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The in-vitro porcine adhesion model is not predictive of the esophageal transit of risedronate tablets in humans

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Abstract

Mucosal damage due to esophageal adhesion of pharmaceuticals is a continued concern to both health care providers and drug manufacturers. As a result of this concern, dosage forms are now being designed to exhibit minimal esophageal adhesion. Previous researchers have used an in-vitro porcine esophageal model to determine the propensity for formulations to adhere to the esophagus as an alternative to human scintigraphy studies. This study used a porcine esophageal adhesion model similar to that used previously to determine the adhesiveness of placebo bisphosphonate formulations. Results are analogous to those obtained by previous researchers, with film-coated tablets showing greater adhesiveness than uncoated tablets. These same tablet formulations were also evaluated previously by a human scintigraphy study, and the results were exactly opposite of those obtained using the in-vitro porcine model. In the human scintigraphy study, the film-coated placebo risedronate tablet had a faster transit time than an uncoated round placebo tablet. In conclusion, the in-vitro porcine esophageal model is not predictive of esophageal transit in man and gamma scintigraphy is the preferred method to evaluate esophageal transit.

Keywords: Esophageal adhesion; Risedronate; In-vitro pig esophageal model

1. Introduction

1.1. Background

When a solid oral pharmaceutical dosage form adheres in the esophagus during oral administration, rapid transference of solid dosage forms to the esophageal cells is likely to occur. This is an undesirable situation which may lead to physical damage to the esophagus. The interior surface of the esophagus is moist rather than wet, and a dosage form in contact with the mucosa will cause partial dehydration at the site of contact as the tablet hydrates, resulting in formation of a concentrated gel between the formulation and the mucosa (Reynolds, 1996). Retention of irritant drugs has been found to cause such problems in the esophagus as damage to the mucosa, ulcera-

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tion, stricture, perforation, and dysphagia (Marvola et al., 1982, 1983; Al-Dujaili et al., 1986). Also, prolonged residence of the tablet in the esophagus may affect drug absorption, as drugs cannot easily pass through the stratified squamous epithelium of the esophageal mucosa (Channer and Roberts, 1985).

The accepted clinical method of detection of esophageal transit is scintigraphic imaging. Scintigraphic imaging is a technique whereby the transit of a dosage form through its intended site of delivery can be imaged through the introduction of an appropriate short-lived gamma-emitting radioisotope (Wilding et al., 1991). The observed transit of the dosage form can then be correlated with the rate and extent of drug absorption. The current studies of esophageal transit using scintigraphic imaging can be time consuming and require the use of human subjects. These studies are therefore used most often as a verification tool in the last stages of the formulation development process.

Esophageal transit of solid pharmaceutical dosage forms is influenced by a variety of physical characteristics of the dosage forms (Perkins et al., 1999). Factors such as shape, size, and surface characteristics of the dosage form can directly determine esophageal transit. A better understanding of the relationship between physical characteristics of tablets and the corresponding adhesiveness of the tablet to the esophageal mucosa may allow an improvement in pharmaceutical tablet design.

A predictive in-vitro test method that would assist in designing oral formulations would therefore be very desirable. An in-vitro method would be faster, less expensive, and would involve no human or animal testing. Due to the above advantages, an in-vitro test method could be used much earlier in the formulation development process as a screening tool.

There have been reports of severe esophageal adverse events in patients treated with the primary aminobisphosphonates pamidronate and alendronate (Lufkin et al., 1994; deGroen et al., 1999) which fit the pattern of pill induced esophagitis or reflux of partially dissolved tablets from the stomach. Risedronate (a pyridinyl bisphosphonate) was formulated as a film-coated tablet to facilitate rapid esophageal transit and to minimize contact with the mucosa.

Previous in-vitro studies of esophageal transit include but are not limited to, a study conducted by Marvola and colleagues which evaluated the use of a porcine esophagus as a predictor of esophageal adhesion in-vivo, Swisher and colleagues who reported adhesion force of pharmaceutical dosage forms using both a pig and dog esophagus, and most recently, Gibson and colleagues who researched the effects of film-coating on esophageal adhesion (Marvola et al., 1982; Swisher et al., 1984; Gibson et al., 1999). The results of Gibson and colleagues indicate that film-coated oval risedronate tablets (Actonel® 30 mg) produced a detachment force significantly higher than uncoated oval tablets of the same shape and size. However the predictive nature of the in-vitro model has never been established. In this study the in-vitro adhesion force of a film-coated oval placebo risedronate tablet, an uncoated oval placebo risedronate tablet, and an uncoated round placebo tablet (similar in size and shape to the Fosamax® 10 mg tablet) were evaluated. The predictive nature of the in-vitro porcine model to in-vivo human esophageal transit was also examined.

