ELSEVIER

International Journal of Pharmaceutics 156 (1997) 79–88

international journal of pharmaceutics

Amphiphilic vehicles improve the oral bioavailability of a poorly soluble HIV protease inhibitor at high doses[1]

Bruce J. Aungst *, Nhung H. Nguyen, Nancy J. Rogers, Susan M. Rowe, Munir A. Hussain, Susan J. White, Linyee Shum [2]

The DuPont Merck Pharmaceutical Company, P.O. Box 80400, Wilmington, DE 19880–0400, USA

Received 11 December 1996; received in revised form 28 May 1997; accepted 12 June 1997

Abstract

DMP 323 is the first clinical candidate from a novel series of HIV protease inhibitors having a cyclic urea structural backbone. In dogs dosed with glycol-based vehicles, DMP 323 was approximately 50% orally bioavailable after a 100 mg dose, but bioavailability was almost 10-fold lower when a 350 mg dose was administered. Since DMP 323 has very low water solubility, it was expected that this loss of bioavailability at a high dose was due to drug precipitation in the aqueous fluids of the gastrointestinal tract. Clinically, high doses were desired so as to maximize antiviral effects. Therefore, formulation strategies to improve bioavailability at high doses were examined. Bioavailability of 350 mg doses in dogs was not improved using formulations consisting of glycols with low concentrations of added surfactants, or with solid dispersions of DMP 323 in PEG or PEG/PVP matrices. An alternative approach was to use formulations comprised primarily of an amphiphilic material such as Gelucire 44/14 (a mixture of glycerides and PEG esters). Bioavailability was increased to 50% using a semi-solid Gelucire 44/14 formulation, compared with the 6% or lower value observed at this dose with glycol vehicles. Gelucire 44/14 increased DMP 323 aqueous solubility and dissolution rate, as did other amphiphilic materials with high HLB values. Oral bioavailability of DMP 323 is determined by the DMP 323 dose, the solubilization afforded by the vehicle when diluted into an aqueous environment, and the vehicle dose. Amphiphilic vehicles such as Gelucire 44/14 could be used to administer high doses of DMP 323 without compromising oral bioavailability. A limitation of this approach is the volume of vehicle required. © 1997 Elsevier Science B.V.

Keywords: DMP 323; HIV protease inhibitor; Bioavailability; Formulation; Solubility; Gelucire

* Corresponding author. Tel.: +1 302 6957063; fax: +1 302 6951460; e-mail: AUNGSTBJ@A1.LLDMPC.UMC.DUPONT.COM

[1] Portions of this work were presented at Journeé Galéniques de Saint-Rémy de Provence, May 1994, and at the AAPS Eastern Regional Meeting, June 1994.

[2] Current address: Quest Pharmaceutical Services, Newark, DE 19711, USA.

PII S0378-5173(97)00189-0

1. Introduction

HIV protease inhibitors prevent replication of the HIV virus. Based on the clinical trials of the most advanced HIV protease inhibitors, these now represent a very promising approach to the treatment of HIV infections and AIDS (Kitchen et al., 1995; Markowitz et al., 1995). HIV protease inhibitors functionally and structurally mimic the peptidic natural substrate of the enzyme. Recently a novel category of nonpeptide HIV protease inhibitors having a cyclic urea structural scaffold was discovered (Lam et al., 1994). The first clinical candidate from this series was DMP 323 (Fig. 1). DMP 323 had reasonably good oral bioavailability in animals when administered at low doses using non-aqueous vehicles (Grubb et al., 1994). But bioavailability decreased with increasing doses. Our goals were to identify formulation approaches that might be used to provide acceptable oral bioavailability at high doses, as well as at low doses. It was anticipated that high doses would be required for effective therapy in order to maintain plasma and tissue concentrations much greater than the concentrations known to inhibit viral replication in vitro. Maximization of drug levels is required to reduce or delay the emergence of drug-resistant viral mutants, which is foreseen as a limitation of these drug therapies (Condra et al., 1995).

Since DMP 323 has low water solubility (≤ 10 μg/ml), precipitation in the gastrointestinal tract was suspected to cause the observed loss of bioavailability at higher doses. Many strategies to improve the oral absorption of poorly water soluble drugs are described in the literature. These include particle size reduction, addition of low concentrations of surfactants as solubilizing agents, formation of solid dispersions or solid solutions, formulation in oils or in emulsion vehicles, and complexation with solubilizing agents such as cyclodextrins. An approach with features similar to several of the aforementioned, is the use of formulations comprised primarily of amphiphilic excipients. One amphiphilic excipient that has been used for this purpose is Gelucire 44/14, a brand of polyglycolyzed glycerides (consisting of glycerides and polyethylene glycol esters). Gelucire 44/14-based vehicles were previously shown to improve the dissolution and oral bioavailability in dogs and humans of α-pentyl-3-(2- quinolinylmethoxy)benzenemethanol, a poorly water soluble lipoxygenase inhibitor (Serajuddin et al., 1988; Sheen et al., 1991).

