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Bioavailability of riboflavin from a gastric retention formulation

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Abstract

A gastric retention formulation (GRF) made of naturally occurring carbohydrate polymers and containing riboflavin was tested in vitro for swelling and dissolution characteristics as well as in fasting dogs for gastric retention. The bioavailability of riboflavin, a drug with a limited absorption site in the upper small intestine, from the GRF was studied in fasted healthy humans and compared to an immediate release formulation. It was found that when the GRF is dried and immersed in gastric juice it swells rapidly and releases its drug content in a zero-order fashion for a period of 24 h. In vivo studies in dogs showed that a rectangular shaped GRF stayed in the stomach of fasted dogs for more than 9 h, then disintegrated and reached the colon in 24 h. Endoscopic studies in dogs showed that the GRF hydrates and swells back to about 75% of its original size in 30 min. These in vivo results correlated with in vitro results. Pharmacokinetic parameters determined from urinary excretion data from six human subjects under fasting conditions showed that bioavailability depended on the size of the GRF. The biostudy indicated that bioavailability of riboflavin from a large size GRF was more than triple that measured after administration of an immediate release formulation. Deconvolved input functions from biostudy data suggest that the large size GRF stayed in the stomach for about 15 h.

Keywords: Riboflavin; Gastric retention; Bioavailability; Swellable systems

1. Introduction

Several different approaches have been developed to achieve an extended gastrointestinal (GI) transit time of oral drug delivery systems, and more particularly to sustain their residence time in the stomach. Gastric retentive dosage forms may provide the best results for drugs that act locally in the stomach or that may be absorbed primarily in the stomach or from the upper regions of the GI tract (i.e., drugs with absorption windows) such as riboflavin (Bates, 1997), ranitidine (Williams et al., 1992), and nitrofurantoin (Hwang et al., 1998). A number of investigators have tried to confine dosage forms to the stomach by employing a variety of concepts. Passage delaying agents have been used to slow down the normal motor activity of the stomach in order to increase gastric residence time (GRT) of drugs (Moes, 1993). A number of floating dosage systems involving various technologies, carrying their own advantages and limitations, have been developed, such as single- and multiple-unit hydrodynamically balanced systems (HBS) (Sheth and Tossounian, 1984; Khattar et al., 1990), single- and multiple-unit gas generating systems (Michaels et al., 1975), hollow microspheres (Kawashima et al., 1992), floating bilayer tablets (Wek et al., 2004), floating bilayer capsules (Oth et al., 1992), and floating ion exchange resins (Atyabi et al., 1996). More recently a floating dosage form made of freeze dried calcium alginate beads has been developed and was found to increase the bioavailability of riboflavin under fasted conditions when citric acid solution was used as an administering vehicle (Stops et al., 2006). Bioadhesive systems that adhere to the mucus were also reported to cause moderate increase in gastric residence time of drugs (Park and Robinson, 1984; Khosla and Davis, 1987; Longer et al., 1985; Harris et al., 1990) and also to cause ulcerous side effects if the released drug is irritating to the mucosa (Moes, 1993). In one study the bioavailability of riboflavin and furosemide was significantly increased when administered in adhesive microspheres compared to when non-adhesive microspheres were used (Akiyama et al., 1997). Expandable gastroretentive dosage forms (GRDFs) have been developed for the past three decades. These formulations are made small enough for easy swallowing but expand upon contact with gastric juice to a size sufficient to cause retention of the formulation in the

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stomach. After drug release their dimensions are minimized with subsequent evacuation from the stomach. Regarding the retention size of these formulations, some studies indicate the size could start around 7 mm and range up to higher than 10 mm (Timmermans and Moes, 1993). There are available data, on the other hand, that confirm that even oral dosage forms as large as 10–15 mm in diameter are not retained in the fasted stomach (Khosla and Davis, 1990). In one study in humans, it has been reported that objects thicker than 2 cm and longer than 5 cm tend to lodge in the stomach (Webb, 1995). GRDFs usually take different sizes and shapes such as rings, disks, and strings (Cargill et al., 1988; Caldwell et al., 1988). Silicone dosage forms having shapes like minimatrices, extruded rods, and moulded slabs were also screened in humans (using scintigraphy) and tested for gastric retention (Kedzierewicz et al., 1999). GRDFs are usually made of swellable expanding agents such as swellable resins and hydrocolloids (Shalaby et al., 1992) and more recently chitosan–poly(acrylic) acid complex has been tested for gastric retention (Torrado et al., 2004). Lately superporous hydrogel (SPH) and SPH composite (SPHC)-based drug delivery systems have been developed to increase GRT of drugs (Dorkoosh et al., 2004). SPH and SPHC are reported to swell to near equilibrium in a very short time (10 min) when immersed in aqueous fluids (Gemeinhart et al., 2001), to superswell and to have high mechanical strength, three properties necessary for gastric retention (Chen et al., 2000). Novel complex gastro-retentive dosage forms (GRDFs) made with different rigid polymeric matrices that expand by unfolding multilayer polymeric films attached to each other by methylene chloride or ethyl alcohol were found to prolong gastric residence time of narrow absorption window drugs, such as riboflavin, furosemide, and levodopa in fasted dogs and in humans only in presence of food (Klausner et al., 2002a, 2003a). In one study, large-dimension DFs lacking rigidity had similar gastro-retentivity as a non-disintegrating tablet (10 mm) as they did not withstand the peristalsis and mechanical contractility of the stomach (Klausner et al., 2003b).

