Evaluation of the *in Vivo* Disintegration of Solid Dosage Forms of a Bile Acid Sequestrant in Dogs Using γ-Scintigraphy and Correlation to *in Vitro* Disintegration

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Purpose. To evaluate the *in vivo* disintegration behavior of tablets and capsules of a bile acid sequestrant, DMP 504, in beagle dogs and to assess the significance of the *in vitro* disintegration of the dosage forms on subsequent *in vivo* behavior in order to draw possible *in vitro–in vivo* correlations.

Methods. Tablet and capsule formulations of a bile acid sequestrant, DMP 504, were formulated with samarium oxide and neutron activated to produce radioactive ^{153}Sm to noninvasively evaluate their *in vivo* behavior in beagle dogs by γ-scintigraphy. A four-way crossover design was completed ($n = 4$) in which (a) tablets from two different batches were administered under the fasted condition and manufactured using different lots of drug substance where one batch exhibited relatively faster *in vitro* disintegration time (30 min) than the other tablet batch, which resulted in slower disintegration (45 min), (b) a capsule formulation was administered to fasted beagles, and (c) the tablet having slower *in vitro* disintegration was also administered in the fed state, and its *in vivo* disintegration was compared to that observed in the fasted state.

Results. Tablets manufactured using a lot of DMP 504 having relatively fast *in vitro* disintegration (~30 min) resulted in relatively rapid *in vivo* disintegration time (15 min) in the fasted condition. This *in vivo* disintegration time was comparable to the *in vivo* disintegration of the capsules (17 min) even though the *in vitro* capsule disintegration time was considerably faster (2 min). Tablets prepared using a drug substance that provided a longer *in vitro* disintegration time (~45 min) resulted in a slower *in vivo* disintegration (63 min). There was no difference observed in the *in vivo* disintegration behavior in fasted and fed dogs for the tablets that provided slower *in vitro* disintegration.

Conclusion. *In vivo* disintegration of tablets of the bile acid sequestrant DMP 504 correlated with *in vitro* disintegration times. γ-Scintigraphy continues to be a good tool to use during early stages of product development to investigate *in vivo* performance of dosage forms. The results of this study provided evidence that the physical chemical specifications of the drug substance may not always be indicative of *in vitro* or *in vivo* performance of tablet dosage form, even when formulation and process are not changed.

KEY WORDS: bile acid sequestrant; DMP 504; γ-scintigraphy; tablets; *in vitro* disintegration; *in vivo* disintegration.

INTRODUCTION

Cholesterol is the major and probably the sole precursor of bile acids. Bile acids are synthesized in the liver, stored in the gallbladder, and then delivered into the intestinal tract to facilitate the absorption of dietary lipids. To a large extent, bile acids are reabsorbed from the intestinal tract, predominantly in the terminal ileum, and returned to the liver through the enterohepatic circulation; bile acid sequestrants interrupt this enterohepatic circulation (1).

DMP 504 (Fig. 1) is a strongly basic anion-exchange polymer. The polymer contains randomly distributed primary, secondary, tertiary, and quaternary amine groups in their hydrochloride salt form. The alkylammonium groups that comprise this polymer form a random network containing a high level of branching and a low level of cross-linking (2,3). DMP 504 complexes with bile acids and is intended for oral use as a nonsystemic cholesterol-lowering agent (2,3). Physicochemical properties of this compound were previously reported (4). During the development stage, dosage forms manufactured using some lots of DMP 504 drug substance showed slow *in vitro* disintegration and consequently a slow *in vitro* drug release rate as measured by a reverse-binding method (5). Interestingly, tentative physical and chemical specifications of these lots of DMP 504 drug substance including wet and dry particle size analysis, surface area, density, swell ratio (unpublished data), and binding capacity (6) were not different. To determine if these differences in the *in vitro* disintegration were also present following *in vivo* administration, γ-scintigraphy was used to determine the time of *in vivo* disintegration.

The use of γ-scintigraphy to examine the *in vivo* behavior of oral dosage forms has been previously reviewed (7,8).