This report presents a comparison of several formulation approaches to improve DMP 323 oral bioavailability at relatively high doses. Bioavailability studies were done in dogs. Formulation approaches tested included aqueous suspensions, glycol-based solutions, polyethylene glycol (PEG) solid dispersions, vehicles containing solubilizing agents, and various amphiphilic vehicles. The most promising approach was with amphiphilic vehicles such as Gelucire 44/14, Labrasol, and PEG-60 hydrogenated castor oil. Both Gelucire 44/14 and Labrasol are polyglycolyzed glycerides with HLB values of 14, Gelucire 44/14 being solid at room temperature and Labrasol being liquid. A number of variations of formulations containing these excipients were examined in vivo. Dissolution and solubility studies were also performed to help understand the effects of these vehicles and factors influencing the oral absorption of this new drug candidate.

2. Materials and methods

2.1. Materials

DMP 323 was prepared at DuPont Merck as described previously (Lam et al., 1994). Gelucire 44/14 and Gelucire 35/10 are two types of saturated polyglycolyzed glycerides. Differences in the composition of Gelucire 44/14 and Gelucire 35/10 result in different melting temperatures (44° and

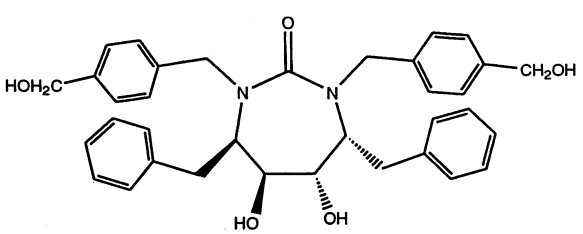

Fig. 1. Structure of DMP 323.

35°, respectively) and HLB values (14 and 10, respectively). Gelucires, propylene glycol laurate (Lauroglycol), and Labrasol (saturated polyglycolyzed C8-C10 glycerides) were provided by Gattefossé (Saint Priest, France). PEG-60 hydrogenated castor oil (Cremophor RH60) and poloxamer 188 (Pluronic F68) were obtained from BASF (Parsippany, NJ). Caprylic/capric triglycerides (Miglyol 812) was supplied by Hüls America (Somerset, NJ). PEG-4 laurate (Emerest 2620) and PEG-8 laurate (Emerest 2650) were obtained from Emery (Mauldin, SC). PEG-8 stearate (Myrj 45) and PEG-40 stearate (Myrj 52) were supplied by ICI Americas (Wilmington, DE). Choleth-24 (Solulan C-24) was obtained from Amerchol (Edison, NJ).

2.2. Oral bioavailability

Oral bioavailability of DMP 323 was evaluated in various treatment groups selected from a colony of 20 beagle dogs. Dog weights ranged from approximately 7.5 to 11 kg. Each treatment group generally consisted of three or four dogs, although for some treatments of greatest interest there were more replicates. Most dogs received several of the treatments. A 2 week washout period separated any two treatments.

Dogs were fasted overnight before dosing. One treatment was i.v. DMP 323 administration. The i.v. dose was 5 mg/kg, administered as a 50 mg/ml solution in PEG 400. Oral doses were either approximately 100 or 350 mg/dog. All oral doses were administered in hard gelatin capsules filled with the various vehicles. Vehicles included solids, solid dispersions, liquids, and semi-solids. Semi-solids were filled into capsules as melts. The drug concentrations in the vehicles varied from group to group and are specified in Section 3. For most liquid and semi-solid vehicles, drug concentrations were near the estimated solubility in the vehicle, so that the volume of vehicle dosed would be minimized. Most vehicles were simple mixtures of drug and excipients, prepared at room temperature or at approximately 60°. These are described further in Section 3. Solid dispersions of DMP 323 in PEG 3350 or PEG 3350:polyvinylpyrrolidone (PVP 40000) mixtures were prepared by solvent evaporation. DMP 323 and excipients were dissolved in 50% ethanol:50% methylene chloride, followed by evaporation of the solvent. All formulations were dosed within 24 h of preparation.

Blood samples were collected by jugular venipuncture and plasma was separated and frozen until analyzed. A validated analytical method was used to determine plasma DMP 323 concentrations. Briefly, the internal standard, a DMP 323 analog, was added to a 1 ml plasma aliquot. This was extracted into 30% methylene chloride:25% hexane:45% ethyl acetate. The organic phase was removed, dried, and redissolved in mobile phase. The HPLC mobile phase consisted of 38% acetonitrile:62% water:0.1% phosphoric acid. A 25 cm octylsilane column was used. Detection was by uv absorbance at 229 nm. At a 1.1 ml/min flow rate, the retention times of DMP 323 and the internal standard were 11 and 20 min, respectively.

