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An investigation into the efficacy of the pectin based anti-reflux formulation-Aflurax

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Abstract

The properties of the new pectin-based anti-reflux agent Aflurax (Ferrosan) were studied in vitro and in vivo. Aflurax had a significantly higher in vitro raft strength than the placebo which was matched to the active except for the pectin (4.66 ± 2.10 and 0.22 ± 0.04 g, respectively). In the modified Rossett and Rice test, the pectin raft remained above pH 3 for 130 min, whereas the pH in the acid phase remained unchanged. A modification to the stirring speed of the Rossett and Rice test was required to obtain a neutralisation profile for the placebo. The neutralisation profiles for the Aflurax and placebo were the same since both contained 5 mEq of base per tablet. In the in vivo study, subjects were randomly assigned to two groups, which either received radiolabelled food and unlabelled formulation, or unlabelled food and radiolabelled formulations. A pH probe was passed naso-gastrically and placed 5 cm from the cardia, and a small gamma detector was placed on the chest wall, coincident with the pH probe. The subjects received the test meal after an overnight fast. The pectin formulation or placebo was administered 30 min later. Each part of the study was performed as a single-blind two-way cross over with the active versus placebo. The reflux of radiolabel and acid was monitored for three hours postprandially. Aflurax reduced the H^+ concentration (total refluxed hydrogen ion index for Aflurax $= 3.5 \times 10^3 \pm 2.1 \times 10^3$, placebo $= 29 \times 10^3 \pm 16 \times 10^3$) and amount of radiolabelled food reaching the oesophagus (total refluxed count index of food in counts $\times 1000$ min^{-1} Aflurax $= 19.2 \pm 2.3$, placebo $= 525 \pm 423$). The mean time for which the oesophageal pH fell below pH 4 was 2.58 ± 1.0 and 0.86 ± 0.4 minutes for the placebo and Aflurax groups, respectively. The total amount of radiolabelled formulation which reached the oesophagus was 1000 ± 660 for the placebo and 621 ± 580 for the Aflurax.

Keywords: Antacid; Anti-reflux agent; Gastro-oesophageal reflux; Pectin; pH monitoring alfurax

1. Introduction

Anti-reflux agents are specifically advocated for the treatment of gastro-oesophageal reflux. They require the presence of gastric acid both to react with sodium bicarbonate and produce bubbles of

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carbon dioxide and to gel the sodium alginate by converting it to alginic acid. The gel entraps the bubbles of carbon dioxide making it a buoyant structure. This 'raft' then floats on the gastric contents and acts to suppress gastro-oesophageal reflux of food and acid (Washington, 1990). Traditionally, anti-reflux agents have relied on alginates to provide the cohesive gel raft. The structure and cohesiveness of the raft is critically important in its ability to suppress reflux events. Problems have arisen when trying to improve the buffering capacity of the anti-reflux agents since aluminium and magnesium based antacids reduce the cohesiveness of the raft.

Aflurax is a new anti-reflux formulation which contains pectin as the gelling agent. It has been shown that Aflurax localises in the fundal region of the stomach and it thus has appropriate in vivo behaviour for an anti-reflux agent (Washington et al., 1988). It has been shown that Gaviscon (Reckitt and Colman Pharmaceuticals) suppresses the reflux of both food and acid into the oesophagus in both provoked reflux in normal volunteers and in patients with endoscopically proven gastro-oesophageal reflux (Washington and Denton, 1995; Washington et al., 1998).

The aim of this study was to investigate the efficacy of the raft formed by Aflurax versus a placebo containing the same amount of base in suppressing gastro-oesophageal reflux. In addition, reflux of the raft into the oesophagus was also studied.

2. Materials and methods

The dose of Aflurax or placebo (Ferrosan A/S, Denmark) used was 2.432 g which was equivalent to the weight of two tablets; the formulations were in powder form. The Aflurax contained 15.8% pectin (w/w), whereas the placebo contained 15.8% sodium caseinate (w/w), which was selected due to its similar mouth feel to Aflurax, and its inability to form a raft in vitro. The Aflurax and placebo contained identical amounts of all other ingredients, including 19.6% magnesium carbonate (w/w) and 7.6% potassium bicarbonate (w/w).

