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The effect of high and low fat meals on the absorption of rifampicin from the gastrointestinal tract in volunteer subjects

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Abstract

A combination of gamma scintigraphy and pharmacokinetic analysis has been used to investigate whether the variable effect of food on bioavailability of rifampicin could be attributed to lymphatic absorption, or to alteration of the gastrointestinal transit time of the drug. Seven healthy subjects participated in the study and were given a ^{99m}Tc-labelled meal and two rifampicin capsules containing ^{111}In-labelled resin and the gastrointestinal transit of both labels was measured. Rifampicin levels were measured in plasma by HPLC. Increasing the fat content of the meal did not alter the gastric emptying of the meal or the resin (T_{50} emptying = 2.99 h low fat meal, 5.83 h high fat meal; T_{50} = 3.14 h resin low fat meal, 2.70 h high fat meal), but it did significantly delay the time for the meal to reach the colon (T_{50} arrival = 9.11 h low fat meal, 10.99 h high fat meal). Surprisingly, it did not affect the arrival time of the co-administered indium-labelled resin (T_{50} arrival = 8.91 h low fat meal, 8.43 h high fat meal). The increased fat present in the meal did not alter the pharmacokinetic parameters for rifampicin.

Key words: Gamma scintigraphy; Pharmacokinetic analysis; Rifampicin; Bioavailability

1. Introduction

Rifampicin is a semi-synthetic derivative of rifamycin B and is well established for the treatment of tuberculosis. The pharmacokinetics of rifampicin and its interactions with other drugs have been widely studied since rifampicin is a potent inducer of cytochrome P450. There are conflicting reports in the literature on the effect of food on the bioavailability of rifampicin. Initially, Proven and Canetti (1968) reported that absorption of rifampicin was not affected by food, but subsequently several groups reported that food decreases rifampicin bioavailability (Binda et al., 1971; Siegler et al., 1974; Gill, 1976; Polasa and Krishnaswamamy, 1983).

Animal studies have suggested that when fats and oils are used as vehicles for rifampicin, bioavailability is enhanced (Takada et al., 1987). There are a number of possible reasons for this effect; rifampicin is poorly water soluble, al-

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though its aqueous solubility is slightly enhanced below pH 6. It has a log P of 2.4, indicating that it is quite fat soluble, and hence it was proposed that fat in the diet promoted lymphatic absorption of the drug; however, this effect has not been proven in man. Probucol and ethylene oestradiol are known to be absorbed by this route, but these materials have a log P in excess of 5. It is also possible that fat in the diet promotes enterohepatic recirculation of rifampicin.

The objective of this study was to investigate whether the effect of food on bioavailability could be attributed to the fat content of the meal, and hence promotion of lymphatic absorption or enterohepatic recirculation. Alternatively, if the bioavailability was independent of the meal composition, the reduction in absorption may be due to alteration of the gastrointestinal transit time of the drug produced by the meal. This was tested by administering rifampicin with either a high or low fat meal in a two-way crossover, and measuring plasma levels; both the rifampicin capsules and the meal were radiolabelled to allow their transit to be studied using gamma scintigraphy.

2. Materials and methods

2.1. Preparation of radiolabelled resin

Each 300 mg capsule of rifampicin was radiolabelled with [^{111}In]indium chloride to provide activity of 0.5 MBq per capsule at the time of dosing. The [^{111}In]indium chloride was dried onto 5 mg micronised Amberlite IRP-69 resin (Sigma Chemical Co.). The capsules were opened and the resin added.

2.2. Preparation of the radiolabelled meals

2.2.1. Low fat meal

The low fat meal consisted of an omelette containing tomato, onion and mushroom, fol-