Oral bioavailability was estimated by comparing dose-adjusted area under the plasma concentration vs time curve (AUC) values after oral and i.v. administration. These were calculated using the trapezoidal method. Results are presented as mean $\pm$ S.E. Statistical comparisons of treatments were performed using t-tests. For some treatment groups, the initial results obtained for two dogs indicated very low bioavailability, and these were not repeated. Individual values are reported for these groups.

2.3. Solubility and dissolution

Initial estimates of DMP 323 solubility in various vehicles were made by determining the amounts of weighed compound dissolving in measured amounts of the vehicle. These were done at room temperature or at approximately 60°. DMP 323 solubility was also determined in aqueous solutions containing various concentrations of the excipients used for the bioavailability studies, to simulate dilution of those vehicles in an aqueous environment. Excess DMP 323 was added to the solvent and mixed for 24 h at room temperature. Filtrates were analyzed by HPLC using methods similar to those described above. Formulation

dissolution studies were done using the rotating basket method. Hard gelatin capsules containing 100 mg DMP 323 in various vehicles were used. The dissolution medium was 900 ml of 0.1 or 0.5% sodium lauryl sulfate in water, at 37°. Rotation speed was 100 rpm. DMP 323 concentrations in filtrates of the medium were determined by uv absorbance.

3. Results

3.1. Bioavailability from glycol solutions and amphiphilic vehicles

DMP 323 bioavailability studies were conducted in dogs. One group of dogs was administered DMP 323 intravenously, and this i.v. data was used for all bioavailability calculations. The $AUC_{0\text{-}\infty}$ after a 5 mg/kg i.v. dose was 3.46 ± 0.15 μg h/ml, and the terminal half-life was 2.7 ± 0.2 h (mean $\pm$ S.E., $N = 3$). DMP 323 had fairly good oral bioavailability ($49.6 \pm 19.7\%$, $N = 8$) when 100 mg doses were administered in PEG 400 (at a concentration of 100 mg/ml DMP 323). This bioavailability value is consistent with those reported previously for dogs administered 5 or 10 mg/kg DMP 323 using non-aqueous dosing solutions (Grubb et al., 1994). To examine bioavailability at a higher DMP 323 dose, a 360 mg dose was administered using a 200 mg/ml DMP 323 solution in 75% PEG 400:25% propylene glycol. A more concentrated dosing solution containing propylene glycol as a cosolvent was utilized, because throughout these studies we wanted to minimize the volume, size, or number of capsules required for dosing. Bioavailability was $5.2 \pm 1.8\%$ ($N = 4$), which was much lower than that for the 100 mg dose. Average plasma concentrations were actually lower after the 360 mg dose than after the 100 mg dose. These results clearly showed that an alternative delivery approach was needed to efficiently administer higher oral doses of DMP 323.

As an alternative vehicle, 86% Gelucire 44/14: 14% PEG 400 was used to administer 85 and 350 mg DMP 323 doses. These dosage forms were prepared by dissolving DMP 323 at 75 mg/g in a molten Gelucire 44/14:PEG 400 mixture at approximately 60° and filling hard gelatin capsules with the solution. This vehicle was semi-solid upon returning to room temperature. Oral bioavailability was $68.9 \pm 13.0\%$ ($N = 10$) after the 85 mg dose. This was not significantly different ($p > 0.05$) from bioavailability after the 100 mg dose using the PEG vehicle. However, a distinct advantage of the Gelucire-based vehicle was seen when the 350 mg dose was given. Bioavailability of DMP 323 was $49.5 \pm 4.2\%$ ($N = 3$), which was significantly ($p < 0.05$) greater than that after the 350 mg dose administered in a glycol solution vehicle. The plasma concentration vs. time profiles are compared in Fig. 2.

Gelucire 44/14 is a surfactant with an HLB value of 14. Other surfactants with similar HLB values were also examined as vehicles for high dose DMP 323 administration. The vehicles administered to dogs were Labrasol (saturated polyglycolyzed C8-C10 glycerides, HLB value = 14) and 86% PEG-60 hydrogenated castor oil (HLB value = 15–17):14% PEG 400. Dogs were dosed with 350 mg DMP 323 using vehicles containing 75 mg/g drug. DMP 323 bioavailability in dogs dosed with Labrasol was $33.0 \pm 7.2\%$ ($N = 3$), and for dogs dosed with PEG-60 hydrogenated castor oil:PEG 400, bioavailability was $36.5 \pm 6.1\%$ (N = 4). These values were also significantly ($p < 0.05$) greater than bioavailability at this dose with the glycol solution dosing vehicle. Thus, these amphiphilic vehicles were similar to Gelucire 44/14 in providing reasonably good DMP 323 bioavailability at this high dose.