Unlike other reported GRDFs that suffer from a number of disadvantages such as the complexity of their manufacture, the use of organic solvents in their preparation, the need of presence of food in the stomach to ensure gastro-retentivity, or the intervention with normal gastrointestinal motility, we report a novel simple gastric retention formulation (GRF) made of naturally occurring food polymers that demonstrate gastro-retentivity in both dogs and humans under fasting conditions using riboflavin as a model drug. The absorption of riboflavin is reported to occur by an active transport mechanism that is saturable, particularly in the upper duodenal area, so that little riboflavin is absorbed in the lower intestine (Levy and Jusko, 1966; Christensen, 1973). The biological half-life of riboflavin is about 66–84 min and clearance data indicated that urinary excretion of riboflavin contributes to one-half of the overall removal of riboflavin from plasma (Zempleni et al., 1996). When a GRF containing riboflavin resides for longer periods in the stomach and slowly releases the drug close to the duodenum, the bioavailability would be expected to improve compared with that of an immediate release formulation.

2. Materials and methods

2.1. Materials

Riboflavin, Locust bean gum (LBG), polyvinyl pyrrolidone (PVP) K26-35, and polyethylene glycol (PEG) 400 were purchased from Sigma Chemical Co. (St. Louis, MO). Xanthan gum (XG) was purchased from Spectrum Chemical Mfg. Corp. (Gardena, CA) and sodium lauryl sulfate (SLS) was purchased from Matheson Coleman & Bell (Cincinnati, OH). Inert, white, radio-opaque barium impregnated polyethylene spheres (BIPS), 1.5 mm in diameter, (Chemstock Animal Health Ltd., New Zealand) and radio-opaque threads (Veterinary Teaching Hospital, OSU) were used as radiodense tracers in animal studies. All other chemicals were of reagent or HPLC grade and used as received.

2.2. Preparation of riboflavin GRF

GRF was prepared by dissolving PVP (0.5%), LBG (0.5%), SLS (0.15%), and XG (0.75%) in deionized water. PVP, LBG and XG formed the hydrogel. SLS was added to enhance the dissolution of riboflavin from the swellable system due to formation of a more porous structure and it produced more flexible dried films that were easier to fit into capsules when compared to films from formulations without SLS. The increased flexibility helped in fitting large GRF in capsules. The resulting solution was heated to a temperature of 85 °C and 6 ml of PEG 400 was then added to the heated viscous solution. The pH of the resulting solution was about 5.5. Accurately weighed riboflavin in powder form was added to the hot viscous solution with constant stirring to produce a homogenous mass. The solution was then poured into different shaped molds. The resulting gel was left to cool for 4 h at room temperature and then dried in a vacuum oven at 50–55 °C for 16 h. The process of drying produced flexible films that are easily rolled and fitted into capsules. The GRF, consisting of a capsule containing the dried gel (film) with riboflavin, was used for the different studies. Each GRF contained 100 mg riboflavin. The content of riboflavin in GRF was determined by dissolving riboflavin in the dried film and the resulting solution is filtered and assayed for riboflavin at 446 nm using a HP 8453 diode array spectrophotometer. Four different shaped GRFs incorporated in size "0" capsules were used for dissolution and animal studies (Table 1). Three different sizes rectangular GRFs fitted in three different size capsules were used in human studies. The sizes of the capsules were '000', '00', and '0' size capsules (Rudnic and Schwartz, 2000).

Table 1
Shapes and dimensions of the four GRFs tested in dogs

Shape	Dimensions (cm)
Cubic	1.5 × 1.5 × 1.5
Rectangular	3.0 × 1.5 × 1.0
Short cylinder	2.2 × 1.7
Long cylinder	5.5 × 1.0

2.3. Dissolution studies

Dissolution studies were carried out according to the United States Pharmacopoeia (USP) XXII paddle method at 37 °C and 50 rpm for 24 h. Dissolution medium consisted of 900 ml USP simulated gastric fluid (SGF) without added enzymes. Samples were collected at 1, 2, 4, 6, 8, 10, 12, 16, 20, and 24 h. Samples were assayed for riboflavin at 446 nm using a HP 8453 diode array spectrophotometer.

2.4. Animal studies

Two mixed-breed dogs (aged 2.5 and 5 years and weighing 20 and 26 kg) were used to study the GRT of four different shaped GRFs incorporated in size "0" capsules (Table 1). All GRFs contained radio-opaque threads for X-rays visualization. Radio-opaque threads cut into small pieces, about 4–5 mm in length, were added and mixed with the hot viscous solution before pouring the solution into molds, so that each GRF contained no less than 20 pieces. All animal studies were approved by the Oregon State University Institutional Review Board (IRB) for the animal studies. The dogs were maintained on a canned protein diet (d/d Hills) for 2 weeks. The two dogs were fasted overnight and one of the four different shaped GRFs was administered orally early in the morning with 4 oz of water to each dog. Each shape was given in a separate day and tested in both dogs at least once. The next shape was only administered to the dog the next day after an X-ray showing the previous dosage form had left the stomach. The whole study lasted 2 weeks.