2. Materials and methods

2.1. Materials

Because the propensity for adhesion is determined primarily by the external properties of a formulation, placebo risedronate tablets were used in this study. Uncoated oval placebo risedronate tablets, film-coated (containing hydroxypropylmethylcellulose and hydroxypropylcellulose) oval placebo risedronate tablets, placebo round uncoated tablets (designed to match the size and shape of round Fosamax® 10 mg tablets) were supplied by Procter & Gamble Pharmaceuticals. Gelatin capsules (positive control, Capsugel), and non-hydrating plastic tablets

Table 1
Physical characteristics of the placebo formulations

Formulation	Dimensions (mm)	Weight (mg)
Film-coated oval placebo risedronate tablet	11.7 × 5.8	247
Uncoated oval placebo risedronate tablet	11.7 × 5.8	240
Uncoated round placebo tablet	9.5 diameter	200
Gelatin capsule (size 1)	length — 18.6	75.5
Risedronate shaped plastic tablet	11.8 × 5.8	256
Round shaped plastic tablet	9.5 diameter	203

(negative controls, Elizatab, Carbide Die Company) were also evaluated in the in-vitro model. The physical characteristics of the placebo formulations are shown in Table 1.

2.2. In-vitro model

2.2.1. Test model development

A method was modeled after the method used by Marvola and more recently by Gibson and colleagues (Marvola et al., 1982; Gibson et al., 1999). An improvement over the previous method used by Marvola was the use of an Instron Universal Testing Instrument (Instron Corporation, Model 4201 Serial # 1000), which was used to measure the force of adhesion of the film-coated tablets to a porcine esophagus.

The model consisted of a porcine esophagus suspended in a fluid bath. A schematic diagram of the in-vitro esophageal model is shown in Fig. 1. All porcine esophagi were obtained from a porcine Yorkshire/Hampshire cross breed weighing 240–290 lb. In the esophageal model, the esophagus (6 in. in length, 1.5 in. in width) was rinsed using Tyrode buffer solution (Marvola et al., 1982), and allowed to drain for a set amount of time prior to each tablet insertion. Due to the structure of the Instron a moving crosshead was

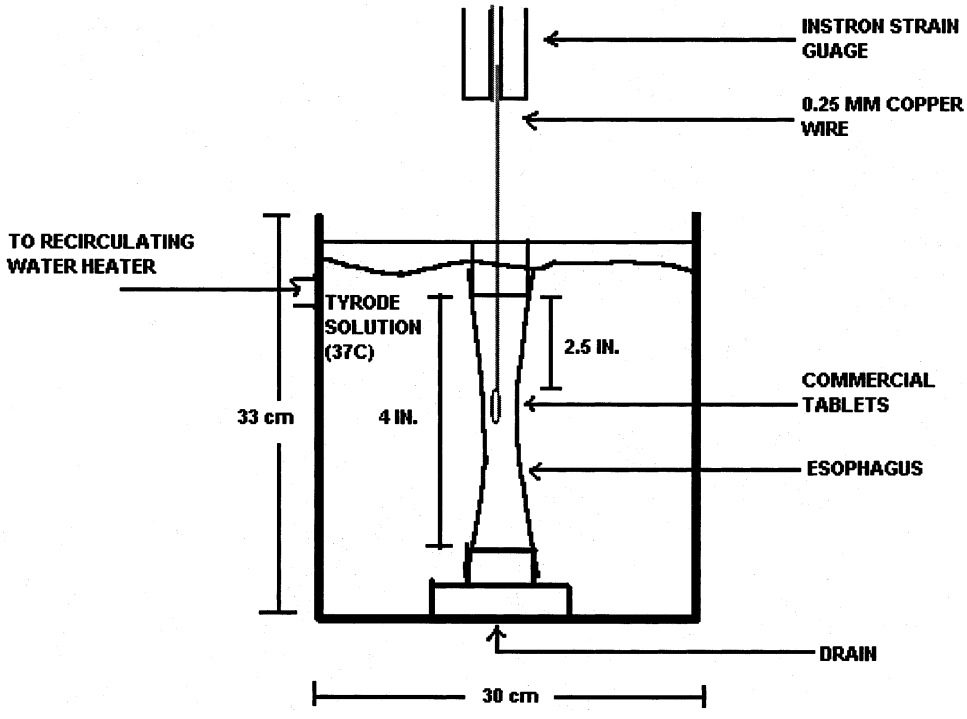

Fig. 1. Schematic of the In-vitro porcine esophageal model.