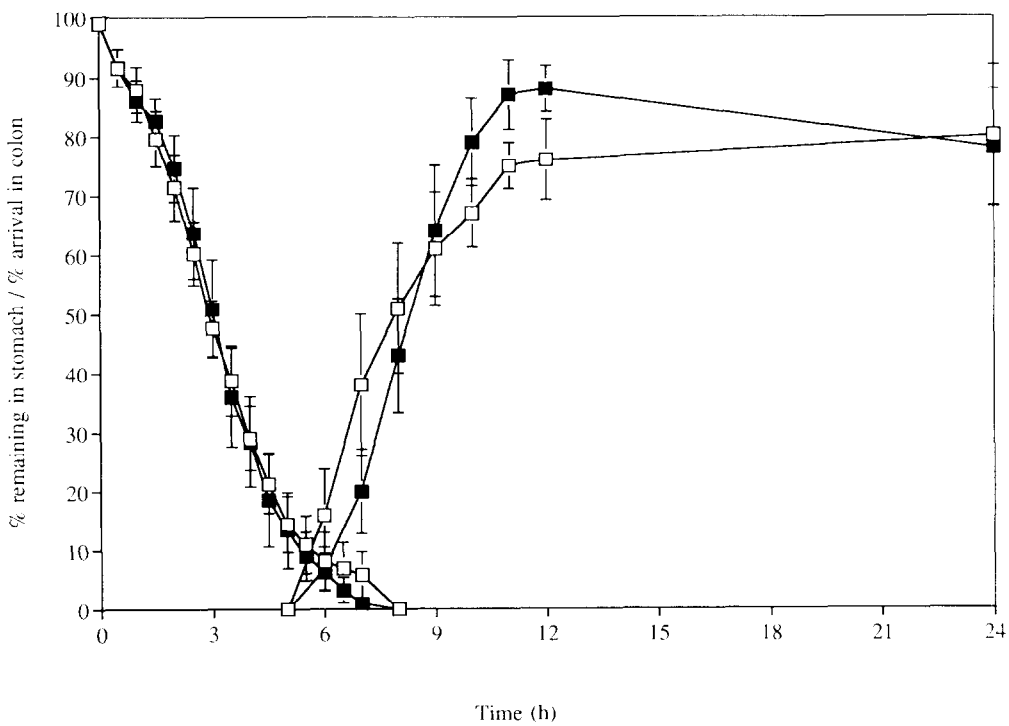

Fig. 1. Mean interpolated gastric emptying and colon arrival times for a low fat meal (□) and radiolabelled resin (■).

lowed by bread and honey, apple pie, stewed apple and sultanas, decaffeinated coffee and plain biscuits. This produced a total calorific value of 999 kcal (4098 kJ), with a nutritional breakdown of 29 g protein (11.6%), 32 g fat (28.4%) and 159 g carbohydrate (60%).

2.2.2. High fat meal

The high fat meal consisted of an omelette containing tomato, onion, mushroom, and cheese followed by bread and butter, apple pie and cream, and decaffeinated coffee. This produced a total calorific value of 1004 kcal (4118 kJ), with a nutritional breakdown of 32 g protein (12.4%), 74 g fat (66.3%) and 57 g carbohydrate (21.3%).

Both the high and low fat meals were radiolabelled by inclusion of 3 MBq ^{99m}Tc sulphur colloid to the eggs prior to cooking. This method has been previously evaluated and provides an accurate indication of the gastric emptying and transit of the test meal using scintigraphic detection (Feldman et al., 1984; Washington et al., 1987).

2.3. Study population

Seven healthy volunteers (six male, one female), age range 19–23 years, were recruited from the University of Nottingham student population. The subjects were screened prior to entry to the trial by a physician. Routine haematology and clinical chemistry tests were carried out. Exclusion criteria included weight outside the range of $\pm 10\%$ of normal mean weight, consumption of medications within the previous 2 weeks, tobacco consumption, excessive alcohol intake or participation in a similar study within the previous 12 months. Vegetarians were excluded since this diet is known to alter gastrointestinal transit times. Female subjects were excluded if there was any reason to suspect that they may be pregnant, or if they used oral contraceptives.

The subjects gave written, informed consent prior to entry into the trial and they were advised that they were free to withdraw from the study at any time. Medical cover was available at all times

