The Effect of Absorption Enhancers on the Oral Absorption of the GP IIB/IIIA Receptor Antagonist, DMP 728, in Rats and Dogs

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INTRODUCTION

DMP 728 (cyclic-[D-2-aminobutyryl-L-N2-methyl-L-arginylglycyl-L-aspartyl-3(aminomethyl)-benzoic acid], methanesulfonic acid salt) is a platelet glycoprotein IIb/IIIa receptor antagonist (1). The GPIIb/IIIa complex is the final common pathway for all agonist-induced platelet aggregation. DMP 728 *in vitro* inhibits ADP-induced platelet aggregation (IC_{50}, 46 nmol/L) and the GPIIb/IIIa receptor (IC_{50}, 0.6 nmol/L) in humans (2). Despite its efficacy, both *in vitro* and after intravenous dosing *in vivo*, the development of DMP 728 has been hampered by low and variable oral absorption. The oral bioavailability of this compound in rats, dogs, baboons and in healthy, male humans is 2–4 (3), 5–12 (4), 3–9(5), and 2–5%(6), respectively. Measurement of the intestinal permeation of this compound *in vitro*, via Caco2 (human colon adenocarcinoma) cells demonstrated that DMP 728 does not permeate well (7). The *in vitro* permeation rate in rat intestinal preparations was also observed to be low (8).

There have been many studies performed investigating methods to improve the absorption of peptides and proteins, which are often poorly orally absorbed (9). Some success has been found by administering compounds with absorption enhancers. Examples of this include the improvement in absorption seen when administering cefoxitin with the long-chain acyl carnitine, palmitoylcarnitine chloride (PCC) (10). The presence of an enteric coating, with the PCC, was found to further increase the absorption of cefoxitin. Sodium caprate (NAC), a long-chain fatty acid, has been reported to enhance intestinal absorption of fenoximethyl penicillin via the paracellular route (11). Lauroylcholine chloride (LCC) is another absorption enhancer which produces an increase in intestinal permeability (12).

Current studies investigated the effect of the absorption enhancers NAC, PCC and LCC on the oral absorption of DMP 728, in both rats and dogs.

MATERIALS AND METHODS

Materials

DMP 728 was synthesized by The Du Pont Merck Pharmaceutical Company (DMPC), Wilmington, Delaware. The control dose for rats was prepared in capsules containing neat powder only. For dogs the control dose was the clinical formulation which included microcrystalline cellulose and lactose. The NAC formulation consisted of NAC (40.2%), PEG 400 (13.4%), PEG 1450 (13.4%), water (26.8%) and DMP 728 salt (6.3%). The PCC and LCC formulations each included five parts enhancer to one part DMP 728 (5:1, w:w). DMP 728 in the clinical formulation was provided by Pharmacy Research and Development, DMPC. Gelatin capsules used in the dog studies were purchased from Hanna Pharmaceutical Supply (Wilmington, Delaware). Size 9 microcapsules, used in the rat studies, were supplied by Torpac (Fairfield, New Jersey). These capsules were administered with a specially designed dosing syringe purchased from Hanna Pharmaceutical Supply (13).

Male CD rats (250–300 g) were obtained from Charles River Laboratories (Kingston, New York) and were housed in metabolic cages and acclimatized for 3–5 days prior to administration of drug. The animals were fasted overnight prior to dosing; water was allowed *ad libitum*. Cannulas were surgically implanted in the jugular vein for blood collection, and the animals were allowed to recover overnight before commencement of the study.

Male beagle dogs (8–10 kg) were obtained from White Eagle Laboratories (Doylestown, Pennsylvania) for the NAC study and from Marshall Farms (North Rose, New York) for the PCC/LCC study. The dogs were housed in stainless steel metabolic cages, and fed once daily, approximately 8 hours after dosing. Water was allowed *ad libitum*. All experiments were conducted in a research facility accredited by the American Association for the Accreditation of Laboratory Animal Care (AAALAC).

