Duodenal ulcer: a model of impaired mucosal defence

R H K Gompertz, A S Michalowski, W K Man, J Spencer, J H Baron

Abstract
There is a new model of chronic duodenal ulcer in which the ulcer is generated by irradiating the lower mediastinum of mice with a single dose of 18 Gy 250 kV x rays. Single ulcers develop in the proximal duodenum of about half the animals. Previous studies have shown a remarkable morphological and behavioural similarity to duodenal ulcer in man. Ulceration occurs because of an imbalance between aggressive and defensive forces within the duodenum and an attempt has been made to elucidate the pathomechanism of this ulcer by determining acid and pepsin secretion. The basal and pentagastrin stimulated secretion of acid, pepsin, and histamine were measured and no changes in acid or pepsin secretion were shown to occur (risk of type II error <1%). It is therefore concluded that this chronic ulcer is a model of impaired duodenal defence.

(Gut 1992; 33: 1044–1049)

Duodenal ulcer is still a common disease and despite recent advances in the understanding of its mechanisms mortality from this disorder is increasing, especially in those over 65 years. The main, unsolved clinical problem is chronic duodenal ulcer. Understanding of the basic pathomechanisms in duodenal ulcer has been hampered by the absence of an experimental model that is truly chronic rather than an acute ulcer in which healing is delayed.

There is a new animal model that seems promisingsly similar to chronic duodenal ulcer in man (Fig 1). Ulcers were found by chance during an experiment on ulcerative oesophagitis after thoracic irradiation in the mouse. Ulcers are induced by irradiation of the lower mediastinum. A single dose of 18 Gy of 250 kV x rays induces an ulcer tendency that leads to the development of ulcers in the proximal duodenum in around 45% of animals by day 9. Both acute inflammation and healing take place simultaneously for three months or more without further treatment and ulcers may cause bleeding, perforation, or pyloric or duodenal stenosis with time. No other model of chronic ulcer shows such morphological and behavioural similarity to the human duodenal ulcer. The ulcer arises as an abscopal effect of irradiation; the duodenum does not receive a significant dose of x rays. The effect is target specific because irradiation of the upper mediastinum (the adjacent portal) never leads to ulceration. Above a threshold dose (14 Gy) the incidence of ulcer rises rapidly to a maximum of 45% at nine days with no increase in frequency with doses above 18 Gy.

There is evidence that the ulcer is mediated by an indirect mechanism rather than being the result of direct irradiation of the duodenum. Firstly, studies of scattered radiation show that a maximum of 2 Gy is absorbed in the duodenal area – a dose insufficient to cause radiation ulcer. Secondly, the induction time for the ulcer is too short to be compatible with a direct radiation ulcer and the anatomical consistency and histological appearances are not those of acute radiation sickness. Thirdly, the irradiation target is specific in that exposure of the adjacent portal (the upper mediastinum) with the same dose of x rays and volume of tissue never causes an ulcer. Fourthly, the dose-response relationship is not a direct one because one would expect that with increasing dose, a 100% yield of ulcer could be achieved, and this is not the case. Fifthly, deliberate additional irradiation of the stomach and duodenum with the doses that might be received by scatter lowers the incidence of duodenal lesions.

Specific effects which occur at a site remote from the target of partial body irradiation are defined as 'abscopal'. Because the duodenal ulcer is generated in mice as an abscopal effect of...
irradiation we call it the ‘abscopal ulcer.’ In this paper the abscopal ulcer is studied as a model of duodenal ulcer in man, but this method of inducing duodenal lesions is also of considerable radiobiological interest.6

Duodenal ulcer arises because aggressive forces outweigh duodenal defences and most attempts to study and treat it focus on acid-pepsin attack. We have therefore studied gastric secretion in this model to test whether ulcer formation is associated with an increased acid-pepsin attack. We have measured basal and pentagastrin stimulated gastric secretion of acid and pepsin and mucosal pepsin levels, and have studied the control of secretion by quantifying luminal and mural histamine levels as well as changes in the activity of histidine decarboxylase to estimate histamine formation capacity (HFC).

