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Comparative bioavailability of L-683,453, a 5α-reductase inhibitor, from a self-emulsifying drug delivery system in Beagle dogs

Bozena Matuszewska*, Lisa Hettrick, Joseph V. Bondi, David E. Storey

Department of Pharmaceutical Research, Merck Research Laboratories WP78-302, West Point, PA, 19486, USA

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Abstract

Bioavailabilities (BA) of the lipophilic compound, L-683,453, from several formulations were determined in fasted and fed purpose-bred Beagle dogs following oral administration and an i.v. reference dose. The compound is poorly soluble in water (~ 0.001 mg/mL) and exhibited very low BA, 0.2%, from suspensions in methyl cellulose, in fasted dogs. Addition of sodium dodecyl sulfate (SDS) into suspensions increased BA significantly to 0.6% in fasted ($P = 0.05$) and to 1.7% in fed animals ($P < 0.01$). A more dramatic enhancement in BA, up to 13.7%, was achieved from a self-emulsifying formulation composed of mono- and di-glycerides of caprylic/capric acids (MDG) and surfactants. It was found that tolerability and efficacy of MDG-based formulations at 16 mg/kg depend not only on the dose but also on the dosing volume. A volume of 2 mL/kg caused emesis, while a volume of 1 mL/kg was well tolerated. In contrast to its effect on suspensions, food had no statistically significant effect on BA of self-emulsifying formulations at dosing volumes of 1 mL/kg and 0.25 mL/kg. However, peak plasma concentrations were achieved faster in fed than in fasted animals.

Keywords: Self-emulsifying system; L-683,453; Bioavailability; Food effect; Volume effect

1. Introduction

L-683,453, N-(2-adamantyl)-3-oxo-4aza-5α-androst-1-ene-17β-carboxamide (Fig. 1), is a potent inhibitor of an enzyme, steroid 5α-reductase, which converts testosterone to dihydrotestosterone, the androgen associated with development of benign prostatic hyperplasia (Steingberg et al.,

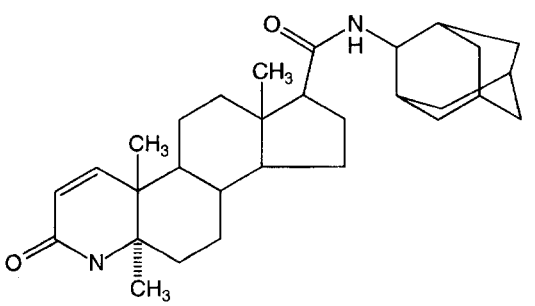

Fig. 1. Chemical structure of L-683,453.

* Corresponding author. Tel.: +215 652 7795; fax: +215 652 5299.

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1991; Cohen et al., 1995). The compound is poorly soluble in water (~ 0.001 mg/mL) which suggests that dissolution and oral absorption from a traditional solid dosage form might not be optimal. In an attempt to enhance the bioavailability of the compound, a novel self-emulsifying drug delivery system (SEDDS) for L-683,453 composed of mono- and di glycerides of caprylic/capric acids (MDG) and non-ionic surfactants was recently developed and characterized (Clarke et al., 1994). Generally, self-emulsifying formulations form a fine emulsion when exposed to aqueous media under conditions of gentle agitation. The resulting oil-in-water emulsions are thermodynamically stable due to the relatively small volume of the dispersed oil phase, the narrow range of droplet size distribution and the polarity of the oil droplets (Groves and de Galindez, 1976; Pouton, 1985a; Pouton, 1985b; Wakerly et al., 1986; Pouton et al., 1987; Wakerly et al., 1987; Shah et al., 1994; Charman et al., 1992). Following oral administration of SEDDS, which can be conveniently encapsulated in soft gelatin capsules, the digestive motility of the stomach and the intestine provide the agitation necessary for self-emulsification (Groves and de Galindez, 1976; McClintic, 1976).

Several publications reported improvement in the extent and rate of absorption of lipophilic compounds from self-emulsifying formulations as compared to more traditional oral formulations (Shah et al., 1994; Charman et al., 1992; Lin et al., 1991; Kelm et al., 1993). The purpose of this study was to evaluate in vivo the performance of SEDDS for L-683,453, administered to Beagle dogs in prefilled gelatin capsules. Bioavailability of the compound from self-emulsifying formulations was compared to that from suspension in methyl cellulose in the absence and presence of sodium dodecyl sulfate (SDS). In addition, the effect of dosing volume of SEDDS and of food on oral absorption of L-683,453, was investigated.