Table 2
In-vitro test conditions used to measure the detachment force

Factor	Standard conditions
Rinse volume	10 ml
Time after rinse prior to tablet insertion	10 s
Number of trials per esophagus	25–30 trials
Load cell displacement rate	2 in./min
Total run time	1 min

positioned above the sample to be tested. Holes (diameter 1 mm) were drilled in each of the tablets, including the plastic negative control tablets and the positive control capsules. A copper wire (diameter 0.25 mm) was inserted into each of the tablets and fixed with acrylic based adhesive. The tablet attached to a wire was inserted in the esophagus using a glass crossbar constructed for the purpose of establishing a standard depth within the esophagus at which to begin the test. The wire was clamped to the moving crosshead and the crosshead was programmed to slowly move upward, measuring the force of resistance of the tablet being pulled through the esophagus. The peak force of resistance exerted as the tablet adhered to the esophageal membrane was measured.

The Instron Strain Gauge gave a readout of pounds force exerted to drag each tablet through the esophagus. Results are reported as detachment force measured in pounds force (lbf), with the maximum force of each trial recorded. Detachment force is equivalent to measuring adherence force. Detachment force is the force necessary to detach the tablet from the esophagus, whereas adherence force is the force with which the tablet adheres to the esophagus.

2.2.2. Study design

Standard test conditions were established through preliminary testing in the in-vitro esophageal model and are listed in Table 2. A crossover design was implemented, where a total of six esophagi were used, with the experimental formulations (uncoated round placebo tablet, uncoated oval placebo risedronate tablet and film-coated oval placebo risedronate tablet) run six times per esophagus and the controls (risedronate shaped plastic tablet, gelatin capsule and round shaped plastic tablet) run four times per esophagus. This resulted in a total of 30 treatment periods. Based on historical data, thirty assessments per esophagus should be sufficient to allow comparison between dosage forms (Gibson et al., 1999). The formulations that were tested on each esophagus, were balanced with respect to the period that they were administered. This helps nullify any effects that order may have upon detachment force. The properties of this experimental design will allow one to know whether an apparent formulation effect is truly a real effect rather than a 'residual' effect of some other formulation or an effect of the period in which it was administered.

2.3. Statistical methods

Detachment force was analyzed using a crossover model with effects for dosage form, period, carry-over, and esophagus. Esophagus was modeled as a random effect. Carry-over accounted for potential residual effects from the previous dosage form, and period accounted for the effect of time. Period was treated as continuous, because of the confounding of period and esophagus. Pairwise comparisons were performed to determine statistical differences between formulations.

3. Results

3.1. In-vitro test results

Summary statistics for detachment force (lbf) by dosage form and Pairwise comparisons are provided in Table 3. The dosage form (P-value = 0.0001), period (P-value = 0.0273), and esophagus (P-value = 0.0001) effects were all significant with respect to detachment force. Carry-over (P-value = 0.9780) did not have a significant effect on detachment force. The detachment force data in the porcine esophagi ranged from 0.39 to 5.48 lbf (Fig. 2).

No statistically significant differences were observed between the uncoated round placebo tablet and uncoated oval placebo risedronate tablets. However, film-coated oval placebo risedronate tablets (mean = 1.81) showed a statistically significant higher detachment force than both uncoated oval placebo risedronate tablets (mean = 1.09) and uncoated round placebo tablets (mean = 0.73). As expected, the positive control gelatin capsule showed the greatest detachment force

Table 3
Results for detachment force (lbf) of the placebo bisphosphonate formulations and pairwise detachment force comparison *P*-values of placebo risedronate formulations

Dosage form	*N*	Mean (lbf)	SD	%RSD
Test formulations				
Uncoated round placebo tablet	36	0.73	0.57	78.1
Uncoated oval placebo risedronate tablet	36	1.09	0.72	66.1
Film-coated oval placebo risedronate tablet	36	1.81	1.38	76.2
Controls				
Risedronate shaped plastic tablet	24	0.39	0.14	35.9
Gelatin capsule (positive control)	24	5.48	4.12	75.2
Round shaped plastic tablet	24	0.59	0.57	96.6
Pairwise detachment force comparison P-values				
Dosage form 1 (Mean)		Dosage form 2 (Mean)		*P*-value 1 versus 2
Uncoated round placebo tablet (0.73)		Uncoated oval placebo risedronate tablet (1.09)		0.4249 (NS)
Uncoated round placebo tablet (0.73)		Film-coated oval placebo risedronate tablet (1.81)		0.0047
Uncoated oval placebo risedronate tablet (1.09)		Film-coated oval placebo risedronate tablet (1.81)		0.0438

NS, not statistically significant.