One way of preparing dosage forms containing a γ-emitting radionuclide is neutron activation (9,10). This technique involves the incorporation of a small amount (milligram levels) of a stable isotope into the formulation before the manufacture of the dosage form, which is then converted to a radioactive isotope by neutron bombardment. The most common stable isotope used is samarium-152, most often introduced as samarium oxide, which is neutron activated to ^{153}Sm. ^{153}Sm has a decay half-life of 46.7 h and emits a γ-ray at 103 keV.

This study discusses the correlation between the *in vitro* disintegration and *in vivo* behavior of DMP 504 tablets. This work also represents an example in which drug substance specification, although tentative, may not be indicative of *in vitro* or *in vivo* behavior of dosage forms, at least as judged from studies in dogs.

MATERIALS AND METHODS

Materials

DMP 504 lots were manufactured by the Chemical Processing Division of DuPont Pharmaceuticals Company, currently Bristol-Myers Squibb Company. Samarium oxide with a purity of 99.999% was purchased from Sigma Chemical Company.

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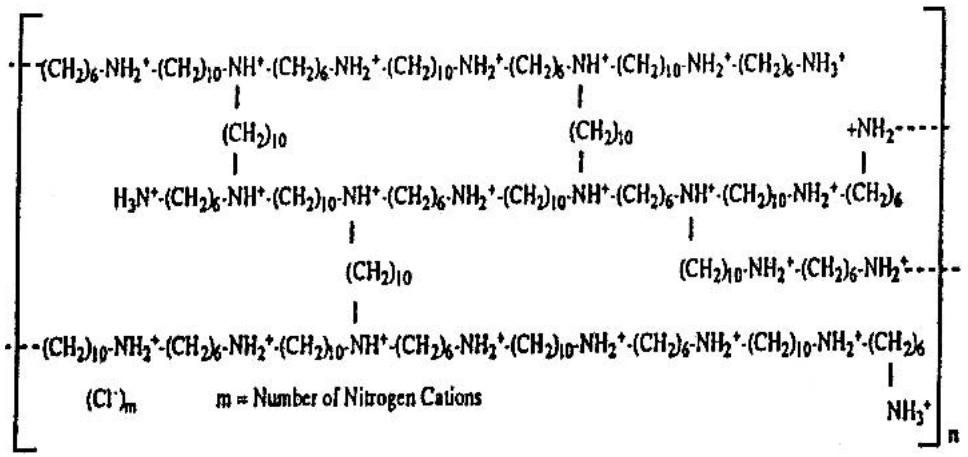

Fig. 1. A representative portion of the network structure or DMP 504.

DMP 504 Capsule Preparation

DMP 504 drug substance was moistened to 7% water content and then blended with small quantity of microcrystalline cellulose (3.6%) and magnesium stearate (1%). The moistening with 7% water was performed because of the hygroscopic nature of DMP 504, which results in capsule brittleness caused by moisture transfer from the capsule shell to the content (11). The powder blend equivalent to 450 mg DMP 504 was accurately weighed out, triturated with 3 mg samarium oxide, and compressed into a tablet using a carver press at 1500 kg force with 0.750″ × 0.320″ capsular shaped tooling. Subsequently, the individual tablets were ground into granules, and the granules were filled into a hard gelatin capsule by hand (size 00).

DMP 504 Tablet Preparation

DMP 504 drug substance was blended with colloidal silicon dioxide (1%), microcrystalline cellulose (17%), citric acid (2%), and magnesium stearate (1%). The powder blend equivalent to 750 mg DMP 504 was accurately weighed out, triturated with 3 mg samarium oxide, and compressed into a tablet using a Carver press at 1500 kg force with 0.750″ × 0.450″ oval-shaped tooling. The tablets were first coated with hydroxypropyl methylcellulose phthalate (1.5% weight gain) and then coated with an aqueous coating solution containing hydroxypropyl methylcellulose, titanium oxide, and polyethylene glycol (Opadry® White, Colorcon, 2.5% weight gain) using a Hi-Coater (model HCT-30, Vector Corp., Marion, Iowa). Because of the swelling properties (12) and fibrous texture of DMP 504 drug substance (12), the seal coating of hydroxypropyl methylcellulose phthalate is required to facilitate the Opadry coating, thereby preventing the premature disintegration in the mouth and to minimize potential swallowing difficulties.