Animal Dosing and Sample Collection

Rat

Four groups of four rats each were given the equivalent of an 8 mg/kg oral dose of DMP 728 in microcapsules. The groups received DMP 728 formulated in either PCC, LCC, NAC, or in the control formulation (neat drug only). Blood samples, ~1.2 mL, were taken from the jugular vein cannulas. The same volume of control donor blood was then transfused to the animal. The samples were collected into heparin containing vacutainers at predose and at 0.25, 0.5, 0.75, 1, 1.5, 2, 4, 6, 8, 10, and 12 h postdose. The plasma was harvested and frozen at −20°C pending HPLC analysis.

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Table I. Mean (±SD) Pharmacokinetic Parameters of DMP 728 in Rats After an 8 mg/kg Oral Dose in NAC, PCC, LCC or in the Control Formulation (n = 4/Formulation)

	Control		NAC	
Parameters	Mean ± SD	%CV	Mean ± SD	%CV
Cmax (μg/ml)	0.070 (±0.020)	29	0.426 (±0.262)[a]	62
Median Tmax (h)	0.375		0.375	
AUC (μg · h/ml)	0.228 (±0.085)	37	0.616 (±0.272)[a]	44
t½ (h)	3.500 (±1.819)	52	3.500 (±1.930)	55
F (%)[c]	2.4		6.0	
	PCC		LCC	
Parameters	Mean ± SD	%CV	Mean ± SD	%CV
Cmax (μg/ml)	0.440 (±0.212)[a]	48	0.435 (±0.160)[b]	37
Median Tmax (h)	0.750		0.500	
AUC (μg · h/ml)	1.379 (±0.585)[a]	42	0.652 (±0.165)[a]	25
t½ (h)	2.775 (±2.125)	77	2.125 (±0.881)	41
F (%)[c]	14.6		6.9	

[a] $p < 0.05$.
[b] $p < 0.01$ between control and formulation with enhancer.
[c] F % obtained by comparison of these AUC values with the AUC of 11.82 μg · h/mL from IV administration of 10 mg/kg DMP 728 to a separate group of rats.

Dog

In a balanced incomplete block design, three groups of four dogs each were administered the equivalent of 2 mg/kg DMP 728 p.o. in capsules. In each study, after a wash-out period of one week, the groups were each given the same dose in a different formulation than the one they received originally, for a total of eight dogs receiving each formulation. Three formulations were used in each of the dog studies. In the NAC study, the animals received NAC, enteric coated NAC, and a control formulation without the enhancer. The PCC/LCC study included PCC, enteric coated LCC, and the control formulation. For the NAC study samples were collected at predose and at 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 8, 12, and 16 h postdose. The PCC/LCC study included the same sampling points, but omitted the 16 h sample because the NAC study results indicated that the terminal half-life could be adequately characterized without this sample. Whole blood samples, ~2 mL, were obtained by repeated venipuncture. The samples were collected into heparin containing vacutainers. The plasma was harvested, and frozen at −20°C pending HPLC analysis.

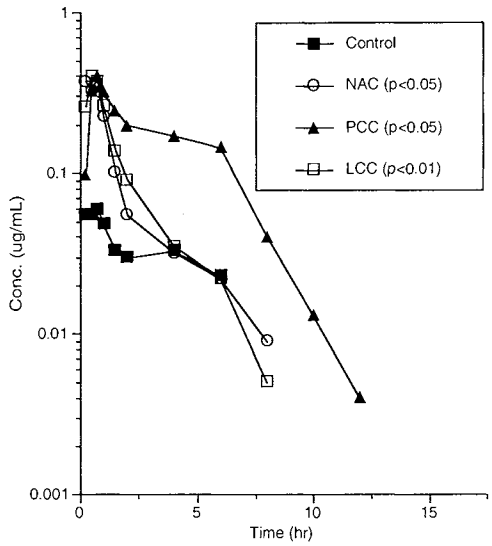

Fig. 1. Mean plasma levels (μg/mL) of DMP 728 in rats after an 8 mg/kg oral dose in NAC, PCC, LCC or in the control formulation (n = 4/Formulation)

HPLC Assay

The plasma samples were analyzed for DMP 728 using an HPLC-fluorescence assay developed earlier (14). This assay included solid-phase extraction, using 0.5 mL of plasma, followed by derivatization for fluorescence detection. The quantifiable limit of the assay was 5 ng/mL.