Methods

IRRADIATION
The source was a vertically downward beam of 250 kV x rays obtained from a Marconi constant potential x ray generator. The measured half value layer of the beam was 1.3 mm copper. The apparatus in which the animals were irradiated was designed to accommodate six animals arranged radially about the central axis of the x ray beam with their heads toward the centre. A minimum of 7 mm lead shielding was used over the central area and over the body of each animal. The central shielding was made in two overlapping sections to allow adequate ventilation and to obtain sharp field definition. Changes in the size and shape of the field were effected by 3-5 mm lead inserts. Any possible variation of dose delivered from position to position was eliminated by rotating the entire assembly at six revolutions per minute. Scattered radiation was reduced to a minimum by raising the platform 30 cm above the supporting table top.

SECRETION STUDIES
Animals were female CFLP mice aged 20–35 weeks, weighing 25–40 g, and obtained from InterFauna UK. Irradiation was administered under barbiturate anaesthesia with a single intraperitoneal (ip) dose of 72 mg kg⁻¹ pento-barbitone sodium (Sagatal, May & Baker). Spontaneous recovery occurred within one hour, during which time the animals were kept warm by infrared heat from a lamp before being returned to the standard conditions of the animal house. Three groups of animals were studied. Control mice (C) were anaesthesised and placed in the irradiation jig but were completely shielded. Animals undergoing upper mediastinal irradiation (U) received a single dose of 18 Gy 250 kV delivered at 1-68 Gy per minute. The field was 10 mm wide and 12 mm long and included the thoracic vertebra T4 cephalad. Mice receiving lower mediastinal irradiation (L) were given the same dose of radiation to the same sized but adjacent field (T5–T10). Since U never leads to ulceration this group of mice was used as irradiated controls. Effects seen in the L mice, the ulcer group, could, therefore, be differentiated from any non-specific effects of thoracic irradiation.

Measurements of gastric secretion were performed on fasted animals (food and bedding removed overnight) but the mice were allowed tap water freely. Anaesthesia for all procedures except initial irradiation (when barbiturate was used) was the ‘CRC cocktail’ which is a mixture of: midazolam (5 mg/ml, Hypnovel, Roche), Hypnorm (fentanyl citrate 0.315 mg/ml and fluanisone base 10 mg/ml, Janssen), and sterile water in a ratio of 1:1:2. The initial dose was 0.01 mg/g ip followed by a subcutaneous (sc) dose of 0.01 ml/g divided between two sites, with further aliquots of 0.005 ml/g/sc as required.

Anaesthetised mice were placed supine on cork boards and steadied by elastic bands around the four limbs. The temperature of the animals was monitored using a rectal thermometer with a digital read out (Comark). Warmth was maintained by radiant heat from lamps. A transverse epigastric incision was made through skin and muscles sparing the epigastric arteries. The stomach was then gently delivered onto the abdominal wall. The pylorus and oesophago-gastric junction were each ligated in continuity with 4/0 Dexon (Davis and Geck). The oesophagus was ligated flush with the wall to spare the vagi. The glandular forestomach was opened to allow gentle removal of the resting contents with forceps and moistened cotton wool before insertion of the cannulae. Polythene tubes (Sterilin; internal diameter 0.75 mm (input) and 2 mm (output)) were inserted; the output catheter had a tip with multiple perforations to allow sump collection of fluid. The stomach was then returned to the abdominal cavity and the wound covered with a moistened cotton wool pad. The inlet cannula was attached to a peristaltic pump (P3, Pharmacia) and isotonic, isothermic saline was perfused through the stomach at a rate of 0.4 ml/minute. Effluent was then delivered into polythene scintillation counter vials (Sterilin) on an ice bath (Fig 2). The vials were changed every

Figure 2: The perfusion circuit, designed so that three mice could be studied at one time, each prepared as above.
10 minutes so that fractions of 4 ml were collected for each time interval. An initial period of washing and equilibration of 30 minutes was allowed. Saline, 0.1 ml, was injected via the tail vein, to act as a control for the subsequent pentagastrin, and a one hour basal collection was then made. Pentagastrin (Peptavlon, ICI), 62.5 μg kg⁻¹ in 0.1 ml saline was then injected. This dose gave a submaximal secretory response for both acid and pepsin in a preliminary dose-response study covering 6.7–500 μg kg⁻¹. The collection continued for a further 90 minutes. At the end of the secretion study each animal was killed by cervical dislocation and the stomach was immediately removed en bloc for tissue assays.