In Vitro Disintegration

The disintegration time for DMP 504 capsules and tablets, before and ~20 days post–neutron activation, was measured using an Erweka disintegration apparatus (Model ZT6-1-D, Erweka Inc., Milford, Connecticut). The disintegration medium used was 0.1 N hydrochloric acid (37°C), and the individual disintegration times were recorded by visual inspection of complete breakdown of dosage forms. The data were reported as the mean ± standard deviation of six individual measurements.

Neutron Activation of Dosage Forms

Each unit dose was placed in a polyethylene envelope and then heat-sealed. Individual tablets were irradiated via neutron activation (thermal flux = 8×10^{13} or 9.36×10^{13} neutrons cm^{-2} sec^{-1}, 6 s) to produce radioactive ^{153}Sm ($t_{1/2}$ = 46.7 h, γ = 103 keV). Before *in vivo* testing, 12 units from each formulation were neutron activated and tested for disintegration *in vitro*, after 10 decay half-lives, to assure that neutron activation did not change the performance of the dosage forms.

Dose Administration and *in Vivo* γ-Scintigraphy

Four female beagle dogs (3 to 4 years old, weight 8.5–12 kg) were used in this study. The protocol adhered to the "Principles of Laboratory Animal Care" (NIH publication 85-23, revised 1985). The study was a balanced, single-dose, complete crossover design involving four dosing periods in which different formulations of DMP 504 were administered, and periods were separated by 2 to 5 days. Treatment A was for DMP 504 capsules utilizing drug substance lot X and administered in a fasting state; treatment B used DMP 504 tablets (tablet 1) manufactured using lot Y and administered in the fasting state; treatment C used DMP 504 tablets (tablet 2) manufactured using lot Z and administered in the fasting state; and treatment D used DMP 504 tablets manufactured using lot Z and administered immediately after feeding of the dogs.

The beagles were comfortably restrained in a standing position (Alice Chatham Sling) and positioned beneath a γ-camera (Siemens BasiCam, Chicago, Illinois) with the camera head located over the back of the beagle. The γ-cameras were equipped with a low-energy parallel-hole collimator, and the pulse analyzer was set to detect the 103 keV γ-ray of ^{153}Sm (15% window width). Dynamic posterior images, each of 2 min duration, were acquired continuously until it was verified that the majority of radioactivity had entered the colon. Doses were administered with 60 ml of distilled water via an orogastric tube following at least 12 h fast for treat-

ments A, B, and C. A slurry of dog food was prepared for treatment D (fed condition) using canned dog food (Big Red Ration; 9% minimum crude protein, 2% minimum crude fiber, 78% maximum moisture) and mixing 2 parts canned dog food with 1 part water. This slurry (60 g), which is equivalent to 40 g of canned dog food and 20 ml of water, was then dosed via an orogastric tube. An additional 20 ml of water was administered after the food slurry to keep fluid content approximately equivalent to that used in the fasted condition. Food and water were allowed after all radioactivity had entered the colon and image acquisition was complete.

Scintigraphic Analysis

The sequential computer-generated images were reviewed for each dog, and regions of interest (ROI) were drawn to represent the stomach, small intestine, and colon (SciWin Scintigraphy Analysis Software, GammaForge, Louisville, Kentucky). All counts were corrected for background and radioactive decay, and dynamic gastrointestinal plots were generated.

Dosage form disintegration was defined as the time when the focal point of the image became less acute and spreading of radioactivity in the stomach was evident.

RESULTS AND DISCUSSION

Bile acid-sequestering agents, which are commercially available (Questran®, Bristol Myers Squibb; Colestid®, Upjohn; and Welchol™, Sankyo), are commonly administered as patient-prepared suspensions and given in relatively high doses (5–9 g, one to six times daily). A solid dosage form containing such a sequestering agent in a reasonably high dose would improve patient compliance because of its ease of administration and improved palatability. Assuming that these agents are most effective when introduced to the stomach in a prehydrated form, it is expected that solid oral dosage forms must disintegrate and hydrate rapidly *in vivo* to be equivalent to the corresponding suspension.