Data Analysis

Cmax is the highest observed plasma concentration. Tmax is the time of the Cmax. The elimination rate constant, λ, is calculated as the slope (by linear regression) of the terminal log-linear portion of the plasma concentration-time curve. The plasma elimination half-life is calculated by 0.693 divided by λ. AUCT is the area under the plasma concentration-time curve from time zero to the last quantifiable time point, determined by linear trapezoidal rule. AUC is the area under the plasma concentration-time curve from time zero to infinity. The AUC is the sum of the AUCT and the area from the last quantifiable time point to infinity. The latter area was estimated from the equation $AUC_{t\text{-}\infty} = C_{p\ last}/\lambda$. The absolute oral bioavailability is determined by dividing the oral AUC by the AUC found after an i.v. dose to a parallel group of animals, normalizing to the same dose, and multiplying by 100. Statistical analyses for dog data were completed by

Table II. Mean (±SD) Pharmacokinetic Parameters of DMP 728 in Dogs After a 2 mg/kg Oral Dose in NAC, Enteric Coated NAC or in the Control Formulation (n = 8/Formulation)

	Control		NAC		NAC-Enteric	
Parameters	Mean ± SD	%CV	Mean ± SD	%CV	Mean ± SD	%CV
Cmax (μg/ml)	0.206 (±0.081)	39	0.578 (±0.222)[a]	38	0.252 (±0.158)[NS]	63
Median Tmax (h)	0.875		0.500		0.625	
AUC (μg · h/ml)	0.698 (±0.359)	51	0.947 (0.223)[a]	24	0.705 (±0.244)[NS]	35
t½ (h)	4.538 (±1.766)	39	4.538 (±0.863)	19	5.150 (±1.283)	25
F (%)[b]	13.0		17.7		13.2	

[NS] $p > 0.05$.
[a] $p < 0.05$, between control and formulation with enhancer.
[b] F % obtained by comparison of these AUC values with that of 5.349 μg · h/mL from an IV administration of 2 mg/kg to a separate group of dogs.

Table III. Mean (±SD) Pharmacokinetic Parameters of DMP 728 in Dogs After a 2 mg/kg Oral Dose in PCC, Enteric Coated LCC or in the Control Formulation (n = 8/Formulation)

	Control		PCC		LCC-Enteric	
Parameters	Mean ± SD	%CV	Mean ± SD	%CV	Mean ± SD	%CV
Cmax (μg/ml)	0.152 (±0.095)	63	0.414 (±0.103)[NS]	25	0.411 (±0.246)[NS]	60
Median T_{max} (h)	1.500		1.000		1.875	
AUC (μg · h/ml)	0.584 (±0.303)	52	1.094 (±0.222)[a]	20	0.912 (±0.351)[NS]	38
t½ (h)	3.813 (±1.421)	37	3.275 (±0.888)	27	2.888 (±0.982)	34
F (%)[b]	10.9		20.5		17.0	

[NS] $p > 0.05$.
[a] $p < 0.05$, between control and formulation with enhancer.
[b] F % obtained by comparison of these AUC values with that of 5.349 μg · h/mL from an IV administration of 2 mg/kg to a separate group of dogs.

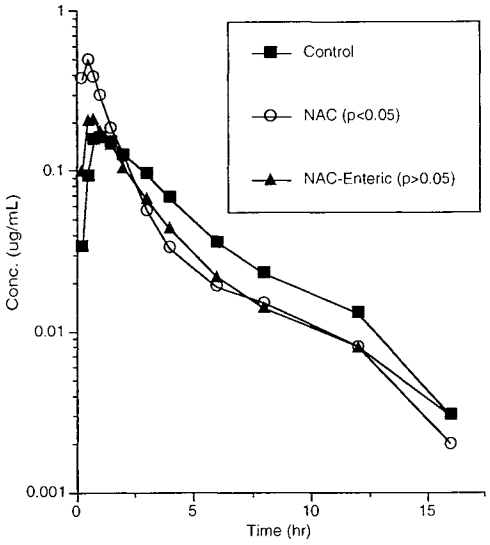

Fig. 2. Mean plasma levels (ug/mL) of DMP 728 in dogs after a 2 mg/kg oral dose in NAC, enteric coated NAC or in the control formulation (n = 8/Formulation).