Results were expressed as basal acid output (BAO), defined as the mean of the last two recordings during the basal collection, and peak acid output (PAO) defined as the highest mean obtainable from two consecutive recordings during the stimulated period. The measurements of luminal pepsin, histamine, and potassium were analysed in the same manner.

ASSAYS

Perfusate

The volume of each of the perfusate samples collected over the course of the experiment was measured, to confirm complete collection without leakage from the circuit, and was then divided for assay of acid, pepsin, and histamine by the following methods:

(i) Acid in 0.1 ml aliquots: titration to pH 7 with 1 mM sodium hydroxide by an automatic titrimeter (Radiometer, Copenhagen);
(ii) Pepsin in 0.1 ml aliquots: spectrophotometry using haemoglobin as substrate;
(iii) Histamine in 2.5 ml of perfusate: fluorometry after extraction via a short Dowex ion exchange resin column;
(iv) Potassium: flame photometry (Corning 405).

Tissue

After removal of the stomach, a 50–100 mg full thickness strip from the oxyntic region was immediately homogenised in 3.5 ml of 0.01 M hydrochloric acid and centrifuged at 2000 g for 15 minutes before removal of the supernatant for the following measurements:

(i) Histamine in 1 ml of supernatant: pipetted in duplicate into tubes containing 0.4 ml 2 M perchloric acid for assay as for perfusate;
(ii) Histidine decarboxylase (histamine formation capacity – HFC) in 1 ml of supernatant;
(iii) Pepsin: as for perfusate.

SECRETION AFTER IRRADIATION

Studies of secretion were carried out three days after irradiation (during the induction period) and seven days after irradiation (at the time that the ulcers appeared) to see whether the lesion was induced or maintained by hypersecretion of acid or pepsin caused by lower mediastinal irradiation. For the initial experiment, at day 7, 70 mice were randomly allocated to one of three groups. Allocation was weighted so that there were 30 controls in order to form a solid baseline and 20 mice each irradiated to the upper or lower mediastinum. For the second series, at day 3, 45 mice were randomly allocated with even weighting so that there were 15 in each group. The size of the experimental groups was calculated to yield a power of 90% to detect a change of biological importance (40%), allowing for some experimental failures and given the SD of data calculated from preliminary studies.

STATISTICS

Data in this study are continuous. Data were examined initially using the Francis and Shapira W² test of normality. Although not normally distributed on initial testing, the data were rendered normal by logarithmic transformation. All statistical comparisons have therefore been made on logarithmically transformed data. Bartlett’s test was performed to confirm equality of variances and data sets were then compared using one way analysis of variance (ANOVA). Where the two control groups (C and U) were sufficiently similar to appear to be drawn from one parent population, these data were pooled and compared with the test group (L) using ANOVA with contrast. Means and 95% confidence intervals (CI) for the mean are used for descriptive statistics and graphs.

Results

SECRETION AFTER IRRADIATION

Technical failures due to anaesthetic deaths, death due to haemorrhage during operation, or leakage from the stomach meant that a number of animals did not yield usable data. Results from 27 controls (C), 17 irradiated controls (U), and 19 ulcer group (L) mice were available for analysis from the first (day 7) series and 15 C, 14 U, and 15 L animals from the second (day 3) series.

ACID

Acid secretion showed a satisfactory response to pentagastrin in each group with a two to threefold rise over basal values. There was no import-
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Figure 4: Basal and pentagastrin stimulated output of potassium at day 3 and day 7 post irradiation for: controls (C), upper mediastinal irradiation (U), and lower mediastinal irradiation (L) mice. Values, mean and 95% CI for the mean.

Figure 6: Tissue pepsin at day 3 and day 7 after irradiation for: controls (C), upper mediastinal irradiation (U), and lower mediastinal irradiation (L) mice. Values, mean and 95% CI for the mean.

The ant difference in either basal or stimulated secretion of acid between any of the three test groups (C, U, L) at either of the time points (day 3 or day 7) (Fig 3).

POTASSIUM
Potassium secretion was unchanged by treatment (C, U, or L), although day 7 concentrations were lower than those of day 3 for both basal and stimulated rates (Fig 4).

PEPSIN
Pepsin secretion doubled after pentagastrin stimulation. No important differences were seen between the test groups at either time point (Fig 5). Secretion in both the basal and stimulated states was less at day 7 than at day 3, an inverse trend to that seen for acid secretion, where basal but not stimulated levels were higher at day 7 (Fig 3). Tissue pepsin values were also unchanged by treatment (Fig 6).