Capsule formulations like the one in this study will usually exhibit rapid *in vivo* disintegration; however, a limitation of capsules is the amount of drug that can be delivered in each unit dose. Thus, a high-dose drug may require several capsules to be swallowed to achieve the desired pharmacologic response. It is possible to increase the amount of drug in each unit dose via compressed tablets, so that fewer dose units are needed to deliver the required dose. A possible limitation of the tablet formulation depends on the disintegration characteristic of the dosage form because *in vivo* disintegration may be delayed if a high-dose drug like this has poor disintegration characteristics.

This study was performed to examine the gastrointestinal behavior of DMP 504 capsules and tablets and to evaluate the significance of slow *in vitro* disintegration observed with some tablets manufactured using some lots of drug substance on the *in vivo* performance using γ-scintigraphy. DMP 504 drug substance lots X and Y resulted in fast *in vitro* disintegration of tablets, whereas tablets manufactured using lot Z resulted in relatively slow *in vitro* disintegration (Table I). Interestingly, no differences were observed in the physical and chemical properties (tentative) of the different lots of drug substance.

Gastrointestinal scintigraphic data are shown in Table II. The second column in Table II lists the time of *in vivo* disintegration, where initial disintegration was defined from the scintigraphic image analysis as the observed spreading of radioactivity away from the dosage form's original focal point. This is a qualitative observation and is only an indication of when the disintegration process started and does not mark the time of complete disintegration. Tablet 1, which had rapid *in vitro* disintegration, had an initial disintegration time of 17 ± 1 min (treatment B) and was comparable to the initial *in vivo* disintegration time of the capsule formulation (15 ± 2 min, treatment A). The more slowly disintegrating tablet 2, which was administered under the fasted and fed conditions (treatments C and D), showed initial tablet disintegration times that were considerably later (63 ± 23 min and 55 ± 29 min, respectively) and were not apparently affected by the presence of food. The time of initial gastric emptying (1st GE) indicated that both tablets began the gastric emptying phase slightly later than the capsule formulation. This observed delay in gastric emptying suggested that the tablet required additional time to hydrate to initiate *in vivo* disintegration, and furthermore, the process of *in vivo* tablet disintegration appeared to be gradual surface erosion. It should be recognized that gastric emptying can be highly variable in the fasted state because intact dosage forms can be rapidly emptied from the stomach during phase III of the migrating motor complex (MMC). Rapid gastric emptying of a single large object is an idiosyncratic event and is dependent on the time of dose administration relative to the phase of the MMC. In fact, such idiosyncrasies were observed in two instances for dog 4 in

Table I. *In Vitro* Disintegration Time of DMP 504 Capsule and Tablets before and 20 Days Post–Neuron Activation

Dosage form	Disintegration time (min) (mean ± SD)	
	Before neutron activation	After neutron activation
DMP 504 capsule (manufactured using lot X and used in treatment A)	2.3 ± 0.1	2.5 ± 0.2
DMP 504 tablet 1 (manufactured using lot Y and used in treatment B)	29.2 ± 3.9	29.8 ± 0.3
DMP 504 tablet 2 (manufactured using lot Z and used in treatments C and D)	44.8 ± 6.9	41.5 ± 6.6

Table II. Disintegration Times and Gastric-Residence Values of DMP 504 Formulations with Omission of Gastric Emptying Data for Beagles That Emptied the Dosage Forms Intact from the Stomach before Disintegration[a]