3.2. Solubility of DMP 323 in various potential vehicles

Whenever DMP 323 was dosed using liquid or semi-solid, aqueous or non-aqueous vehicles containing DMP 323 concentrations exceeding its solubility, very low oral bioavailability was seen. DMP 323 solubility in the vehicle was therefore an important criterion for evaluating potential dosing vehicles. Many of the vehicles of interest, such as Gelucire 44/14-based vehicles, were semi-solid at room temperature. Therefore, solubilities were initially evaluated at 60–70° using a visual

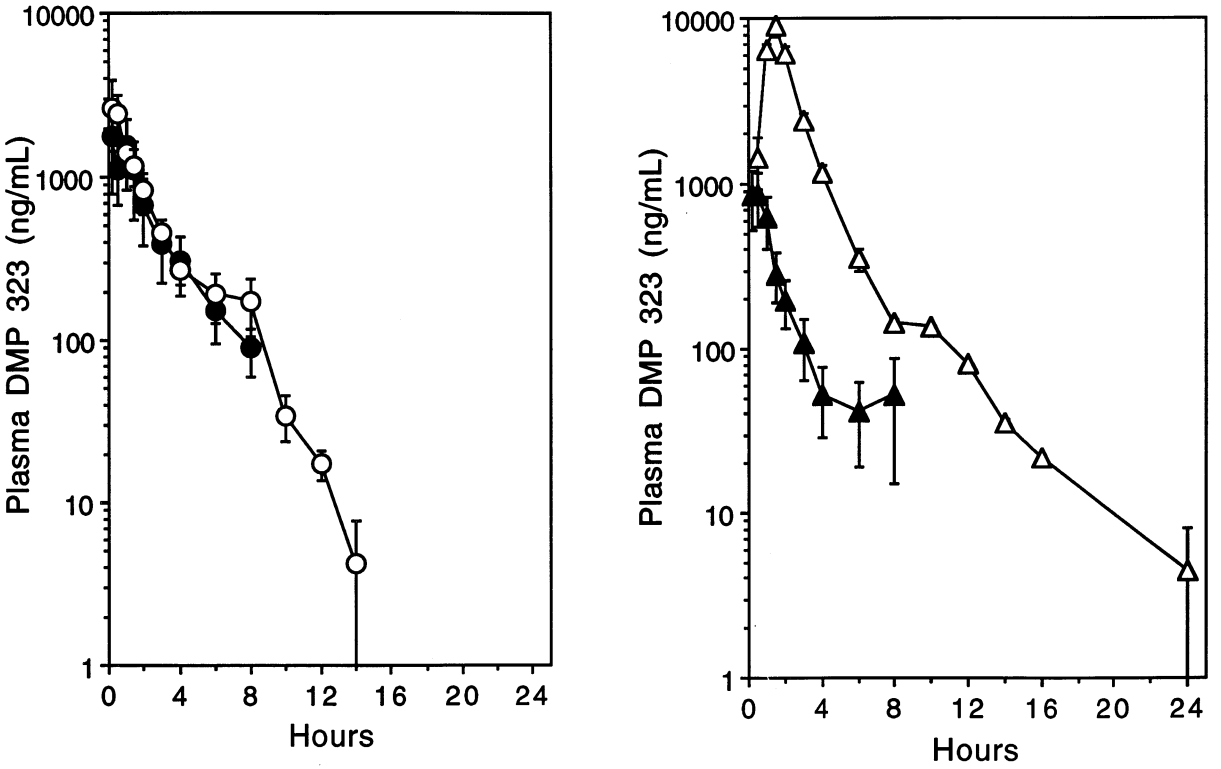

Fig. 2. Plasma DMP 323 concentration vs. time profiles in dogs administered 100 mg (left panel) or 350 mg (right panel) oral doses using glycol solution dosing vehicles (closed symbols) or Gelucire 44/14:PEG 400 vehicles (open symbols).

estimation of solubility; the amount of weighed compound that dissolved in a known amount of the warmed vehicle. Solubility estimates for a number of potential vehicles are given in Table 1. DMP 323 had good solubility in PEG 400, propylene glycol, and glycofurol. PEG and propylene glycol were therefore used as additives in amphiphilic vehicles to increase DMP 323 solubility in the vehicle. For example, as shown in Table 1, PEG 400 and propylene glycol increased the solubility of DMP 323 in Gelucire 44/14. For the amphiphilic vehicles examined, good DMP 323 solubility (≥ 50 mg/ml) was attained only with vehicles having high HLB values, such as Gelucire 44/14, Labrasol, and PEG-60 hydrogenated castor oil. DMP 323 was much less soluble in Gelucire 35/10 and propylene glycol laurate, which have HLB values of 10 and 4, respectively. DMP 323 was also relatively insoluble in the oils evaluated (caprylic/capric triglycerides and oleic acid), so the possibility of using emulsions was excluded from further testing.