2.5. Radiographic studies

Radiographic examinations were performed using a Transworld 360 V X-rays generating unit. X-ray cassettes used were 3 M Trimax 12 paired with 3M ultradetail (1416) film. Radiographic examinations were performed from two angles, a lateral view and a dorsoventral view. Radiography was used to follow passage of GRFs in the GI tract. Radiographs for dogs were exposed at 0 min (just before dosing to ensure an empty stomach), at 5 min (just after dosing to assure that the GRF was in the stomach), at 2, 9, and 24 h. If the GRF was removed from the stomach at the 2 h radiograph, then no more radiographs were taken for the rest of the day. The dogs were fed after the 2 h radiographs. Food was sometimes mixed with BIPS to study the effect of GRF on food emptying from the stomach. Hills d/d diet is known to suspend BIPS. BIPS have a density similar to food but are sufficiently radiodense to show clearly on abdominal radiographs. BIPS mimic passage of food in the GI tract and provide an accurate estimate of the gastric emptying rate and intestinal transit time of food (Chandler and Guilford, 1995). BIPS can be differentiated from radio-opaque threads in radiographs. For the food emptying studies two sizes rectangular shaped GRFs were used. A small rectangular GRF (3.0 cm × 1.5 cm × 1.0 cm) fitted in size "0" capsule and a large rectangular GRF (7 cm × 1.5 cm × 1.0 cm) fitted in size "000" capsule.

2.6. Endoscopic study

Endoscopy was used to allow visual observation of the initial swelling of the GRF in fasted stomach. One dog was used for this study. The animal was fasted 16 h prior to dosing. The dog was dosed while awake. The small rectangular GRF was used in this study. The animal was induced with ketamine (259 mg) in combination with diazepam (7.5 mg) given intravenously. The animal was intubated with a cuffed endotracheal tube and maintained under general anesthesia with isoflurane gas and oxygen. Following attainment of a suitable anesthetic plane, a flexible fiber optic endoscope (Olympus GIF XP20 with a 7.9 mm outer diameter and 1.025 m scope, Olympus Corporation, Lake Success, NY) was introduced into the mouth and esophagus and guided to the stomach. A camera attached to the endoscope monitored the GRF and the process of expansion was recorded on videotape over a period of 45 min. Once fully recovered the animal was returned to its normal housing.

2.7. Design of human studies

The study protocol was approved by the Oregon State University Institutional Review Board (IRB) for the protection of human subjects. All human subjects (four male and two female) gave informed written consent for participation. All subjects were judged to be healthy on the basis of medical history and were not taking any medication including multiple vitamins or riboflavin. Subjects were also asked to avoid eating certain foods known to contain appreciable amounts of riboflavin (such as liver, milk, eggs, and riboflavin-enriched food such as cereals, corn products and noodle products) for at least 48 h prior to and during the study. The study consisted of four treatments under fasting conditions. Bioavailability of riboflavin from the different sizes rectangular GRFs was compared to an immediate release formulation containing the same dose of riboflavin. The different treatments for the human studies were as follows:

1. Large GRF capsules (LGRF): were size '000' capsules filled with dried films containing 100 mg riboflavin. The dimensions of incorporated gel before drying were 7 cm × 1.5 cm × 1 cm.
2. Intermediate GRF capsules (IGRF): were size '00' capsules filled with dried films containing 100 mg riboflavin. The dimensions before drying were 5 cm × 1.5 cm × 1 cm.
3. Small GRF capsules (SGRF): were size '0' capsules filled with dried film containing 100 mg riboflavin. The dimensions before drying were 3 cm × 1.5 cm × 1 cm.
4. Immediate release (IR) capsules: were size '1'capsules filled with lactose as the principal excipient (200 mg) and 100 mg riboflavin.

Each of the six subjects ingested an IR capsule (Treatment A), a LGRF capsule (Treatment B), an IGRF capsule (Treatment C) and a SGRF capsule (Treatment D) in a randomized crossover design with a washout period of at least 1 week. Capsules were ingested with 200 ml of water. All subjects were asked to fast at least 10 h before the study and no food was allowed for 2 h after

dosing. A breakfast consisting of a bagel with tea or coffee was provided 2 h after dosing. Lunch was offered 4 h after breakfast and consisted of a chicken sandwich and French fries. No restriction on water intake was imposed during the study and no soft drinks were allowed. Subjects provided their own dinner.

2.8. Collection of urine samples

Fasted subjects emptied their bladder and provided a zero-time urine sample prior to dosing, then ingested a formulation. Subjects collected the contents of their bladders in 16 oz disposable plastic containers at 1, 2, 3, 4, 6, 8, 10, 12, and 24 h post dosing. Volume and time elapsed since vitamin ingestion was recorded directly after voiding for each urine sample. Aliquots were frozen at −20 °C until analyzed for riboflavin. Urine samples were protected against exposure to light because of light sensitivity of riboflavin.

2.9. HPLC procedure

Urine was analyzed for concentration of riboflavin by HPLC. The column was a reverse-phase micro-particulate C_{18} (μBondapak C_{18}, particle size 10 μm, 30 cm × 4 mm, Waters Associates, Milford, MA) preceded by a C_{18} guard cartridge (ODS, 4 mm × 3 mm, Phenomenax, CA). Assay procedure followed that described by Smith (1980). The mobile phase was prepared by mixing exact volumes of 0.01 M potassium monobasic phosphate solution (adjusted to pH 5 with 1N sodium hydroxide) and methanol at a flow rate of 1.2 ml/min. The detector was a fixed-wavelength fluorescence spectrofluorometer (Gilson Spectra/Glo Fluorometer, Middleton, WI). Excitation wavelength was 450 nm. The wavelength range for the emission filter was 520–650 nm. Peak areas were determined with a Shimadzu integrator (CR501 Chromatopac, Shimadzu Corp., Kyoto, Japan). All solutions were protected from light. Retention time of riboflavin was about 6 min. Sensitivity of assay was good at 1 μg/ml with a linear relationship between peak areas and riboflavin concentrations of 1–10 μg/ml ($R^2 = 0.9971$).