2. Materials and methods

2.1. Materials

L-683,453, an investigational lipophilic compound, was synthesized by Merck Research Laboratories (Rahway, NJ). Medium chain mono- and di-glycerides of caprylic and capric acids (Imwitor 742) were obtained from Huls UK. Ltd. (Bucks, UK.), and Polysorbate 80 (Tween 80) and sorbitan ester 80 (Span 80) were obtained from ICI (MacClesfield, UK). Methyl cellulose, Methocel A4C Premium, was purchased from Dow Chemicals (Midland, MI).

2.2. Methods

2.2.1. Preparation of capsules and suspensions

Self-emulsifying formulation in MDG/Polysorbate 80/Span 80 was prepared by first dissolving the compound in MDG, followed by addition of Polysorbate 80 and Span 80. The formulation was encapsulated in size 12 hard gelatin capsules (fill volume 7.4 mL) supplied by Torpac (New York, NY) which were more suitable for the large volumes used in the study than soft gelatin capsules.

Suspensions were prepared by mixing the compound with 0.5%, w/v methyl cellulose solution employing a mortar and pestle. Whenever the SDS was incorporated, the compound was first mixed with a small volume of SDS solution and then methyl cellulose solution was slowly added. In all suspension studies, the drug particle size was controlled to 95% less than 25 microns (as assessed by optical microscopy).

2.2.2. Oral bioavailability study in dogs

2.2.2.1. Animals. Four purpose bred male Beagle dogs, 2.5–3 years old and weighing 9.0–15.5 kg, were used in the study. The same dogs were used throughout all experiments. Washout time between treatments was at least 2 weeks. All animals were cared for and used in accordance with the rules and guidance of the Merck Research Laboratories Institutional Animal Care and Use Committee and the Guide for the Care and Use of Laboratory Animals (DHHS Publication No. (NIH) 85-23, revised 1985).

2.2.2.2. The i.v. bolus reference study. Four fasted Beagle dogs were dosed with a 1 mg/kg i.v. bolus of L-683,453 dissolved in propylene glycol:ethanol (7:3, v/v). The dosing volume was 1 mL/kg. Blood

samples, 5 mL, were withdrawn prior to dosing and at appropriate time intervals after dosing. Plasma was stored at −70°C prior to analysis, and concentrations of L-683,453 in plasma were assayed by HPLC as described below.

2.2.2.3. Oral absorption from methyl cellulose suspensions. The absorption of L-683,453 either formulated as a suspension in 0.5%, w/v, methyl cellulose or 0.5% methyl cellulose containing 0.02% SDS (w/v), was examined in a three-period crossover study. Doses of 80 mg/kg of L-683,453 in 25-mL dosing volumes were given orally, via gavage, to the four dogs fasted overnight. The dose was followed by 25 mL of water.

Experiments with fed animals at the same dose 80 mg/kg (suspension with SDS) were performed as a separate experiment using three dogs. Dogs were fasted overnight and fed with regular dog chow ~30 min prior to dosing. Blood was withdrawn prior to dosing and at appropriate time intervals after administration. Concentrations of L-683,453 in plasma were determined by HPLC.

2.2.2.4. Oral absorption from SEDDS. Oral absorption of L-683,453 at 16 mg/kg dose and at dosing volumes of either 1 mL/kg or 0.25 mL/kg SEDDS was examined in a four-period crossover study using the four dogs under fasting and fed regimens. In the fed arm, animals were fasted overnight and fed ~30 min prior to dosing. The dose, administered in hard gelatin capsules prepared shortly before dosing, was followed immediately by 50 mL of water. Blood was collected and analyzed for L-683,453 as described below.

It should be mentioned that a self-emulsifying formulation containing dose of 80 mg/kg of L-683,453 in a dosing volume of 2 mL/kg was not tolerated by the animals. It was subsequently determined that both the dose and the dosing volume affect the tolerability. In vivo screening revealed that the highest tolerated dose was 16 mg/kg in a 1 mL/kg dosing volume. Since such a high volume of formulation (selected to support the toxicology studies in dog) may not be relevant to humans, a dosing volume of 0.25 mL/kg, which represents the limit of solubility for a 16 mg/kg dose, was also included in the study.

2.2.2.5. Assay for L-683,453 in dog plasma. The assay is a modification of a previously published assay for finasteride in human plasma (Constanzer et al., 1991). A Waters Assoc. HPLC system equipped with a 746 data module, a 600E system controller, an Ultra Wisp 715 automatic injector and a 6000A chromatographic pump (Waters-Millipore, Milford, MA) were used for all analyses. As a detector, a variable-wavelength ultraviolet (UV) detector Spectroflow 783 (Kratos, Ramsey, NJ) was employed. Standards of L-683,453 ranging from 10–1000 ng/mL were prepared by addition of 25 μL aliquots of L-683,453 (dissolved in methanol) into 1 mL of blank plasma, (which had previously been centrifuged for 10 min at 2500 rpm). Samples, diluted with 3 mL of water, were vortexed briefly and loaded onto 1-mL Bakerbond cyano cartridges (J.T. Baker Inc. Philipsburg, NJ), which had been activated sequentially with 1 mL of methanol, 1 mL of acetonitrile, 1 mL of methanol, and finally washed with 3 mL of water. After plasma was applied, the cartridge was washed with 3 mL of 10% acetone (in water) and then with 3 mL of water. L-683,453 was eluted with two 250 μL aliquots of methanol.