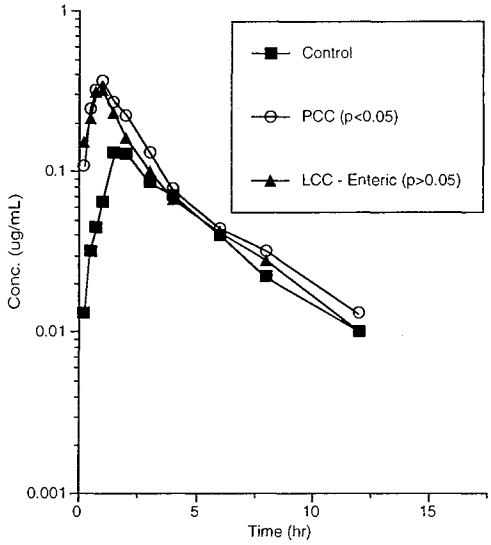

Fig. 3. Mean plasma levels (μg/mL) of DMP 728 in dogs after a 2 mg/kg oral dose in PCC, enteric coated LCC or in the control formulation (n = 8/Formulation).

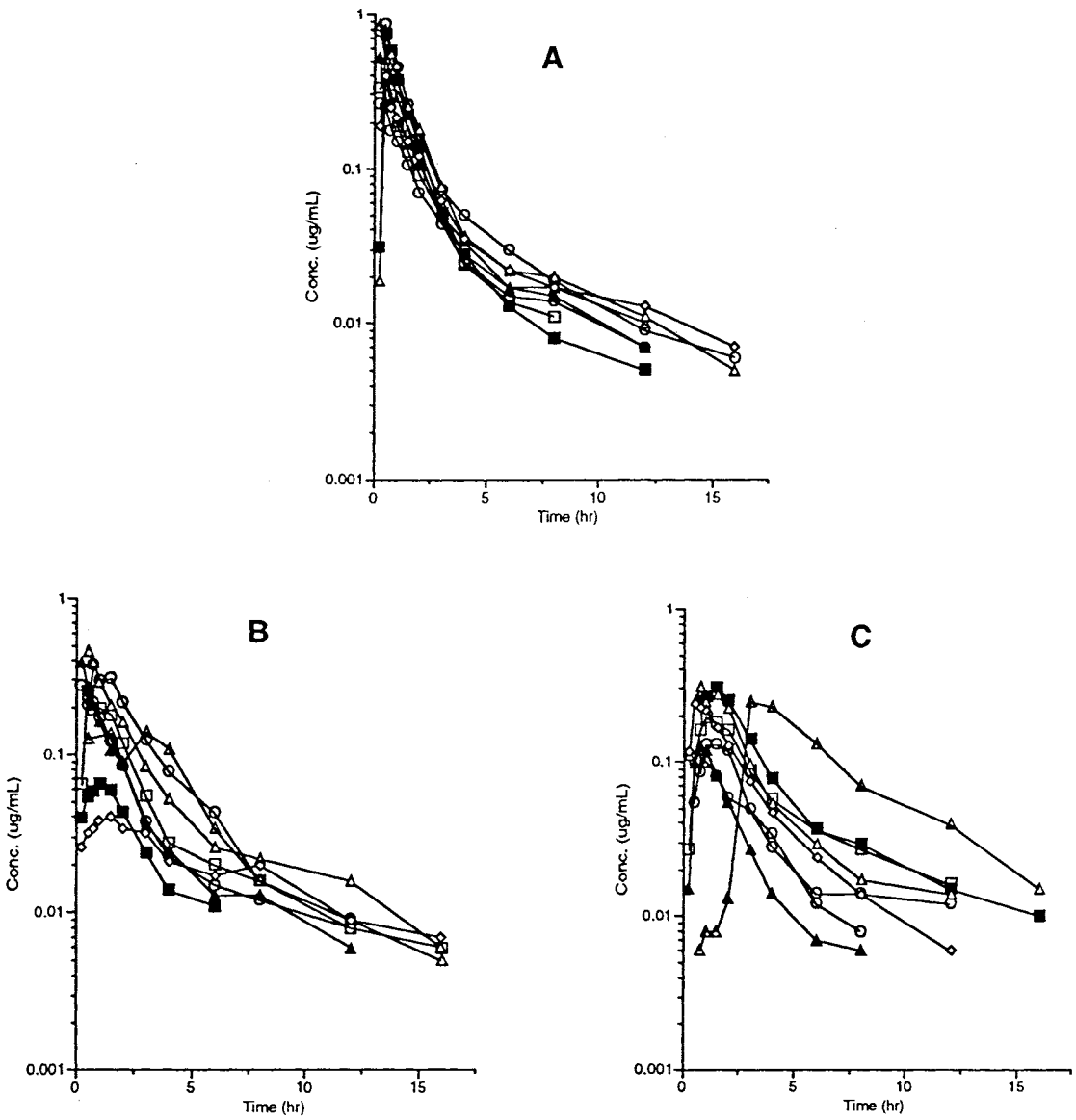

Fig. 4. Plasma levels (ug/mL) of DMP 728 in dogs after a 2 mg/kg oral dose in NAC (A), enteric coated NAC (B), or in the control formulation (C).

analysis of variance (ANOVA) on individual, log-transformed, data. Rat data were statistically compared by multiple t-test, on individual, log-transformed, data, for significance at the 95% confidence level.

RESULTS AND DISCUSSION

The results of the rat study are summarized in Table I and Figure 1. Mean Cmax (μg/mL) values were 0.07, 0.44, 0.44, and 0.43 for the control, PCC, LCC, and NAC, respectively. Mean AUC (μg·h/mL) values were 0.23, 1.38, 0.65, and 0.62, respectively. The data showed that Cmax and AUC values after PCC, LCC, and NAC were all significantly greater than control ($p<0.05$).

Data from the dog studies are summarized in Tables II–III and Figures 2–3. Results of the NAC study (Table II) indicated that the mean Cmax values were 0.21, 0.58, and 0.25 μg/mL for the control, NAC and enteric NAC formulations, respectively. Mean AUC values were 0.70, 0.95 and 0.71 μg·h/mL, respectively. The data showed that Cmax and AUC values after NAC were significantly greater than control ($p<0.05$) while the enteric coated NAC was not different from control ($p>0.05$).

The PCC/LCC study in dogs (Table III) produced mean Cmax (μg/mL) values of 0.15, 0.41, and 0.41 for the control, PCC and LCC, respectively. Mean AUC (μg·h/mL) values were 0.58, 1.09, and 0.91, respectively. The PCC formulation was better than control ($p<0.05$) when comparing AUC, but not Cmax ($p>0.05$). The data for the LCC formulation were not different from control ($p>0.05$).

DMP 728 is not significantly metabolized and it is stable to gastrointestinal peptidases (2, 15). In *in vitro* assays, intestinal permeation and transport of DMP 728 across Caco-2 cell monolayers is low (7,8). Our results indicated that administration of DMP 728 with absorption enhancers signifi-