2.10. Analyses of samples

Approximately 10 ml of urine was centrifuged at 4000 rpm for 10 min. A portion of the supernatant (150 μl) was transferred to HPLC tubes and 50 μl injected onto the HPLC column. Endogenous riboflavin was taken into account by subtracting the area obtained from analysis of zero time urine sample from standards and samples.

2.11. Pharmacokinetic analysis

The different treatments were compared in terms of urinary recovery of riboflavin during the first 24 h after administration, $\text{Recovery}_{0-24\,\text{h}}$, maximum urinary excretion rate ($R_{\max}$), and the time ($T_{\max}$) required to reach $R_{\max}$. All parameters were determined from the individual urinary excretion rate–time curves, a plot of urinary excretion rate against the mid-point of a urine collection interval. Urinary excretion rate for each time point of urine collection was calculated by multiplying the concentration of drug in urine for each time point (as determined from the standard curve) by the volume of urine collected to get the amount of unchanged drug excreted in urine during this time interval (D_u). This amount was then divided by the time interval for collection of urine sample to obtain the urinary excretion rate (D_u/t). Graphs were constructed by plotting (D_u/t) versus the midpoint of collection period (t^*). $\text{Recovery}_{0-24\,\text{h}}$ was determined from the individual cumulative urinary drug excretion–time curves, a plot relating the cumulative unchanged drug excreted (D_u) to the collection time. Urinary excretion data can be used to estimate bioavailability because the cumulative amount of drug excreted in urine is directly related to the total amount of drug absorbed and then excreted through a first-order elimination process (Wagner, 1979; Shargel and Yu, 1985).

2.12. Statistical analysis

Differences between each of the three GRF capsules (LGRF, IGRF, and SGRF) and the immediate release (IR) capsule in pharmacokinetic parameters were examined using a multiple comparison procedure (Dunnett's test). This test controls Type I experiment wise error ($\alpha = 0.05$) for comparisons of all treatments against a control. Differences between pairs of means were performed on $\text{Recovery}_{0-24\,\text{h}}$, $R_{\max}$, and $T_{\max}$ for urinary recovery data, and 95% confidence intervals (CI) were computed.

2.13. Deconvolution analysis

Deconvolved input functions from biostudy data were determined using computer software PCDCON (Gillespie, 1992). Deconvolution generates an input function (cumulative amount absorbed in vivo versus time) from an input response and the drug's characteristic impulse response function. The cumulative drug input over time predicted by deconvolution was used to estimate the GRT of the different size GRFs. GRT was calculated from the deconvolved curve as the time observed when absorption stops, based on the assumption that input continued as long as the formulation was in the stomach, and stopped when the formulation passed the absorption window. The input response used was urinary excretion rate of riboflavin from different formulations, while the impulse response used was a literature-derived elimination rate constant as determined from an intravenous bolus dose of riboflavin (Zempleni et al., 1996).

3. Results and discussion

The mean riboflavin content in GRFs was found to 98 ± 5%. Riboflavin is known to be stable to heat in solution from pH 1 to 6.5 (Vanderveen and Vanderveen, 2000). These results indicate that there was no loss of riboflavin during the manufacturing process. Dissolution testing of the GRF showed that when the capsule containing the dried film is immersed in SGF, the capsule shell rapidly dissolves and releases the dried film which then hydrates and swells in few minutes. It took about 30 min for the rectangular GRF to swell back to about 80% of its original size.

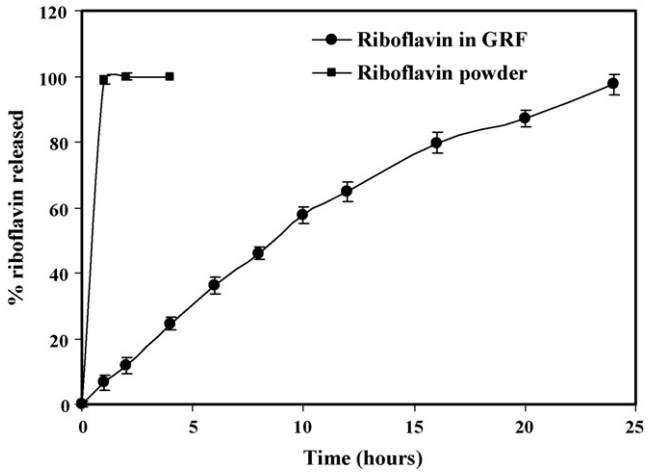

Fig. 1. Release of riboflavin from rectangular GRF compared to riboflavin powder. Error bars represent standard deviations ($n = 3$).

The other shapes took 45–60 min to swell back to about 75% of their original size. In preliminary studies it was found that the drying time and temperature affect the rate of swelling. Initial swelling is a very important factor in development of GRF to avoid early gastric emptying by the strong contractions of the housekeeper waves. The different shapes, however, continued to increase in size slowly over time but never reached the original size before drying, even after at 24 h. Theses results are consistent with published data reporting that compression exerted on dried gels to fit into capsules may affect the dimensional stability of the gel (Gemeinhart et al., 2001). Different shaped GRFs released 60–75% of their riboflavin content in 12 h in an almost zero-order fashion and retained their integrity for 24 h in SGF. Fig. 1 shows the release profile of riboflavin from a rectangular shaped GRF (3 cm × 1.5 cm × 1 cm) encapsulated in a "0" size capsule compared to an immediate release capsule containing the same amount of riboflavin.