Finally, 200 μL of water were added to the extract and 200 μL of this diluted extract were injected to the HPLC column: Altex RP-8 (15 cm x 0.46 cm, 5 μm) equipped with Brownlee PRP-8, 5 μm, 3 cm, guard cartridge. The mobile phase was composed of acetonitrile: methanol: water (7:6:5, v/v/v, all HPLC grade from Fisher Scientific, Fair Lawn, NJ) and was run at a flow rate of 1 mL/min. Detection was performed at 210 nm. Standard curves were linear over plasma concentrations of 10–1000 ng/mL (data not shown). The limit of quantification was 10 ng/mL and the limit of detection was 2 ng/mL. Assay accuracy for dog plasma standards indicated readback values within 5.8 ± 2.1% (mean ± S.D.) of nominal values. Recovery of L-683,453 from dog plasma was calculated by comparison of the peak areas of

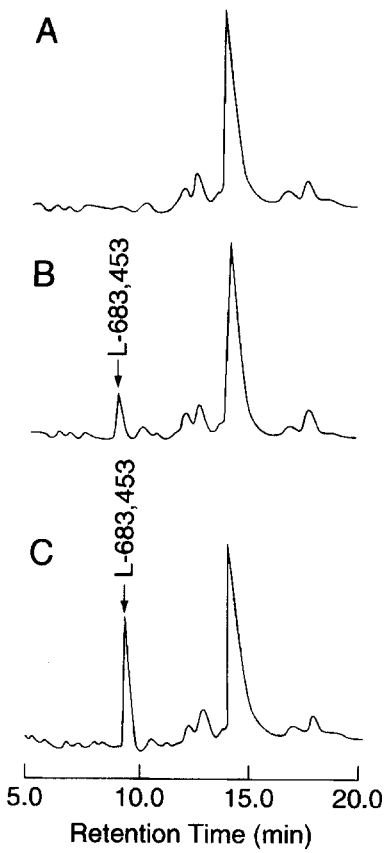

Fig. 2. Representative chromatograms of L-683,453 in dog plasma. (A) Blank control plasma; (B) control plasma spiked with 100 ng/mL of L-683,453; (C) post-dose plasma sample of fed dogs 2 h after dosing with 16 mg/kg at dosing volume 0.25 ml/kg; the concentration of L-683,453 is equivalent to 308.7 ng/mL.

L-683,453 extracted from plasma to that of the injected standards. Recovery of the drug was very high (94.3–98.1%) and was effectivelly independent of concentration. Representative chromatograms for the blank, a control plasma spiked with L-683,453, and post-dose plasma samples are presented in Fig. 2.

2.3. Calculations and analysis of data

Pharmacokinetic parameters were calculated from the plasma concentrations of the parent compound following each administration in individual animals. The maximum plasma concentration, C_{max}, and the time of its occurrence, T_{max}, were compiled from the concentration-time data. The area under the curve, AUC, was calculated by the linear trapezoidal rule from zero to the last plasma concentration above the limit of quantification (10 ng/mL). The absolute bioavailabilities of L-683,453 in the oral formulations were calculated from AUC data relative to that for the i.v. administration (correcting for the difference in dose) according to standard pharmacokinetic procedures.

Data was analyzed by two-way analysis of variance (unless stated otherwise). Values reported are means $\pm$ SEM and 'P' represents significance probabilities associated with pairwise differences among means (2-sided, least-significant difference test). Data were considered statistically significant if $P \leq 0.05$.

3. Results and discussion

Plasma concentration profiles of L-683,453 administered to fasted beagle dogs as an i.v. refer-

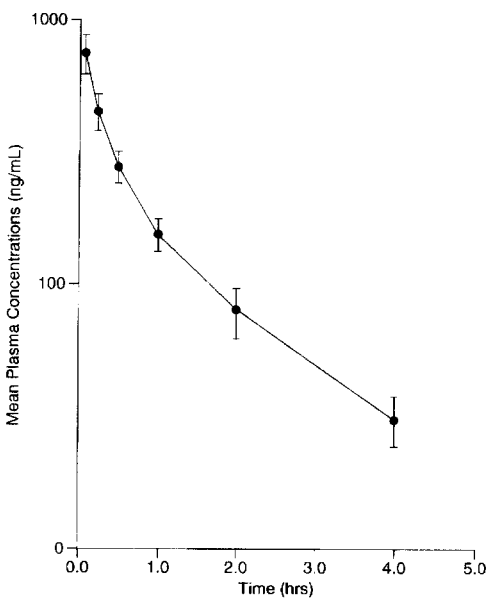

Fig. 3. Average plasma concentration vs. time profile for L-683,453 administered as an i.v. solution in propylene glycol:ethanol (7:3, v/v) to four fasted dogs.