Beagle	Disintegration (min)	1st GE (min)	GE50 (h)	GE duration (h)	AUC_{stom}	MGRT (h)
Treatment A: fasted condition, capsule, drug lot X						
1	14	28	0.65	0.53	0.66	0.35
2	14	52	1.00	0.40	0.97	0.51
3	18	58	1.05	0.53	1.08	0.55
4	Omit, capsule emptied intact at 5 min					
Mean	15	46	0.90	0.49	0.90	0.47
SD	2	16	0.22	0.08	0.22	0.11
Treatment B: fasted condition tablet 1, drug lot Y						
1	16	96	2.00	1.33	2.05	1.11
2	18	70	1.76	1.33	1.73	0.94
3	Omit, tablet emptied at 18 min after initial tablet disintegration					
4	16	30	0.98	1.53	1.01	0.66
Mean	17	65	1.58	1.40	1.60	0.90
SD	1	33	0.53	0.12	0.53	0.23
p-value (B vs. A)	0.42	0.42	0.11	0.0003	0.11	0.04
Treatment C: fasted condition, tablet 2, drug lot Z						
1	86	96	1.59	0.67	1.66	0.85
2	64	78	1.70	1.07	1.75	0.92
3	40	50	1.63	0.67	1.57	0.84
4	Omit, tablet emptied intact at 14 min					
Mean	63	75	1.64	0.80	1.66	0.87
SD	23	23	0.06	0.23	0.09	0.04
p-value (C vs. A)	0.023	0.15	0.005	0.09	0.005	0.004
Treatment D: fed condition, tablet 2, drug lot Z						
1	51	58	1.28	1.20	1.35	0.75
2	40	54	1.28	1.60	1.43	0.82
3	96	100	2.29	2.13	2.52	1.34
4	32	44	1.06	1.60	1.30	0.80
Mean	55	64	1.48	1.63	1.65	0.93
SD	29	25	0.55	0.38	0.58	0.28
p value (D vs. A)	0.057	0.32	0.15	0.004	0.09	0.044

[a] 1st GE, first gastric emptying of radioactive marker from stomach; GE50, time for 50% of the radioactivity to empty from the stomach; GE duration, duration of gastric emptying equal to time of complete gastric emptying minus time of initial gastric emptying; AUC_{stom}, area under the gastric emptying curve (trapezoidal rule); MGRT, mean gastric residence time equals $AUMC_{stom}$ divided by AUC_{stom}.

treatments A and C before *in vivo* disintegration; thus, disintegration occurred in the small intestine at approximately 27 min (treatment A) and 6 min (treatment C) after gastric emptying. Dog 3 in treatment B emptied the tablet shortly after the start of *in vivo* disintegration. Because the dosage forms emptied from the fasted stomach before dosage form disintegration in these three instances, data from these subtreatments were not included in the statistical analysis. The net result is gastric emptying values that are variable, and as a consequence, any differences between treatment conditions are difficult to demonstrate with statistical significance. It is still possible to predict trends and tendencies from the scintigraphic image analysis whether or not statistical significance is demonstrated in this small sample population ($n = 4$).

The remaining gastric residence values in Table II (GE 50, GE duration, AUC_{stom}, and MGRT) indicate that the tablet formulations remain in the stomach for a longer time than the capsule formulation (some comparisons show statistical significance, $p < 0.05$, paired t test, refer to Table II). Collectively, these results confirm that relatively large tablets that disintegrate gradually will remain in the beagle stomach longer than rapidly disintegrating formulations as long as the MMC does not enter phase III before complete dosage form disintegration. Food did not alter the longer *in vivo* disintegration time observed with tablets manufactured using lot Z (treatment D).

It is interesting to note the disparity between rapid *in vitro* capsule disintegration (2 min, Table I) and a later *in vivo* disintegration time (15 min, Table II). Furthermore, the *in vivo* disintegration times of the capsule and tablet 1 were similar (15 vs.17 min). Gelatin capsules typically exhibit faster *in vitro* disintegration than tablets because hard gelatin capsules contain loosely packed granules or powders, and in this case, the drug is a super disintegrant (12), and under the *in vitro* disintegration condition, the capsule shell is completely surrounded in an aqueous environment. Conversely when in the stomach, it is common for a hard gelatin capsule to initially float on top of the swallowed liquid, and as a consequence only part of the capsule is in contact with water where conditions like this can result in later disintegration time as compared to that *in vitro*. Compared to capsules, compressed tablets are typically denser, and when administered *in vivo*, the dense tablet can land in the antrum of the stomach, where it is more likely to be covered in gastric fluids. Thus, the disintegration process is likely to initiate closer to the *in vitro* disintegration time than that observed with a capsule.

In conclusion, the *in vivo* disintegration of DMP 504 tablets in dogs correlated with the *in vitro* disintegration times in a rank order manner, and the presence of food appeared to have little or no effect on the *in vivo* disintegration time of the more slowly disintegrating tablet. It was also demonstrated that γ-scintigraphy provided a useful tool to interpret the *in vivo* significance of unexpected changes to disintegration times during early stages of product development.

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