Of all the shapes tested in dogs, only the rectangular shape was found to stay in the stomach for at least 9 h. The other three shapes were emptied from the stomach in less than 2 h. These results are consistent with in vitro results in that of all shapes tested in vitro, the rectangular shape was found to swell back faster and to a larger extent than the other shapes. These results are also consistent with other published works in which rectangular shaped GRDFs were reported to show gastro-retentivity in dogs (Klausner et al., 2002b). Radiographs at 24 h indicated the absence of radio-opaque threads in the stomach for the rectangular shaped GRF and its disintegration as indicated by the spread

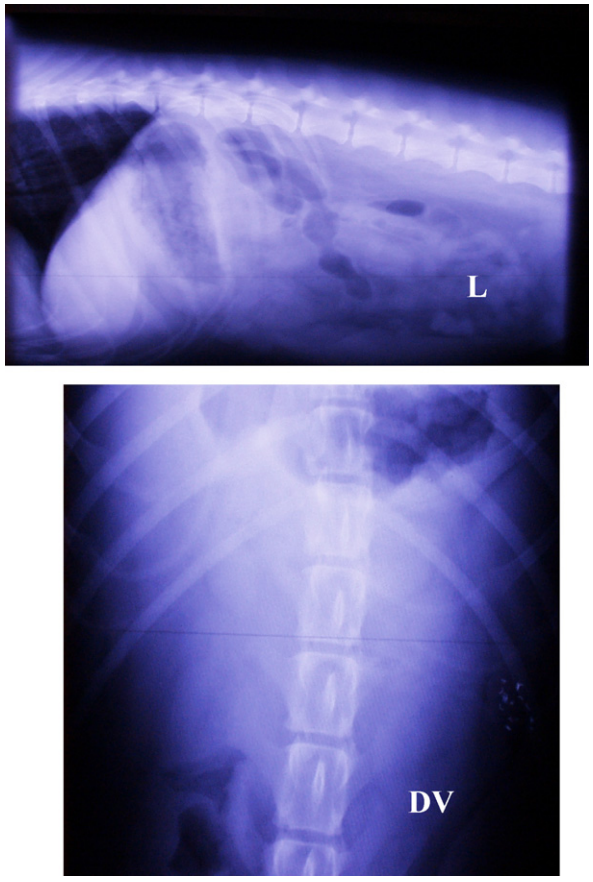

Fig. 2. Radiographs taken early morning just before dosing the dogs to ensure empty stomach. L: lateral view and DV: dorsoventral view.

of threads in the colon. A total of four studies were conducted using the rectangular shaped GRF and in all four studies the GRF was retained in the stomach of dogs. The results of these studies are shown in Figs. 2–6. Radiographs taken 2 h after food mixed with BIPS showed the food has emptied from the stomach while GRFs did not (Fig. 7). This indicates that GRF did not affect food emptying from the stomach and food did not affect swelling of GRF in the stomach as GRF continued to swell after the 2 h radiograph, the radiograph at 9 h showed a fully swollen GRF, which means that the presence of food in the stomach did not prevent the GRF from swelling further and food did not result in the destruction of GRF in the stomach. Similar results were obtained from the larger size rectangular GRF indicating that there was no blockade of pyloric sphincter by GRF (Fig. 7B). Based on these results, the rectangular shaped GRFs were cho-

Table 2
Mean PK parameters of riboflavin after oral administration of 100 mg in immediate release or GRF capsules to six fasted volunteers

	Treatments			
	IR	SGRF	IGRF	LGRF
Recovery from 0 to 24 h (mg)	5.3 ± 1.7	4.1 ± 1.7	9.3 ± 5.3	17.4 ± 9.7
Maximum urinary excretion rate (mg/h)	1.4 ± 0.4	1.1 ± 0.6	2.0 ± 0.9	2.5 ± 0.9
Time of maximum urinary excretion rate (h)	2.5 ± 0.6	2.3 ± 0.9	3.3 ± 1.1	5.1 ± 2.4
Mean residence time (h)	4.7 ± 0.8	5.9 ± 1.0	5.3 ± 1.7	7.0 ± 1.2

Data are mean values ($n = 6$) ± S.E.

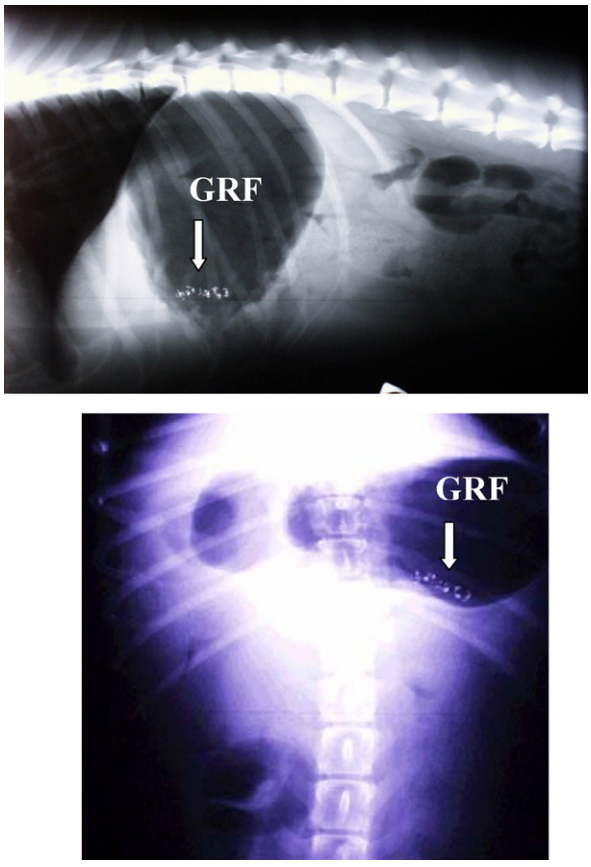

Fig. 3. Lateral and dorsoventral views showing the GRF in the stomach right after dosing the dogs.

sen to test in humans. Endoscopic views showed the location of GRF in the stomach (Fig. 8A). It was observed that the capsule shell dissolves in few minutes and the GRF is released. The swelling of GRF occurred gradually over a period of 30 min (Fig. 8B). After 45 min the gel was recovered from the stomach to compare its dimensions with in vitro results. It was found that the recovered gel reached about 75% of its original "before drying" dimensions. Thus, these in vivo results correlated with in vitro results.