The test meal consisted of 2 eggs (120 g), 50 g cheese, 30 g each of mushrooms, onions and tomatoes, 10 g butter, one slice bread, 250 ml lemonade, one small apple pie, 50 g ice-cream and one cup of coffee. It was served as a Spanish omelette with lemonade, followed by apple pie and ice-cream, and coffee. The total calorific value was 1060 kcal. The meal has previously been shown to provoke reflux in normal volunteers (Washington, 1990).

Technetium-99 m (Tc-99 m) pertechnetate was converted to sulphur colloid (Mallinckrodt Diagnostica UK Ltd), and diethylenetriaminepentaacetic acid (DTPA) (Amersham) chelate using commercial kits. The omelette was radiolabelled by addition of 2 MBq of Tc-99 m sulphur colloid to the eggs before cooking; the validity of this method has previously been established (Velasco, 1982). The liquid phase of the meal was labelled by addition of 2 MBq of Tc-99 m DTPA to the lemonade.

Both the Aflurax and the placebo were labelled with 3 MBq of indium-113 m (In-113 m) chloride, which was mixed to a paste with 2.432 g of either Aflurax or the placebo. The mixture was then made up to 10 ml with distilled water.

2.1. Integrity of radiolabel for placebo

1.5 MBq of In-113 m was added to 2.432 g placebo. This was added to 125 ml simulated gastric juice (USP formulation) at 37°C. The mixture was stirred to form a suspension. The 2 ml samples of the mixture were removed at intervals over a period of 4 h and centrifuged at 3000 rpm. The pellet was washed by resuspending it in distilled water and recentrifugation. Samples of the pectin, washings, and supernatant were counted to assess the retention of the radiolabel by the formulation.

2.2. Raft strength of aflurax and placebo

Granulate equivalent to one tablet of each formulation was made up to a paste with 5 ml of distilled water, and added to 125 ml of 0.03 M HCl at 37°C. The beakers were swirled and then placed in a waterbath at 37°C for 10 min to allow

raft formation. A raft strength test was then performed using a purpose-built apparatus to measure the force required to pull a wire probe through the floating layer (Washington et al., 1986). Values found were corrected for the force needed to pull the probe the same distance through water.

2.3. Neutralisation characteristics of the formulations

This was assessed using the standard Rossett and Rice test with a stirring speed of 400 rpm (Rossett and Rice, 1954) and a modified form of as described by Washington and co-workers (Washington et al., 1985) which is suitable for the evaluation of anti-reflux agents. The 1.216 g of each formulation was added to 100 ml of 0.03 M HCl, which was kept at 37°C in a water bath. A pH probe measured pH either within or below the raft until the pH returned to baseline.

2.4. In vivo anti-reflux behaviour of the formulation

Twelve healthy male and non-pregnant females were recruited from the University student population. Students with a weight outside the range of $\pm 10\%$ group mean weight, a history of diabetes, gastrointestinal disorders, or allergies to any of the test meal ingredients were excluded. Other criteria for exclusion were smoking, participation in a gamma camera study within the last 12 months, consumption of medicines which could influence the results of the study, or any suspicion of pregnancy. All the female subjects were asked the date of their last menstruation on each study day.

The study was carried out according to the guidelines for the Declaration of Helsinki. Written, informed consent was obtained from all volunteers prior to entry to the study. They were advised that they were free to leave the trial at any time. Approval from the University of Nottingham Ethical Committee was granted. Permission to administer radioisotopes to volunteer subjects was obtained from the Department of Health. The subjects who took the radiolabelled meal received a dosimetry of 0.17 mSv, whereas the subjects taking the radiolabelled formulation received a dosimetry of 0.1844 mSv of radioactivity.