3.3. Unsuccessful alternative vehicles

Table 2 summarizes dog bioavailability results for various alternative formulation approaches that did not afford good DMP 323 bioavailability at approximately 350 mg doses. The addition of low concentrations of the surfactants, sodium lauryl sulfate or choleth-24, to glycol vehicles resulted in 5% or lower oral bioavailability, similar to that after the surfactant-free glycol dosing solution. A PEG 400:ethanol vehicle containing 30% polysorbate 80 provided only slightly higher DMP 323 bioavailability. A semi-solid vehicle comprised of 50% poloxamer 188:50% PEG 400 also produced low bioavailability. Glycofurol is similar to PEG 400 or propylene glycol in that it is a good solvent for DMP 323. But as with other

Table 1
Approximate solubility at 60–70° of DMP 323 in various possible dosing vehicles

Vehicle	Visually estimated DMP 323 solubility (mg/g)
PEG 400	185–205
Glycofurol	>200
Gelucire 44/14	50–60
86% Gelucire 44/14:14% PEG 400	75–90
50% Gelucire 44/14:50% PEG 400	130–145
86% Gelucire 44/14:14% propylene glycol	115–130
Labrasol	120–150
PEG-60 hydrogenated castor oil	65–90
50% Poloxamer 188 (HLB = 29):50% PEG 400	>150
Gelucire 35/10 (HLB = 10)	<35
Propylene glycol laurate (HLB = 4)	<10
Caprylic/capric triglycerides	<5
Oleic acid	<5
40% PEG 400:30% ethanol:30% polysorbate 80	200–230

glycol solution vehicles, oral bioavailability of DMP 323 was low when glycofurol was used as a dosing vehicle. Also tested were several PEG-based solid dispersions, which were prepared by a solvent evaporation method. DMP 323 bioavailability was very low from each of these formulations. Each of these formulation approaches was tested using vehicles containing high DMP 323 concentrations. These approaches may have been more successful if lower drug concentrations and higher vehicle doses had been used. But since our goal was to be able to administer high doses with minimum dosing volumes, they were not examined further.

3.4. Minimizing the dosing vehicle volume

Results presented so far indicate that high DMP 323 doses were adequately bioavailable only when the vehicle was comprised primarily of an amphiphilic material with a high HLB value. Low proportions of glycols could be added to these vehicles to increase DMP 323 solubility. Various attempts to minimize the dosing volume and maintain oral bioavailability were performed using Gelucire 44/14 and Labrasol-based vehicles. Bioavailability results are presented in Table 3, which also includes for comparison the results that were previously discussed using 75 mg/g drug concentrations. Increasing the DMP 323 concentration in the 86% Gelucire 44/14:14% PEG 400 vehicle to 250 mg/g, at which it did not completely dissolve when heated, gave very low bioavailability. With any increase in the drug concentration in the vehicle, the volume of vehicle dosed was reduced. For example, for a 10 kg dog administered 350 mg DMP 323, the vehicle dose would be 4.7 g using 75 mg/g DMP 323, and 1.4 g using 350 mg/g DMP 323. Increasing the proportion of PEG 400 to 40% to increase DMP 323 solubility, and using a DMP 323 concentration of 125 mg/g, resulted in only 7% bioavailability.

Table 2
Oral bioavailability (F, %) of DMP 323 in dogs after 350 mg doses using various dosing vehicles

Vehicle	Vehicle DMP 323 conc. (mg/g)	DMP 323 F (%)	N
1% Na lauryl sulfate:49% PEG 400:25% PEG 3350:25% PG	150	4.7 ± 1.1	3
10% choleth-24:90% PEG 400	150	2.6 ± 0.9	3
30% polysorbate 80:40% PEG 400:30% ethanol	150	7.1 ± 1.9	3
50% poloxamer 188:50% PEG 400	150	2.1 ± 0.2	3
Glycofurol	200	4.0 ± 2.3	3
PEG 3350 solid dispersion	333	0.2 ± 0.0	3
50% PVP 40000:50% PEG 3350 solid dispersion	333	0.3 ± 0.1	3
1% Na lauryl sulfate:99% PEG 3350 solid dispersion	333	0.05, 0.04	2