Table 2 summarizes mean pharmacokinetic (PK) parameters for the different treatments in six subjects obtained from the biostudy. The largest mean value for $Recovery_{0–24\,h}$ was observed for LGRF capsule, followed by IGRF capsule, IR capsule, and SGRF capsule. The mean $Recovery_{0–24\,h}$ estimate from the LGRF capsule (17.4 mg) was determined to be 225% larger and statistically significantly different (95% CI is 3.691–20.383) relative to the mean from IR capsule (5.33 mg). Mean $Recovery_{0–24\,h}$ estimate from SGRF capsule (4.1 mg) was less but not statistically significantly different (95% CI is −9.593 to 7.099) relative to the mean from the IR capsule (5.33 mg). The mean $Recovery_{0–24\,h}$ estimate from the IGRF capsule (9.3 mg) was higher but not statistically significantly different from the IR capsule (95% CI is −4.338 to 12.354). This could be due to prolonged gastric residence time of the formulation in only two of the volunteers (subjects 1 and 2 only had significantly higher urinary $Recovery_{0–24\,h}$ from IGRF capsule when com-

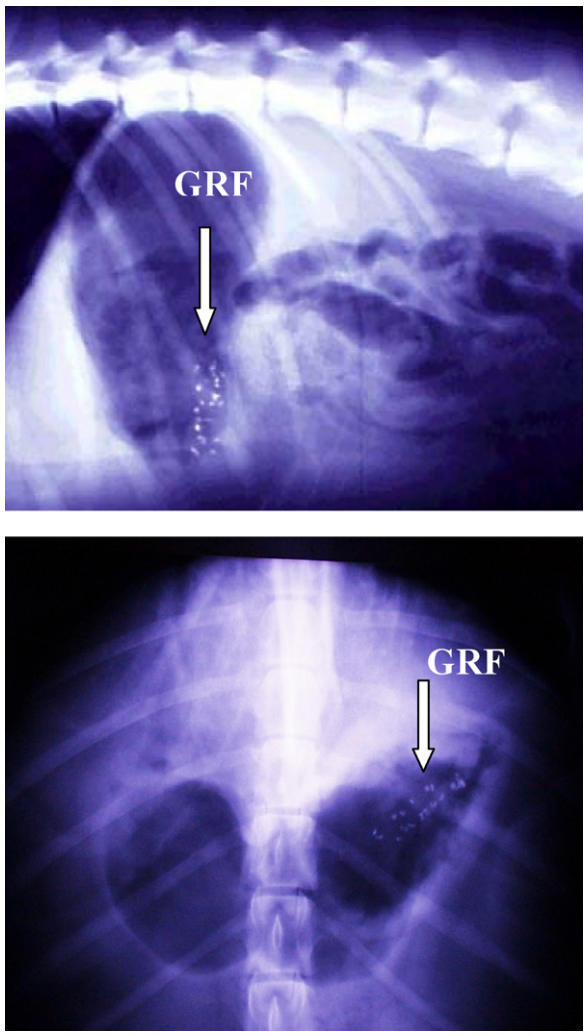

Fig. 4. Lateral and dorsoventral views showing the GRF in the stomach 2 h post dosing. The GRF has swollen as indicated by larger volume occupied by the radio-opaque threads.

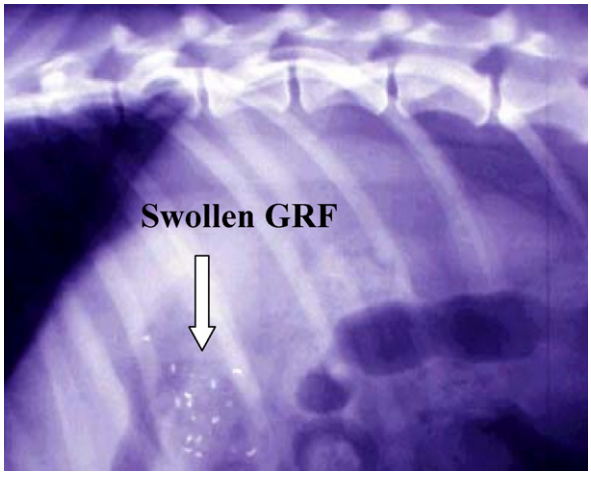

Fig. 5. A lateral view showing the fully swollen GRF still in the stomach 9 h post dosing.

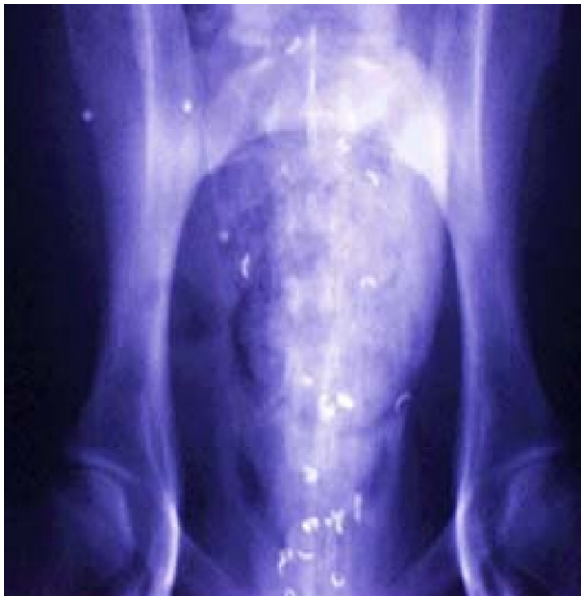

Fig. 6. Radiograph 24 h post dosing showing the GRF has disintegrated and reached the colon as indicated by spread of radio-opaque threads in the colon.