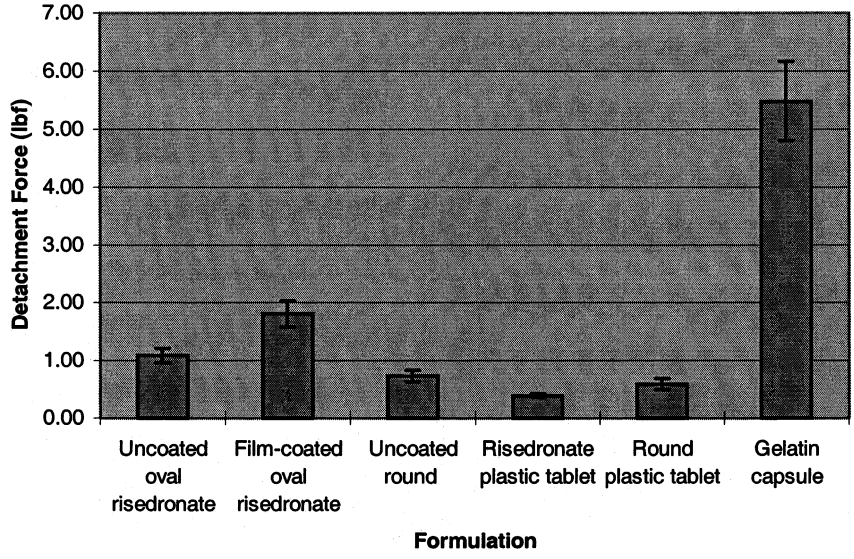

Fig. 2. Histogram of the detachment force in the porcine esophagi.

Table 4
Human esophageal transit results of the two formulations ingested with 30 and 50 ml of water (Perkins et al., 2000)

Esophageal transit (s)	30 ml water		50 ml water	
	Film-coated risedronate tablet	Uncoated round tablet	Film-coated risedronate tablet	Uncoated round tablet
Mean ± SEM	3.2 ± 0.3	65.2 ± 32.8	2.3 ± 0.2	3.4 ± 0.4
Median	2.8	3.8	2.0	2.8
P (for median difference)		$P = 0.002$		$P < 0.001$
Stasis (transit > 20 s)	0 subjects	5 subjects	0 subjects	0 subjects

(mean = 5.48), and the non-hydrating plastic tablets (oval mean = 0.39, round mean = 0.59) showed the lowest detachment force.

Results show that the rank order of detachment force is gelatin capsule > coated tablets > uncoated tablets, which is consistent with results obtained in previous studies (Marvola et al., 1982; Al-Dujaili et al., 1986; Gibson et al., 1999).

4. Discussion

While the results of this study are consistent with conclusions drawn from studies using this in-vitro model, they are in stark contrast to what is observed when these same formulations are tested in human trials. A human scintigraphy study previously compared the esophageal transit of a film-coated oval placebo risedronate tablet to that of an uncoated round tablet similar in size and shape to the round Fosamax® 10 mg tablet (Perkins et al., 2000). Tablets were ingested with 30 and 50 ml of water, as these volumes will detect formulations prone to esophageal adhesion. A total of 31 healthy postmenopausal women with a mean age of 63 years (range 55–74 years) were randomized into this four-way crossover study. Subjects swallowed a radiolabeled placebo tablet with 30 or 50 ml of water and scintigraphic image acquisition was conducted. Results are shown in Table 4 (Perkins et al., 2000).

The esophageal transit time of the film-coated placebo risedronate tablet was significantly shorter than that of the uncoated round tablet for both volumes of water, a result contraindicating the conclusion found using the in-vitro system. In three subjects who swallowed the uncoated round tablet with 30 ml of water, the esophageal transit was greater than 10 min, the duration of imaging. Under conditions where the uncoated round tablet caused esophageal stasis (transit time > 20 s) in five of 30 subjects, no stasis was observed with the film-coated risedronate tablet. (Perkins et al., 2000). The results obtained for the uncoated round tablet (similar in size and shape to the round Fosamax® 10 mg tablet) are consistent with the reported delayed esophageal transit if swallowed with minimal volumes of water (deGroen et al., 1999). Clearly the combination of film-coating and oval shape promotes esophageal transit compared to an uncoated round tablet.

H. Al-Dujaili described in-vitro studies of esophageal adhesion as ex-vivo experiments that cannot replace studies in human subjects, but they can indicate potentially abnormal behavior (Al-Dujaili et al., 1986). Results of this in-vitro study support those obtained by Gibson and colleagues, in which it was shown that film-coated commercially available risedronate tablets (Actonel® 30 mg) showed a statistically significant higher detachment force than uncoated placebo tablets (Gibson et al., 1999). However, these results do not correlate with the results of a recent study in which esophageal transit in humans was evaluated using scintigraphy (Perkins et al., 2000).

These results indicate that the porcine esophageal adhesion model is not predictive of esophageal adhesion in humans. Though a better understanding of the relationship between physical characteristics of tablets and the correspond-

ing adhesiveness of the tablet to esophageal mucosa may allow an improvement in pharmaceutical tablet design, this model does not provide that predictive property. The general conclusion that can be drawn from the preceding data is that esophageal transit testing needs to be conducted in-vivo in humans, as in-vitro methods which have been developed to date do not correlate with in-vivo tests.

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