The 12 subjects were randomly divided into two groups; Group I were given the radiolabelled meal but unlabelled Aflurax or placebo, and Group II were given unlabelled meal but radiolabelled formulation. Subjects in both groups attended for two study days; administration of Aflurax and placebo were crossed-over.

The pH probe (Radiometer, Copenhagen) was calibrated, and then marked 5 cm from the end using approximately 1 MBq Tc-99 m dried onto a square of filter paper, and secured using waterproof tape. The probe was sterilised before use by immersion for 10 min in a cyclohexidine solution ('Hibiscrub', ICI Pharmaceuticals, UK).

Subjects fasted overnight if the study was carried out in the morning or for 6 h for an afternoon study. On arriving in the department, the pH probe was passed naso-gastrically into the stomach. Using the change in pH between the stomach and oesophagus, the tip was positioned approximately 5 cm from the cardia. The subjects then swallowed half a mug of water, labelled with approximately 0.5 MBq of Tc-99 m DTPA, whilst standing in front of the gamma-camera in order to outline their stomach. The pH probe was then adjusted to position the Tc 99 m tag on the probe 10 cm above the cardia, i.e. the tip was 5 cm above the cardia.

A small external source of Tc-99 m was used to locate a position on the chest wall approximately 5 cm from the cardia (i.e. coincident with the tip of the pH probe). The detector was placed in this position and the source removed; the detector was secured using a belt. Both the pH probe and the gamma detector were connected to a two channel solid state recorder (Novo Memolog; Vertec Scientific Ltd., Reading). Baseline values were recorded for 20 min.

Subjects in Group I ingested the test-meal, labelled with 4 MBq of Tc-99 m distributed between the solid and liquid phases. Thirty minutes later, they were given either unlabelled Aflurax or placebo. Subjects in Group II ingested the unlabelled test-meal. After 30 min, they were given

either Aflurax or the placebo, which was labelled with 3 MBq In-113 m.

Recording of data took place for approximately 3 h, whilst the subject was ambulant. After the recording period, the data was transferred to an Apple Macintosh computer for analysis. At the end of the three hours, the pH probe and gamma detector were removed and the calibration of the pH probe was checked; any drift was assumed to be linear and a correction was made. On the second occasion the crossover between Aflurax and the placebo was performed.

3. Results

3.1. In vitro studies

Greater than 86% of the In-113 m chloride label remained associated with the pectin phase of the Aflurax after 150 min, and 98% of the label remained associated with the solid phase of the placebo after incubation in hydrochloric acid. The raft strength of Aflurax was 4.36 g $\pm$ 2.10 (mean $\pm$ S.D.) ($n = 4$), and that of the placebo was 0.22 g $\pm$ 0.04 ($n = 4$). An unpaired t-test was performed giving a significant value of $P = 0.008$; thus, Aflurax forms a significantly stronger raft than the placebo. The pH within the raft of Aflurax remained above pH 3 for 130 min ($n = 5$), whereas the pH below the raft dropped to below pH 3 after 8 min ($n = 3$) (Fig. 1). Thus, although the raft itself remains at a high pH for some time, the bulk acid phase was not neutralised by Aflurax. The placebo formulation did not have a significant effect on the pH in the modified Rossett and Rice test, i.e. the pH did not rise above pH 3 ($n = 3$). When the stirring speed was increased to 400 rpm, i.e. the raft did not form, the Aflurax and placebo produced similar neutralisation profiles. Two tablets of either formulation increased the pH to a maximum of 5.7 with a time above pH 3 of 55 min.

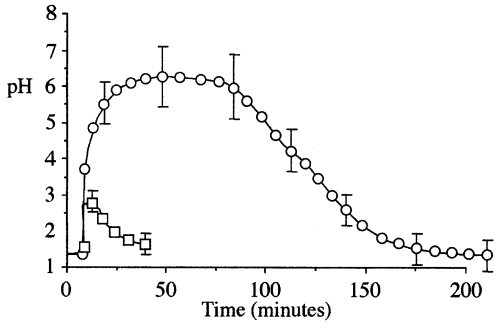

Fig. 1. pH profile of Aflurax, within (○) and below raft (□) in the modified Rossett and Rice test

3.2. In vivo studies

A typical trace for pH and counts is shown in Fig. 2. Full sets of data were obtained from all subjects.