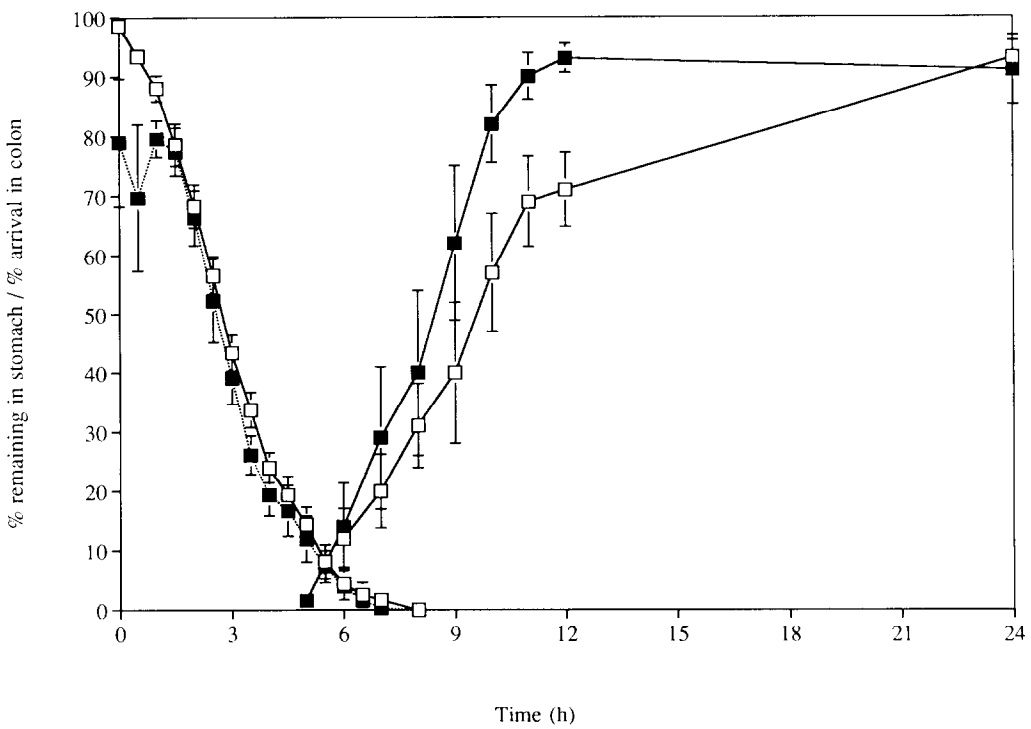

Fig. 2. Mean interpolated gastric emptying and colon arrival times for a high fat meal (□) and radiolabelled resin (■).

during the study. Ethical approval was obtained from the Nottingham University Medical School Ethical Committee and permission to administer radioisotopes was obtained from the Department of Health. The effective radiation dose equivalent to each subject for the complete study was estimated to be 0.75 mSv. The study was performed in accordance with the guidelines of the declaration of Helsinki (Venice Amendments), 1984.

2.4. Protocol

The subjects were fasted from 9 p.m. on the evening prior to each study day. At 7.45 a.m. on the study day a pre-study blood sample was taken. At 8.00 a.m. the subjects were given either the high or low fat radiolabelled breakfast. The subjects were randomly assigned to two treatment groups and the cross-over study was performed after a 1 week washout period. All subjects were given two ^{111}In-labelled rifampicin capsules with 50 ml of water immediately after ingestion of the meal. Radioactive anterior and posterior markers enclosed in plastic tape were attached to the chest opposite the stomach to allow accurate alignment of the sequence of images. Anterior and posterior static images of 30 s duration were recorded every 15 min whilst the meal was in the stomach and then every 30 min until the meal reached the colon. Thereafter, images were recorded hourly until the 16 h post-dose blood sample. Single anterior and posterior static images were recorded at 24 h post-dose.

Blood samples (5 ml) were taken at 0 h (pre-dose) and then at 1, 2, 3, 4, 6, 8, 10, 12 h and 24 h post-dose. Samples were collected either via an indwelling cannula or by serial venipuncture. The plasma was separated from the blood samples and stored at −20°C. Plasma rifampicin assays were performed by HPLC at the Merrell Dow Research Institute, Gerenzano, Italy.

The subjects were provided with decaffeinated coffee or fresh orange juice and biscuits at 11.30 a.m.; lunch at 1.00 p.m. which consisted of two filled rolls, one packet of crisps, piece of fruit or cake, coffee or tea; decaffeinated coffee or tea