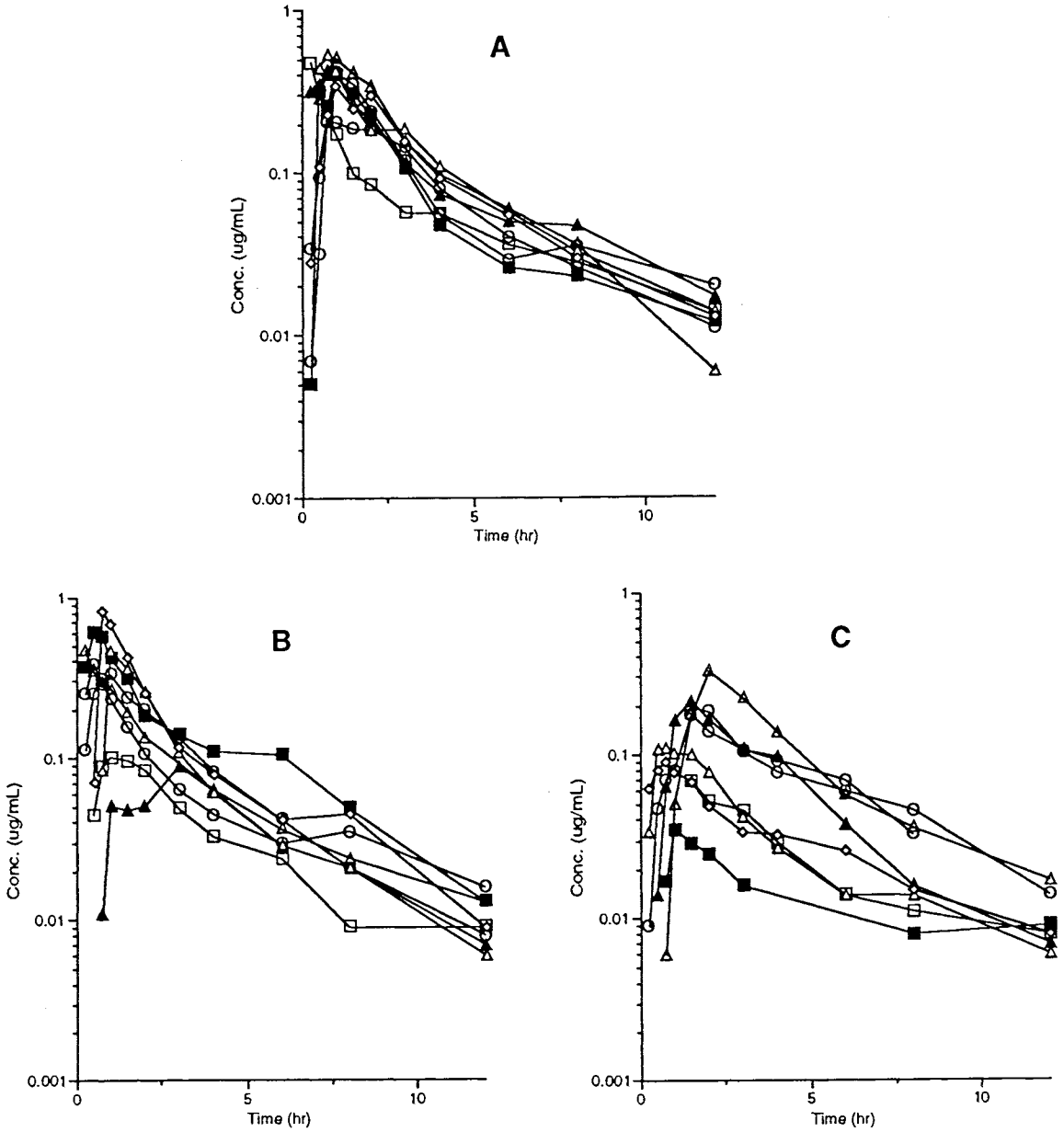

Fig. 5. Plasma levels (ug/mL) of DMP 728 in dogs after a 2 mg/kg oral dose in PCC (A), enteric coated LCC (B), or in the control formulation (C)

cantly increased the oral bioavailability of this compound, and yielded a reduction in the variability, as observed by a decrease in the %CV of the AUC values in dogs. NAC (11) and PCC (16) appeared to increase intestinal permeation, via the paracellular route, of fenoxymethylpenicillin (NAC) and hydrophilic marker compounds, including lucifer yellow and ruthenium red (PCC). The absorption enhancer LCC has also been shown to improve the intestinal absorption of polypeptides (12). The presence of an enteric coating with PCC has been shown to improve the bioavailability of cefoxitin in dogs (10). However, administration of DMP 728 and an absorption enhancer, with an enteric coating, did not improve the bioavailability when compared to non-coated DMP 728 with enhancer. Administration of DMP 728 with NAC demonstrated a 2-fold improvement in bioavailability in rats, and a 60% improvement in bioavailability in dogs, compared to control. The bioavailability in rats increased 6 times when DMP 728 was administered with PCC as compared to control. The absorption of DMP 728 with PCC in dogs was 2 times better than control. Administration of DMP 728 with LCC yielded a 3-fold improvement in the absorption in rats. Although an insignificant improvement was seen in dogs, when compared to control, the variability in the AUC values appeared to be reduced. This reduction in variability is also apparent when comparing NAC and PCC to control and enhancer with enteric coating (Figures 4 and 5). In conclusion, the results indicated that administration of DMP 728 with the absorption enhancers NAC, PCC, or LCC produced a significant increase in the oral absorption of this compound. The data also demonstrate a

reduction in the variability of the absorption of this compound in dogs.

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