The total refluxed hydrogen ion index was defined as the sum of the hydrogen ion concentrations corresponding to the oesophageal pH throughout the experiment. This could be calculated directly from the pH data and provided a measure of the total amount of hydrogen ions refluxed into the oesophagus. This does not correspond to the total quantity of refluxed acid since the volume of refluxed acid cannot be measured. The values of the index were not normally distributed and hence the significance of the difference between control and treated groups was calculated using a non-parametric Wilcoxon rank sum test. Standard deviation is thus not meaningful and has not been calculated. The rank sum for the treated group was 130 (12 subjects) while the sum for the placebo group (ten subjects) was 146. This demonstrated greater than 90% significance in the reduction of acid reflux in the presence of Aflurax.

The performance of the anti-reflux agent was also evaluated by measuring the total time for which the oesophageal pH remained below pH 4 and 5. Again, this data proved not to be normally distributed. The mean times below pH 5 were 4.64 and 1.62 min for the placebo and treated groups, respectively. The significance of this by the signed rank test was 87%. The mean times below pH 4 were 2.58 and 0.86 min for the placebo and active groups, respectively. The significance of this by the Wilcoxon's signed rank test was 86%.

The data obtained from the gamma probe normally showed a slowly varying component due to

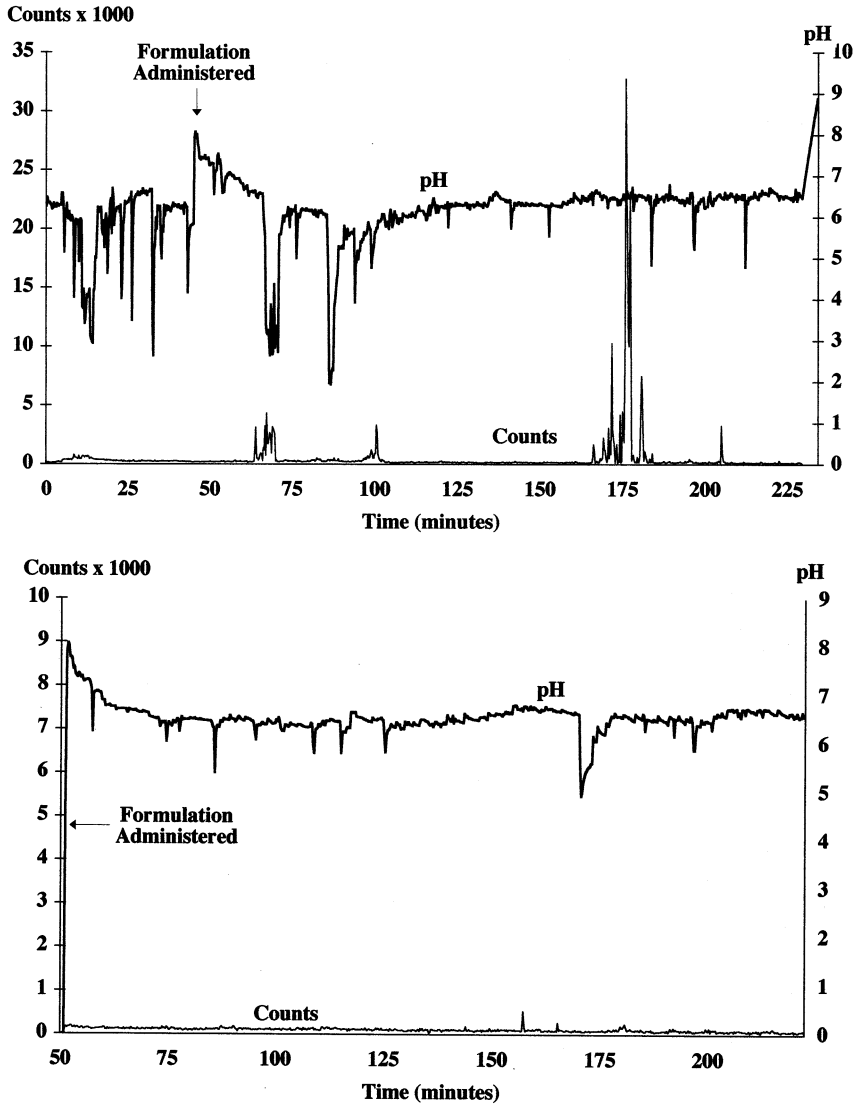

Fig. 2. A typical trace after radiolabelled food with (a) placebo and (b) Aflurax. pH trace, showing acid reflux (grey); counts trace, showing food reflux (black).

background from the stomach; superimposed on this were rapid fluctuations due to reflux events. It was necessary to define a reflux count index which was only sensitive to this rapidly varying component. This was performed by a conventional differential technique in which the instantaneous rate of change of the signal was taken to quantify the rapidly varying component. The absolute value of this derivative was then summed over the duration of the experiment to define the total reflux count index. When radiolabelled food was administered the total reflux count index was 525 after placebo and 19.2 after Aflurax corresponding to a 98% significance in the reduction of refluxed food. When labelled anti-reflux agent or placebo were administered the total reflux count indices were 621 and 1000, respectively, giving a significance of 75%. This demonstrated that the placebo was refluxed to a greater extent than the Aflurax (Table 1).

4. Discussion