Table 3
DMP 323 bioavailability in dogs at 350 mg doses using Gelucire 44/14 or Labrasol vehicles and the effects of reducing the dosing vehicle volume (or increasing drug concentration)

Vehicle	DMP 323 (mg/g)	F (%) Mean ± S.E.	N
86% Gelucire 44/14:14% PEG 400	75	49.5 ± 4.2	3
86% Gelucire 44/14:14% PEG 400	250	0.9, 1.0	2
60% Gelucire 44/14:40% PEG 400	125	7.0 ± 2.1	3
86% Gelucire 44/14:14% PG	75	31.7 ± 1.1	3
75% Gelucire 44/14:25% PG	150	6.2 ± 0.4	3
67% Gelucire 44/14:25% PG:8% PEG 3350	100	15.7 ± 4.2	3
67% Gelucire 44/14:25% PG:8% PEG 3350	150	6.7 ± 2.0	3
Labrasol	75	33.0 ± 7.2	3
Labrasol	125	21.7 ± 1.5	3

Propylene glycol (PG) could be substituted for PEG 400. With a vehicle of 86% Gelucire 44/14: 14% PG containing 75 mg/g DMP 323, bioavailability was 32%. Increasing the proportion of glycols to $\geq 25\%$, and increasing the DMP 323 concentration, again resulted in reduced oral bioavailability. Using Labrasol, a DMP 323 concentration of 125 mg/g could be used and a fairly good bioavailability of 22% was maintained. Taken together, these results suggest that at this high DMP 323 dose, bioavailability was proportional to the amount of Gelucire 44/14 or Labrasol administered.

3.5. Dissolution and solubilization

To help understand the mechanism of improved DMP 323 bioavailability with amphiphilic vehicles, several vehicles were compared in dissolution studies. All dissolution studies were performed using hard gelatin capsule formulations containing 100 mg DMP 323. The medium contained sodium lauryl sulfate (SLS) as a solubilizer. With a dissolution medium of 0.5% SLS in water, sink conditions were in effect (solubility was 300 mg/900 ml), and dissolution profiles using Gelucire and glycol vehicles were not different (data not shown). However, Gelucire and glycol-based vehicles had markedly different dissolution profiles when non-sink conditions were used (0.1% SLS in water, wherein DMP 323 solubility was 11 μg/ml). Dissolution profiles are shown in Fig. 3. Dissolution of DMP 323 from capsules containing only the solid drug was slow and incomplete. In contrast, DMP 323 dissolved rapidly and completely from the Gelucire 44/14:PEG vehicle. With the PEG:PG vehicle containing 200 mg/g DMP 323, as in the high dose dog study, dissolution was initially rapid but incomplete. DMP 323 concentrations in the dissolution medium filtrates then decreased with time, reflecting further precipitation. When a PEG:PG vehicle containing 75 mg/g DMP 323 was used, as in the low dose dog study, DMP 323 rapidly and completely dissolved, but then gradually precipitated. These dissolution results, obtained under non-sink conditions, are consistent with the dog bioavailability results.

The dissolution results and the bioavailability studies in which dosing volumes were varied, suggested that DMP 323 bioavailability is related to the solubilization effect of the vehicle when diluted in an aqueous environment. Solubility studies were therefore performed in aqueous solutions containing low concentrations of PEG 400, Gelucire 44/14, or Labrasol. This mimics dilution of the vehicle in the aqueous gastrointestinal fluids. For example, if a 1.5 g excipient dose dissolved in 150 mL of gastrointestinal fluid, the excipient concentration would be 1%. PEG 400 at concentrations up to 4% afforded little solubilization of DMP 323 in aqueous media (Table 4). In contrast, 1–4% Gelucire 44/14 or Labrasol greatly increased DMP 323 aqueous solubility. The in-