HISTAMINE
Histamine secretion responded to pentagastrin stimulation in every case. Both basal and stimulated output of histamine were significantly higher in the L group on day 7 compared with the U and C group (p=0.03 ANOVA with contrast). No changes between groups were seen at day 3, although levels decreased by about sixfold in all groups between days 3 and 7 (Fig 7).

Gastric tissue concentrations of histamine and activity of its precursor enzyme histidine decarboxylase (a measure of the histamine forming capacity (HFC) of the mucosa) were unaffected by treatment at day 3. At day 7, however, histamine concentrations tended to be less in the L group than in the C and U groups but this was statistically insignificant (Fig 8).

POWER CALCULATIONS FOR ACID AND PEPSIN
Acid and pepsin secretion were unaffected by treatment to induce duodenal ulcer. Histamine secretion alone differed significantly among the groups. Because this seems to be largely a 'negative' result, in the sense that differences in the secretion of acid and pepsin were not discovered, the confidence that a type II error (a false negative) has not been made was estimated. Power calculations (the power of a study of a given size to detect a particular difference) were applied prospectively to quantify the size of study required to detect a change of 40% with a power of 90% (vide supra). With the results available, by using the actual difference in observed means, the probability that a change of given size might have been overlooked in the study can then be calculated according to the following formula:

\[
\frac{\text{difference from control of observed mean} - \text{difference from control of significant mean}}{\text{SEM for the difference observed}}
\]

The p value is then taken from standard tables of 't' using the appropriate number of degrees of freedom. The difference between mean rates of
secretion of acid and pepsin in control subjects and patients with duodenal ulcer is about 190%. Using the equation above, the probability that a difference of this order has been missed in this series of experiments is less than 1% for both acid and pepsin. These studies therefore show that the ulcer formation is not due to hypersecretion of acid or pepsin.

**Discussion**

The abscopal ulcer is an ideal model of acute to chronic duodenal ulcer both morphologically and in terms of its natural history of chronicity and occasional complication. Yet we have confidently shown that the ulcer is not caused by hypersecretion of acid or pepsin. There was a tendency for the mucosal pepsin values to be higher at day 7 than at day 3, which, although not statistically significant in this study, was also seen in a smaller, preliminary study in which a significant change was shown. The tissue pepsin value changed inversely compared with secreted pepsin, which suggests that pepsin is released from stores more readily, both in the resting and stimulated state, at day 3 than at day 7; although this tendency was seen in all groups.

Histamine secretion was decreased about sixfold in all groups at day 7 compared with day 3. The reason for this is not clear but it may relate to the more recent stress associated with handling and anesthesia for irradiation. Histamine secretion was significantly higher in the ulcer group at day 7 compared with the other groups. The concomitant decrease in tissue histamine suggests increased sensitivity to pentagastrin stimulation similar to that seen for pepsin responses at day 3. Increased histamine secretion was not, however, associated with increased acid or pepsin output at day 7. Histamine has effects other than the stimulation of acid and pepsin secretion, such as marked vasoactivity. The changes in histamine secretion may be important in that they change mucosal blood flow and thus alter mucosal defence.

An incidental byproduct of these studies has been the establishment of a reliable way of measuring several variables of gastric secretion in both the basal and stimulated states in the intact mouse. The mouse has the advantage over the rat in having a 10-fold smaller body volume—thus less substance is needed for each set of measurements. This may be useful when test compounds are being developed and their production in large amounts is difficult and expensive.

The abscopal duodenal ulcer is not mediated by gastric hypersecretion of acid or pepsin. This might, at first sight, seem to reduce the validity of the model for the study of duodenal ulcer in man. However, although the acid secretory rates in the human duodenal ulcer population are nearly double those in non-ulcer subjects, less than half of patients with duodenal ulcer have acid secretion above the upper limit of normal. Furthermore, there is a wide band of acid secretory rates where an individual may or may not bear an ulcer. Acid hypersecretion is not, therefore, the sole factor in ulcer development; mucosal changes must also occur.