In the current study, Aflurax decreased both the amount of food and H^+ ion concentration reaching the oesophagus when compared to placebo. The reduction in food reflux was similar to that reported for Liquid Gaviscon (Reckitt and Colman Pharmaceuticals), i.e. 1.38×10^5 for controls and 2.8×10^4 for Gaviscon (Washington et al., 1998). The reduction in food reflux with Aflurax occurred despite the fact that the placebo possessed an identical neutralisation capacity demonstrating the effectiveness of the raft forming component. The buffering capacity of the placebo was sufficient to neutralise the 100 ml of 0.03 M HCl present as the initial volume in the Rossett and Rice test. Although pH monitoring is generally regarded as the 'gold standard' in the diagnosis of gastro-oesophageal reflux, global results of 24-h monitoring of both oesophageal pH and pressure correlate poorly with symptoms. In addition, a poor correlation between the reflux of radiolabelled food and acid has also been widely reported both using conventional scintigraphy and pH monitoring (Shay et al., 1992; Vandenplas et al., 1992; Tolia et al., 1993) and using a gamma probe (Washington, 1990; Washington and Denton, 1995; Washington et al., 1993, 1998). Gamma scintigraphy or probe measurements will detect the reflux of material which is at pH 4 or above, whereas pH 4 is traditionally chosen as the cut-off point for reporting acid reflux events in pH monitoring. It has been suggested that scintigraphy may reflect histological oesophagitis rather than endoscopic oesophagitis (Fujiwara et al., 1993).

Table 1
Results summary

	Aflurax	Placebo
Total refluxed count index, food (counts × 1000 min^{-1})	19.2	525
Total refluxed count index, formulation (counts × 1000 min^{-1})	621	1000
Total refluxed hydrogen ion index	0.0035	0.029
Time below pH 5 (min)	3.4	4.2
Time below pH 4 (min)	1.1	2.2

Interestingly, both Aflurax and the placebo formulation were refluxed into the oesophagus. It is possible that the Aflurax is acting both as a reflux suppressant and is also being refluxed in preference to the gastric contents. This will serve to locally deliver antacid to the lower oesophagus. However, it can be concluded that it was the mechanical barrier action of the Aflurax raft which resulted in the reduction in total refluxed hydrogen ion index from 29×10^{-3} to 3.5×10^{-3}.

5. Conclusions

Aflurax significantly reduced the amount of food and the acid concentration reaching the oesophagus in vivo and thus should be effective as an anti-reflux agent.

6. Disclaimer

The work has no connection with the author's current employers and was performed at the University of Nottingham.

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