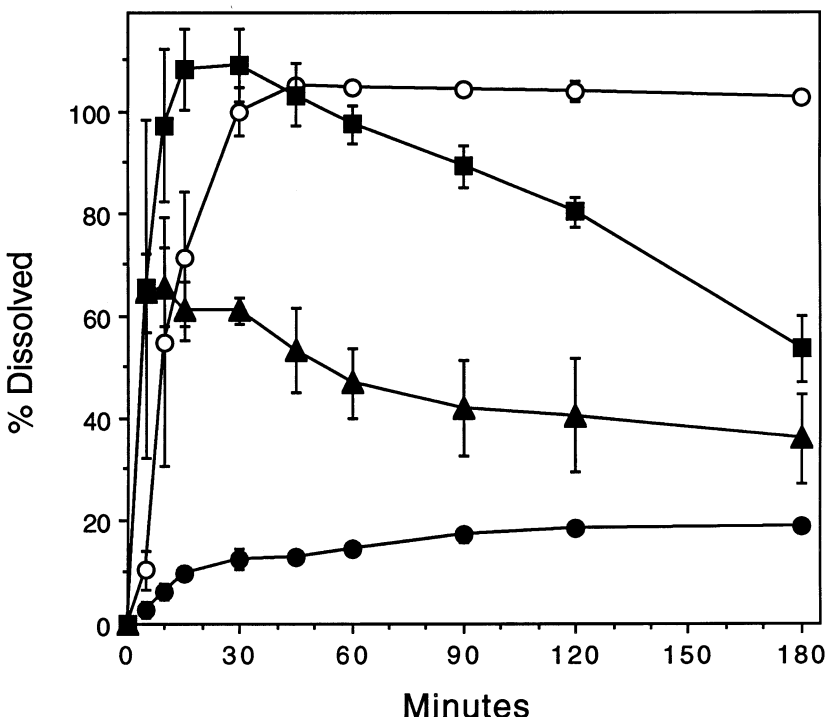

Fig. 3. Dissolution of various capsule formulations of DMP 323 under non-sink conditions using a dissolution medium of 0.1% sodium lauryl sulfate in water. The capsules contained 100 mg DMP 323 as the solid powder (●), a solution of 200 mg/g (▲) or 75 mg/g (■) in PEG:PG, or the Gelucire 44/14:PEG vehicle at 75 mg/g DMP 323 (○).

crease in oral bioavailability observed when these were used as vehicles is probably due to solubilization of DMP 323 in the fluids within the gastrointestinal tract.

Other possible excipients were then evaluated by measuring their effects as solubilizing agents for DMP 323 using low excipient concentrations in water. As shown in Table 4, other amphiphilic vehicles with HLB values of 13–17 were also effective solubilizing agents. Each of these would be expected to be useful for attaining oral bioavailability of DMP 323 at high doses. The solubilization effects of the vehicle depend on the proportion of the amphiphilic component, as can be seen comparing polysorbate 80 and the vehicle containing 30% polysorbate 80. Only the 30% polysorbate 80 vehicle was used to dose dogs, and DMP 323 bioavailability was 7.1%. Amphiphilic vehicles with lower HLB values were much less effective solubilizing agents. Poloxamer 188 has a high HLB value but was ineffective at solubilizing DMP 323. It was also ineffective as a vehicle (at 50% concentration) in the dog bioavailability study reported on Table 2.

4. Discussion

DMP 323 was adequately bioavailable (approximately 50%) in dogs dosed orally with 100 mg doses using glycol-based vehicles. Based on in vitro metabolism studies, high i.v. clearance, and the structure of DMP 323, first-pass elimination was thought to contribute to the incomplete bioavailability under these conditions. Using glycol-based vehicles to administer several-fold higher doses resulted in a drastic reduction in oral bioavailability. DMP 323 has very low water solubility, and precipitation from the dosing solution seemed to be responsible for the loss in bioavailability under these conditions. Since the need for administering high doses clinically was

Table 4
Aqueous solubilization of DMP 323 by low concentrations of excipients

Excipient	DMP 323 Solubility (μg/ml)		
	4% Excipient	2% Excipient	1% Excipient
PEG 400	12	8	5
Gelucire 44/14 (HLB = 14)	758	356	183
Labrasol (HLB = 14)	410	191	57
PEG-60 hydrogenated castor oil (HLB = 15–17)	715	340	211
Polysorbate 80 (HLB = 15)	724	392	186
30% polysorbate 80:40% PEG 400:30% ethanol	182	85	47
PEG-4 laurate (HLB = 9.3)	81	48	17
PEG-8 laurate (HLB = 13)	527	283	136
PEG-8 stearate (HLB = 11.1)	13	44	72
PEG-40 stearate (HLB = 16.9)	718	388	200
Glycerol dilaurate (HLB = 4)	14	11	9
Poloxamer 188 (HLB = 29)	15	12	9

anticipated, the studies presented here were intended to test formulation strategies to improve oral bioavailability at high doses.

The bioavailability and solubility results presented are consistent with the notion that DMP 323 bioavailability in animals is dependent on the amount of drug in solution within the gastrointestinal tract. This can be explained using the theoretical approach for estimating fractional absorption previously described by Dressman et al. (1985). They proposed that fractional absorption (F_a) is proportional to aqueous solubility (S) and the volume of the gastrointestinal fluids (V_g), and is inversly proportional to the dose (D).