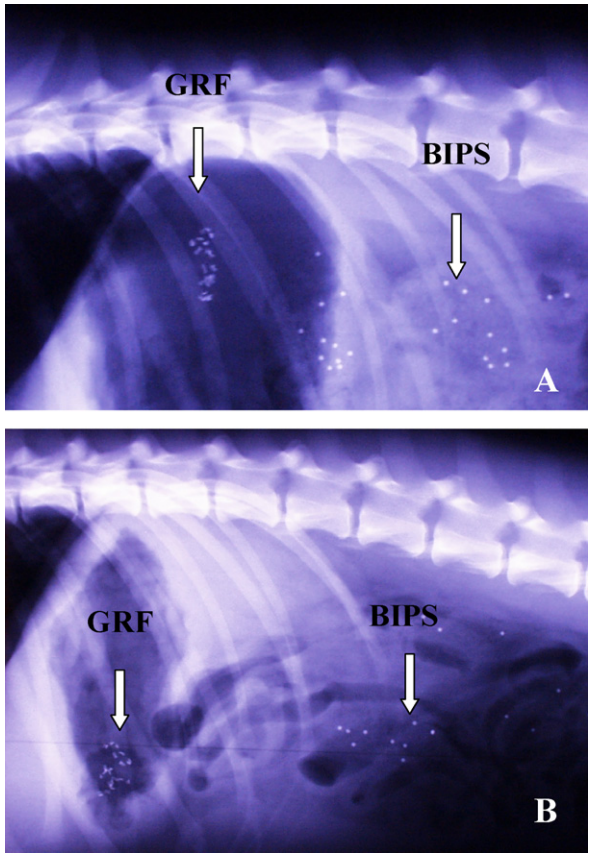

Fig. 7. Lateral views 2 h after giving food mixed with BIPS showing the food has emptied from the stomach while the GRF has not. (A) GRF in '0' capsule and (B) GRF in '000' capsule.

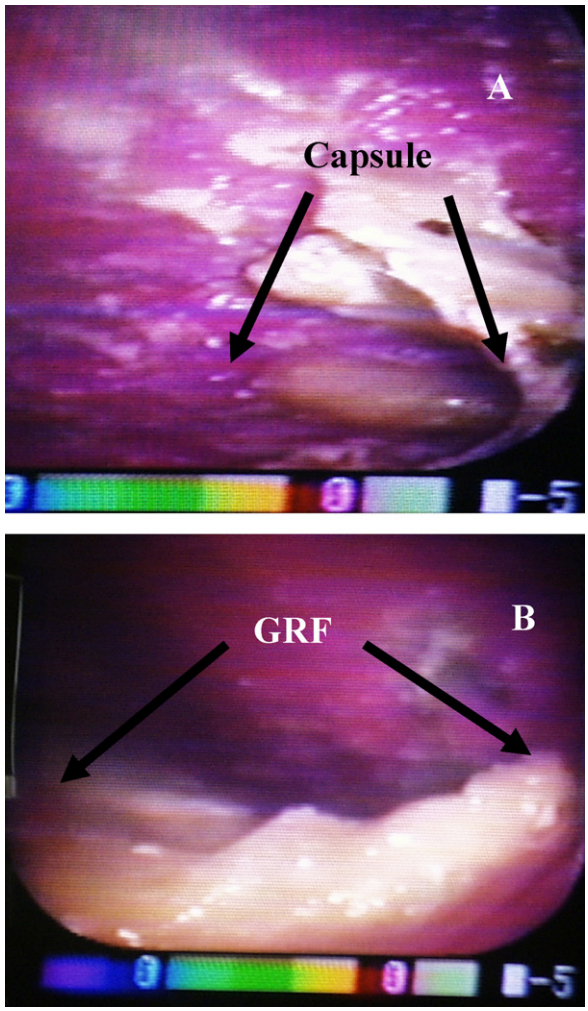

Fig. 8. (A) Endoscopic view of the gastric mucosa showing the capsule in stomach 5 min post dosing. (B) Endoscopic view 30 min post dosing showing the GRF is released from the capsule and is swollen.

pared to the IR capsule). Results of this study are also shown in Fig. 9. Statistical comparison of T_{max} parameter also indicated a significant difference (95% CI is 1.086–6.581) between results from LGRF capsule (5.08 ± 2.4 h) and IR capsule (2.5 ± 0.63 h). Statistical comparison of R_{max} parameter did not indicate a significant difference (95% CI is -0.0558 to 2.1758) between results from LGRF capsule (2.5 ± 0.98 mg/h) and IR capsule (1.36 ± 0.4 mg/h). R_{max} and T_{max} parameters from IGRF and SGRF capsules were not significantly different from the (IR) capsule. These results are shown in Fig. 10. The improved bioavailability of riboflavin from the LGRF capsule (urinary recovery was more than triple that measured after administration of the IR capsule) obtained in this study, suggests that the formulation was retained in the stomach. The LGRF stayed in the stomach for enough time to slowly release its vitamin content and consequently the released vitamin passed gradually through the absorption window and was absorbed more efficiently. Administration of the SGRF capsule, on the other hand, resulted in reduction of riboflavin absorption when compared with the IR capsule. This could be due to the small size of the formulation which was emptied from the stomach with relatively