Although these studies do not reveal the mechanism by which the ulcer is induced, some logical proposals can be made. Since the ulcer occurs as a remote effect of irradiation, it follows that it must be mediated by humoral or neural means. Either possibility is open at the moment but a humoral mechanism seems unlikely in view of the lack of a suitable potential target within the irradiated volume. A neural mechanism seems, by contrast, to be feasible. The spinal cord within the irradiated volume contains the cell bodies of the sympathetic nerves supplying the upper intestine and although nerves are relatively radioresistant, their cell bodies are less so. The cell bodies of the sympathetic nerves to the upper gastrointestinal tract which are located within the irradiated volume thus represent a credible target since they might reasonably be expected to influence the duodenum in such a way that would lead to a susceptibility to ulcer by, for example, reducing mucosal blood flow or bicarbonate secretion, both of which functions are under sympathetic control. Stress and smoking both increase sympathetic drive and both occur in duodenal ulcer, so that changes in sympathetic activity are probably important in the pathogenesis of this disorder. Irradiation seems to be able to mediate these effects in humans since irradiation for testicular seminoma or teratoma, which includes the spine, leads to duodenal ulcer more frequently than does treatment for the same condition without irradiation.

What is the relevance of this ulcer model? Are not the problems of duodenal ulceration, for practical purposes, solved? The modern therapeutic armamentarium includes H2 antagonists, mucosal protective agents, bismuth salts, and anti-Helicobacter pylori chemotherapy, as well as omeprazole which allows complete blockade of acid secretion under which condition all peptic ulcers will heal. Yet, despite all this, duodenal ulcer is still a common disease and the associated mortality is increasing, especially in those over 65 years. Relapse is usual after therapy has ceased and may occur despite treatment. A better understanding of duodenal ulcer might confer the ability to predict risk, to identify resistant ulcers before months or years of ineffective therapy have made them apparent, and to develop new means of treatment. New research into the underlying duodenal mucosal defect is required and since the basic science of duodenal

![Figure 8: Mucosal histamine and histidine decarboxylase at day 3 and day 7 after irradiation: controls (C), upper mediastinal irradiation (U) and lower mediastinal irradiation (L). Values are means, mean and 95% CI for the mean.](http://gut.bmj.com/Downloaded)
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ulcer cannot easily be studied in man, an animal model of chronic duodenal ulcer is required.

There are many models of duodenal ulcer – do we need a new one? Ulcers induced by a deliberate state of hypersecretion,1 such as the histamine in beeswax model, cannot be expected to reveal any new insights into alternative mechanisms. Cysteamine has been used to induce ulcer2 and, although an alternative to other models, the cysteamine ulcer could not accurately represent human chronic duodenal ulcer. This is because its chronicity can be achieved only by delivering a massive insult or repeated dosing so that it takes a long time for the acute ulcer to heal and often leads to the death of the animal in the acute phase. Spontaneous gastric ulcers occur in genetically mast cell depleted mice3 and duodenal ulcer can occur spontaneously in autoimmune mice,4 but the physiological situation is not normal in either case and nor are these suitable comparisons with human ulcers. Models of mucosal damage in which a noxious agent such as ethanol is employed are simply not relevant to chronic duodenal ulcer. What is needed is an ulcer model which is a single, truly chronic, proximal duodenal ulcer. Ideally, such an ulcer would arise in an idiosyncratic way after the induction of an ulcer diathesis, and would behave similarly to human chronic duodenal ulcer, sometimes proceeding to the typical complications of bleeding, perforation, and stenosis and responding to treatment in a way analogous to man. The abscopal model seems to have the potential to fulfil these criteria, although evidence that it mimics the spontaneous healing and recurrence of human duodenal ulcer is so far lacking.

Previous studies3-4 have shown that the abscopal lesion is a proper model of duodenal ulcer in that acute inflammation and healing attempt take place simultaneously – this is true chronic inflammation. The complications of bleeding, perforation, and stenosis may occur. The results of the present studies show that no hypersecretion of either acid or pepsin occurs and we conclude, therefore, that the model is mediated by a diminution of mucosal defence. Further studies will attempt to elucidate the mechanism of this mucosal defect which may help us to understand better the underlying pathogenesis of human duodenal ulcer and to improve treatment.

This work was supported by Medical Research Council Grant No 8709 257 SB. We thank Mr Kang Li for technical assistance.

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Gut 1992 33: 1044-1049
doi: 10.1136/gut.33.8.1044

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