$$F_a \propto S \cdot V_g / D$$

This assumes that membrane permeability is not a limiting factor, which is apparently the case with DMP 323. DMP 323 is also probably subject to first-pass elimination, so the fraction absorbed does not represent the fraction bioavailable. DMP 323 had adequate oral bioavailability when administered in low doses using non-aqueous vehicles, but at high doses bioavailability was greatly reduced. When the solubility limit of DMP 323 in the gastrointestinal fluids is reached, increasing the dose results in decreased fractional absorption. Gelucire 44/14, Labrasol, and PEG-60 hydrogenated castor oil improved DMP 323 solubility in dilute aqueous solutions, as would occur in the gastrointestinal lumen after oral dosing, and increased oral bioavailability at 350 mg DMP 323 doses. Bioavailability was related to the amounts of these excipients administered. Most of the attempts to reduce the volume of vehicle (or number of capsules) dosed resulted in reduced bioavailability. This is consistent with the concentration-dependent solubilization of DMP 323 by these excipients, as shown in Table 4. We have not explored the influence of changing the volume of gastrointestinal fluids, but have assumed it is constant in all of these studies.

Amphiphilic vehicles are thus shown to be useful for administering DMP 323 at doses where glycol vehicles are inadequate. From solubility studies using low concentrations of amphiphilic excipients, we expect that various excipients could be utilized if they have HLB values approximating 13–17. PEG and PEG/PVP solid dispersion formulations, which have been shown to improve dissolution and bioavailability of other compounds with low water solubility (Ford, 1986), did not improve DMP 323 bioavailability. Bioavailability studies varying the proportions of drug and amphiphilic excipient indicated that the solubilization effects of these excipients and the resultant bioavailability depends on the dose of excipient administered. The administration of 350 mg doses required excipient doses of several grams and multiple dosage units. This is one limitation of this formulation approach.

Another possible limitation is the physical stability of formulations based on these amphiphilic excipients. We utilized drug concentrations which were close to or may have even exceeded solubility in the vehicle at room temperature. All formulations were dosed within 24 h of preparation. For those formulations affording greatest DMP 323 bioavailability, it was subsequently shown that there was no drug crystallization within the formulation within 24 h. But over longer periods of time drug precipitation within these vehicles could occur, and lower drug concentrations might be necessary for this formulation approach to be practical.

References

Condra, J.H., Schleif, W.A., Blahy, O.M., Gabryelski, L.J., Graham, D.J., Quintero, J.C., Rhodes, A., Robbins, H.L., Roth, E., Shivaprakash, M., Titus, D., Yang, T., Teppler, H., Squires, K.E., Deutsch, P.J., Emini, E.A., 1995. In vivo emergence of HIV-1 variants resistant to multiple protease inhibitors. Nature 374, 569–571.

Dressman, J.B., Amidon, G.L., Fleisher, D., 1985. Absorption potential: estimating the fraction absorbed for orally administered compounds. J. Pharm. Sci. 74, 588–589.

Ford, J.L., 1986. The current status of solid dispersions. Pharm. Acta Helv. 61, 69–88.

Grubb, M.F., Wong, Y.N., Burcham, D.L., Saxton, P.L., Quon, C.Y., Huang, S.M., 1994. Pharmacokinetics of HIV protease inhibitor DMP 323 in rats and dogs. Drug Metab. Disp. 22, 709–712.

Kitchen, V.S., Skinner, C., Ariyoshi, K., Lane, E.A., Duncan, I.B., Burckhardt, J., Burger, H.U., Bragman, K., Pinching, A.J., Weber, J.N., 1995. Safety and activity of saquinavir in HIV infection. Lancet 345, 952–955.

Lam, P.Y.S., Jadhav, P.K., Eyermann, C.J., Hodge, C.N., Ru, Y., Bacheler, L.T., Meek, J.L., Otto, M.J., Rayner, M.M., Wong, Y.N., Chang, C.H., Weber, P.C., Jackson, D.A., Sharpe, T.R., Erickson-Viitanen, S., 1994. Rational design of potent, bioavailable, nonpeptide cyclic ureas as HIV protease inhibitors. Science 263, 380–384.

Markowitz, M., Saag, M., Powderly, W.G., Hurley, A.M., Hsu, A., Valdes, J.M., Henry, D., Sattler, F., La Marca, A., Leonard, J.M., Ho, D.D., 1995. A preliminary study of ritonavir, an inhibitor of HIV-1 protease, to treat HIV-1 infection. N. Engl. J. Med. 333, 1534–1539.

Serajuddin, A.T.M., Sheen, P.-C., Mufson, D., Bernstein, D.F., Augustine, M.A., 1988. Effect of vehicle amphiphilicity on the dissolution and bioavailability of a poorly water-soluble drug from solid dispersions. J. Pharm. Sci. 77, 414–417.

Sheen, P.C., Kim, S.I., Petillo, J.J., Serajuddin, A.T.M., 1991. Bioavailability of a poorly water-soluble drug from tablet and solid dispersion in humans. J. Pharm. Sci. 80, 712–714.

.