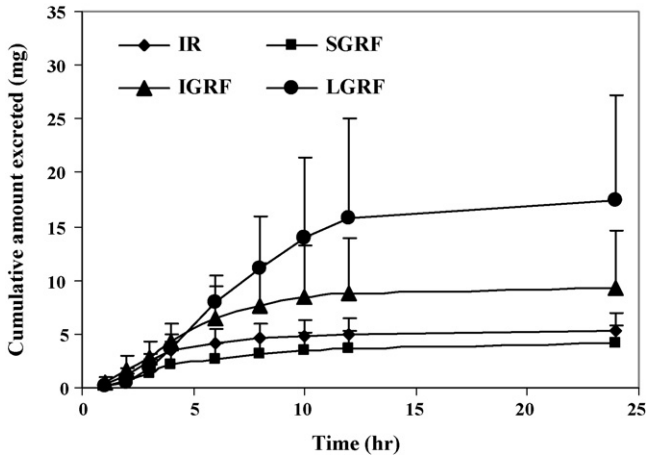

Fig. 9. Average cumulative amount of riboflavin excreted in urine for fasted subjects ingesting immediate release (IR) or GRF formulations. Error bars represent plus standard deviations ($n = 6$).

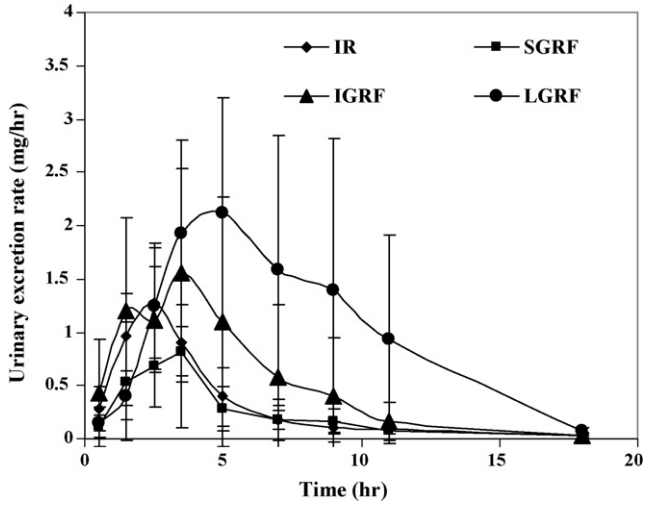

Fig. 10. Average urinary excretion rate of riboflavin for fasted subjects ingesting immediate release (IR) and GRF formulations. Error bars represent standard deviations ($n = 6$).

little drug released. Once the formulation passes the absorption window, no absorption takes place. The size of SGRF is larger than sizes previously reported not to be retained in the stomach (Khosla and Davis, 1990).

Fig. 11 shows the mean cumulative amount of drug absorbed versus time deconvolved from average biostudy data for the IR, SGRF, IGRF, and LGRF capsules. Absorption continued for up to 15 h for the LGRF capsule before it stopped. This may suggest that the LGRF was retained in the stomach and slowly released drug for about 15 h. The absorption from the IGRF capsule continued for about 9 h while absorption from SGRF capsule continued for only 3 h.

Based on these results it can be concluded that the greater bioavailability obtained from LGRF can be attributed to longer GRT of the LGRF. The size and cohesion of the LGRF was large enough to withstand compression pressure in the stomach and to be retained for enough time to slowly release its drug content under the conditions studied.

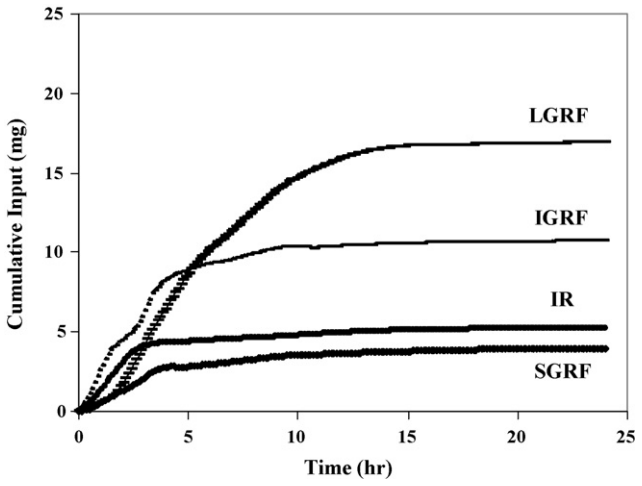

Fig. 11. Deconvolved input functions from average biostudy data for immediate release (IR) and GRF formulations of riboflavin.

Note that subjects fasted overnight and then fasted only an additional 2 h after GRF administration. Since many people will eat within a couple of hours after arising, and then again at 4–6 h intervals, this schedule was considered realistic for most cases. However, in some cases patients may not eat for as long as 6 or more hours following dosing and the migrating myoelectric complex (MMC) activity also known as the "housekeeper waves" occurring at the end of the digestive phase may occur multiple times during the fasting period. Thus, the GRF should be studied with longer intervals before eating following GRF administration in order to define need for timing of eating following GRF administration.

4. Conclusions

We conclude that a GRF made of naturally occurring carbohydrate polymers increased the gastric residence time of drugs under fasting conditions in man. The results are preliminary and further studies under different conditions such as varying conditions of food intake and longer fasting times should be conducted to show that the GRF could be retained in the stomach under more challenging circumstances.

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