the microscopical injury caused by acid diffusion into the duodenal mucosa. These events may lead to the development of an acute duodenal ulcer. If, on the other hand, they only result in hyperchlorhydria without ulceration, then a suddenly superimposed higher magnitude of adrenergic hypothalamovagal pathway stimulation, such as personal threat, can depress the blood flow of the duodenal mucosa producing a degree of hypoxia or ischaemia that generates free radicals. The duodenal mucosa is thus rendered susceptible to damage by the hyperchlorhydria and ulceration may be produced. It follows that an initial event of high magnitude adrenergic hypothalamovagal stimulation can result in a degree of mucosal ischaemia in the duodenum capable of yielding ulceration despite normochlorhydria (20–22). Free radicals were recently shown in the rat and in man to be implicated in peptic ulceration and scavenging them stimulates the healing of this ulceration (6–10).

Helicobacter Pylori is found in the gastric antrum of more than 90% of patients with duodenal ulceration compared with only a minority of matched controls (11–15). Relapse of duodenal ulcer disease is significantly reduced when this bacterium is eradicated and ulcer recurrence after eradication is almost always preceded by re-infection (14, 15). The present study shows that when antral infection with Helicobacter Pylori persists, almost half of the duodenal ulcers which had been healed will recur within 12 months.

The mechanism by which Helicobacter Pylori contributes to the development of duodenal ulceration remains a topic of vigorous debate largely because the bacterium only colonises gastric type mucosa. The argument that the bacterium may increase gastric acid secretion via enhancing gastrin release, the gastrin link, cannot be sustained in view of the fact that Helicobacter Pylori occurs in over 90% of duodenal ulcer patients and not all of them have hyperchlorhydria (18, 19, 23). In this study, ulcer recurrence was associated with Helicobacter Pylori suggesting a direct link between infection and relapse. The observation that maintenance therapy with the H_2-receptor antagonist cimetidine did not completely prevent recurrence of duodenal ulceration, suggests that gastric acid secretion is not the primary mechanism behind the role of Helicobacter Pylori in ulcer relapse.

It is well known that Helicobacter Pylori colonises patches of gastric metaplasia which develop in the duodenum of patients who have duodenal ulcer disease and may, therefore, directly induce local damage, the leaking roof hypothesis (24). In the initial infection hypochlorhydria may occur, but as acid secretion gradually recovers, the ectopic gastric epithelium in the duodenal cap becomes inflamed and infiltrated with inflammatory cells. The oxidative bursts of these cells yield free radicals into their vicinity (4, 5), an action which may lead to tissue injury (4). On the other hand, the mucosal inflammation disrupts its barrier (23) thus allowing acid diffusion into the duodenal mucosa. As previously explained, this effect can also generate oxyradicals. The cytotoxic activity of free radicals may be promoted by further actions of Helicobacter Pylori which impair the defences of the duodenal mucosa. Among these actions is the production of ammonia by a urease activity

(23) and a diminution of the surface mucus, which maintains a pH gradient that protects against acid back diffusion, as a result of the disturbance of enterocyte mucus production (23). Under such circumstances the duodenal mucosa may be so damaged that it ulcerates purely as a consequence to the tissue injury sustained. It must be stressed, however, that any acid in the duodenum can also participate in the development of ulceration by acting on an already attenuated mucosa. The observation made in this study that radical scavengers almost completely prevented duodenal ulcer relapse despite persistence of the Helicobacter Pylori infection suggests that production of oxygen-derived free radicals as a consequence to this infection is a key factor in the relationship between Helicobacter Pylori and duodenal ulcer recurrence.

One of the advantages of this study is that smokers were not included. This arrangement alleviated smoking as a source of oxygen-derived free radicals and avoided results incurred by scavenger activities against radicals produced by smoking. Another advantage is that of studying ulcers healed by the same drug so that differences in the relapse rate produced by the choice of ulcer healing agent could be avoided (25).

DMSO has additional activities besides scavenging hydroxyl radicals which could have participated to some extent in allowing the results attributed to this agent to be realised. DMSO has been shown to inhibit neutrophil functions (26) and these cells mediate mucosal injury by oxidative bursts that yield free radicals (4, 5). Consequently, DMSO might have exerted effects against the production of radicals by inhibiting the neutrophil functions. On the other hand, the observation that neither of the radical scavengers used interfered with the magnitude of inflammatory cell infiltration occurring in response to H. Pylori infection, does not support a role for an anti-inflammatory mode of action. Moreover, since these scavengers did not influence the degree of bacterial infection, it is unlikely that they practiced antibacterial activities.

The prevalence of Helicobacter Pylori increases with age – about one percent per year – and most persons over 65 are actually infected with the bacterium, yet they may not develop any peptic ulceration (14, 16, 23, 24). In fact it has been recently demonstrated that infection of the gastric antrum with Helicobacter Pylori may be associated with an entirely normal mucosa (16). Therefore, it is construed that a balance exists between the host defenses and the virulence of the microorganism and that a disturbance of this balance enables Helicobacter Pylori to change from dormant commensal to the pathogen responsible for the development of peptic ulceration. In view of this impression and the lack of knowledge about the mode of transmission of the bacterium, it may be necessary to treat the whole family if a serious attempt is to be made at preventing duodenal ulcer recurrence.

In conclusion, the results suggest that oxygen-derived free radicals are involved in the relapse of duodenal ulceration in patients infected with Helicobacter Pylori.

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