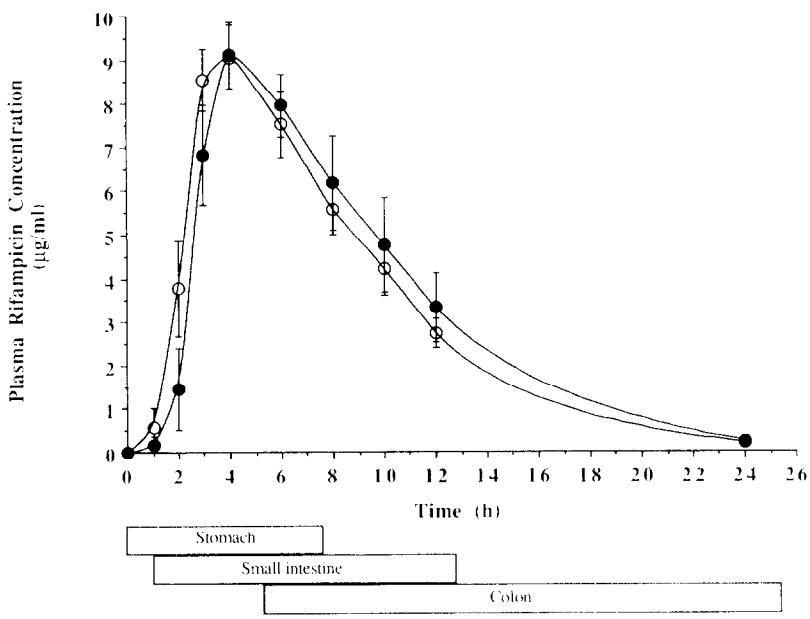

Fig. 3. Plasma rifampicin concentrations in subjects given low (●) and high (○) fat meals, with average dose form locations shown beneath the x-axis.

and biscuits at 4.00 p.m., and an evening meal at 7.00 p.m. consisting of steak, chips, peas, cold sweet, decaffeinated coffee or tea.

2.5. Data evaluation

The gamma camera images were analysed by identification of the stomach and the large intestine. Templates were then constructed referring the position of the anterior and posterior marker to these organs. Regions of interest (ROIs) were then drawn around the stomach and colon on each image, allowing gastric residence time and colon arrival of the marker to be assessed. Any oesophageal adhesion of the rifampicin capsules was noted.

The counts in the stomach and fundus were corrected for background counts and decay of the radioisotope. The ^{99m}Tc counts were also corrected for ^{111}In overlap into the technetium channel. The geometric mean of the activity in the regions of interest in the anterior and posterior channels was calculated to correct for tissue attenuation (Hardy and Perkins, 1985). The data from the individual subjects were finally interpolated onto a common time axis in order to calculate the mean gastric emptying and colon filling curves.

Statistical analysis was performed using a Wilcoxon Signed Rank Test.

3. Results

No adverse effects were reported by the subjects, and the labelled capsules were observed to disperse throughout the gastric contents within 30 min. The gastric emptying and colon arrival profiles of the indium-labelled Amberlite resin from the capsules and technetium-labelled meal are shown in Fig. 1 for the low fat meal and Fig. 2 for the high fat meal. The times required for 50% emptying from the stomach and 50% arrival in the colon are shown in Table 1, together with numeric data for the individual subjects. The small intestinal transit time can be estimated by taking the difference between the 50% emptying from the stomach and 50% arrival in the colon values. This was 6.12 and 8.16 h for the low and high fat meals and 5.77 and 5.73 h for the labelled resin, respectively. The dip in the gastric emptying profile with the high fat meal at between 0 and 2 h is due to oesophageal retention of one of the capsules which was observed in three subjects. The activity from the lodged capsules cleared from the oesophagus into the stomach slowly over a 2 h period.

There was no significant difference between the gastric emptying of the two meals or the dose form, as measured by the T_{50} ($p = 0.622$ and 0.189, respectively). The composition of the meal also did not affect the gastric emptying of the dose form ($p = 0.460$, high fat; 0.268, low fat). Further, there was no significant difference between the colon arrival of the two meals, as measured by the T_{50}, ($p = 0.151$), or the arrival of the two dose forms ($p = 0.156$), or the meal and dose form with low fat meal ($p = 0.811$). How-

Table 1
Time (h) for 50% of the ^{99m}Tc-labelled meal and ^{111}In-labelled resin administered in two 300 mg rifampicin capsules to leave the stomach and for 50% of the tracer to arrive in the ascending colon. Small intestinal transit time (S.I.T.T.) is calculated as the difference between these two values

Subject no.	Gastric emptying		S.I.T.T.		Colon arrival	
	Meal	Resin	Meal	Resin	Meal	Resin
Low fat						
1	2.6	2.8	8.2	4.3	10.8	7.1
2	2.8	3.4	4	4.7	6.8	8.1
3	3.5	3.5	8	5.7	11.5	9.2
4	3.1	2.9	7.2	6.6	10.3	9.5
5	4.6	4.9	4.3	5.4	8.9	10.3
6	2.3	2.0	7	9.1	9.3	11.1
7	2.0	2.5	4.2	4.6	6.2	7.1
Mean	2.99	3.14	6.13	5.77	9.11	8.91
SE	0.33	0.35	0.71	0.63	0.75	0.58
High fat						
1	2.8	3.1	5.6	4.7	8.4	7.8
2	2.9	3.1	9	4.7	11.9	7.8
3	2.8	2.8	8.7	6.6	11.5	9.4
4	3.1	2.8	6.8	6.3	9.9	9.1
5	2.9	2.8	8.4	5.9	11.3	8.7
6	3.0	2.0	8.8	7.9	11.8	9.9
7	2.3	2.3	9.8	4	12.1	6.3
Mean	2.83	2.70	8.16	5.73	10.99	8.43
SE	0.10	0.15	0.55	0.51	0.51	0.46

ever, there was a significant difference between colon arrival of meal and labelled resin for the high fat meal ($p = 0.011$).

The mean plasma curves for both treatment groups are shown in Fig. 3. The plasma concentrations reached a peak after 4 h and the half-life was approx. 6 h. The data show that there is large intersubject variation, and as a consequence there was no significant difference between the low and high fat meals at any time point.

4. Discussion

Fatty acids in the duodenum, especially long chain acids, are known to delay gastric emptying. However, the number of calories delivered to the duodenum per unit time is controlled to regulate the rate of delivery of acidic chyme delivered to the small intestine. In this study, the two test meals were matched for calorific value and osmolarity and no significant difference was observed between their rates of gastric emptying or colon filling. This is in agreement with the work of Hunt and Stubbs (1975) who reported that gastric emptying rates correlate with the nutritive density of the meal, i.e., isocaloric concentrations of different foods leave the stomach at approximately equivalent rates. Since the gastric emptying and colon arrival rates of the test meals in the present study were identical, any differences in absorption of the drug could be attributed to the different composition of the meals. The high fat meal in the present study contained 13% fat w/w, which supplied 66% of the total energy of the meal, and the low fat meal contained 5% fat w/w which supplied 29% of the total energy of the meal.

An interesting phenomenon observed in this study is the effect of fat in the small intestinal transit time of the resin and the meal. As the meals were matched for calorific value, the small intestinal transit time of the meal must have been increased in response to the high fat load even though the transit of the co-administered resin was unaffected. The delivery of both meal and resin from the stomach to the small intestine was identical and the discrimination of the two labels appears to have occurred within the small intestine.

The pharmacokinetic values obtained for rifampicin in this study agree with those quoted in the literature for this drug when administered after food (Siegler et al., 1974). The mean plasma curves obtained for rifampicin when administered after the two test meals were not significantly different. This implies that the variability in blood levels observed by other workers after various feeding regimes was not due to lymphatic absorption of the drug, even though the drug would be expected to partition into the lipid present in the meal. The largest difference in bioavailability appears to be between the fasting and feeding states, in which fasting produces elevated serum levels and reduces the time to peak concentration (Siegler et al., 1974; Polasa and Krishnaswamamy, 1983).

A possible explanation for the variable absorption seen for rifampicin after different meals may be the alteration of gastric pH produced by the food. Pure parietal secretion has an H^+ concentration of 0.15 M which is diluted to between 0.02 and 0.06 M by non-parietal cell secretion. In our studies, we have found the resting pH of healthy people to be around 1.8, but this is increased by a meal to between 3 and 5, and food with a high protein content such as milk can raise gastric pH to over 6. Food with a high protein content stimulates acid secretion to the greatest extent. Rifampicin is more soluble in acid media than neutral, and hence it is absorbed from fasting subjects (Polasa and Krishnaswamamy, 1983). Stimulation of acid secretion by histamine has been demonstrated to produce plasma concentrations of rifampicin which are twice those produced if the gastric contents are neutralised with sodium bicarbonate (Acocella, 1979). Consequently pH-controlled dissolution of rifampicin may be rate-limiting. Although rifampicin dissolves easily at low pH, it is highly dissociated and hence is not rapidly absorbed (Maggi et al., 1966). The presence of a meal will decrease solubility of the drug since it will raise pH to an unpredictable degree, which could explain the apparently variable effects of food. Fat in food has been shown to reduce acid secretion (Long

and Brooks, 1965), but in general the acid-secreting response of the stomach to food is known to be highly variable between individuals.

It can be seen by correlating plasma-profiles and position of the formulation that the peak plasma levels occur when the majority of both food and formulation are in the proximal part of the small intestine. This also supports the pH controlled absorption hypothesis, since the acidic chyme from the stomach is neutralised in the small intestine by secretions from the pancreas and Brunner's glands, both of which contain bicarbonate. This would give an effective 'window of absorption' in the upper part of the small intestine. There is no detectable colonic absorption of the drug, which would have been seen as a secondary peak or tail to the plasma concentration-time curve.

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