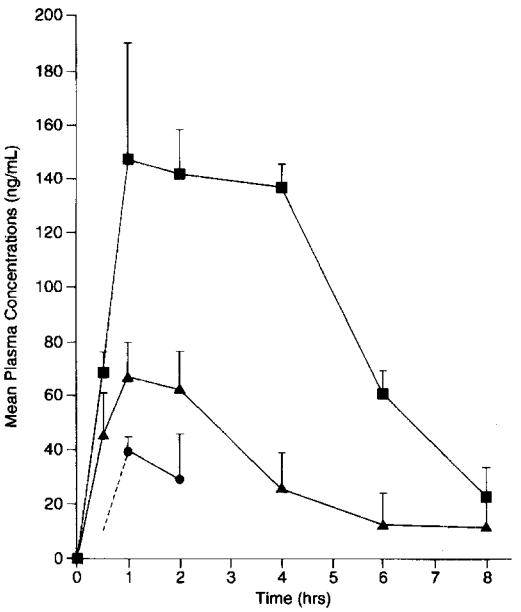

Fig. 4. Average plasma concentration vs. time profiles for L-683,453 (80 mg/kg) administered orally as: (●) a suspension in 0.5% w/v methyl cellulose to fasted dogs (n = 4); (▲) a suspension in 0.5% w/v methyl cellulose with 0.02% w/v SDS to fasted dogs (n = 3); and (■) as 0.5% methyl cellulose cntaining 0.02% SDS w/v to dogs fed ~ 30 min prior dosing (n = 3).

ence formulation are presented in Fig. 3. The average half-life was 1.2 ± 0.3 h and clearance was 11.5 mL/min.kg. The pharmacokinetic parameters following the i.v. administration were consistent between all animals, suggesting that clearance of the compound did not differ significantly between animals at the administered dose.

The plasma concentration profiles for L-683,453 administered as a single oral dose of 80 mg/kg in a form of suspension (with or without SDS) are illustrated in Fig. 4 and the pharmacokinetic parameters are summarized in Table 1. Plasma concentrations of compound administered to fasted animals were very low, mean C_{max} equaled 74 ± 29 ng/mL. The parent compound could not be detected in plasma 4 h post-dosing. The bioavailability was very low, i.e., 0.2%. Incorporation of SDS into the suspension increased the bioavailability to 0.6% (P = 0.05). This small enhancement in absorption can be attributed to the superior quality of the suspension containing the surfactant which acts as a wetting agent, resulting in a finer, more uniform dispersed suspension (Clarke et al., 1994). When administered shortly after a meal, the absorption of L-683,453 measured by AUC, from the SDS-containing formulation was markedly increased, by approximately 3-fold, as compared to fasted animals. The bioavailability reached accordingly 1.7% (P < 0.01, 2-paired, one-way analysis of variance). Although this increase is significant, oral absorption from suspensions given after a meal was still very poor and was considered insufficient for development of the compound as a conventional tablet dosage form.

In an effort to achieve a more substantial increase in absorption of L-683,453, several oil-surfactant formulations were prepared and screened in vitro to select a formulation which forms a fine and stable emulsion upon contact with water (Clarke et al., 1994). Based on these data, the MDG/Span 80/Polysorbate 80 (7:2:1, w/w/w) formulation was selected for in vivo studies.

Data reported above for suspensions and previous experience with an analogous compound (Matuszewska et al., 1993) indicated a significant increase in oral absorption when the dose was given following a meal. Therefore, absorption from SEDDS was measured both in fasted and fed animals. Results are summarized in Fig. 5, where mean plasma concentrations vs. time following administration of a single dose of 16 mg/kg are illustrated. The average pharmacokinetic parameters are given in Table 2.

The superior performance of SEDDS as compared to a suspension is apparent from these data. Bioavailabilities increased under all conditions investigated, from 7.7% in fasted animals at a 1-mL/kg dosing volume to 13.7% in fed animals at 0.25 mL/kg which represents a significant increase by 4.5–8.1 fold as compared to the highest value in suspension, i.e. 1.7%. The differences between bioavailabilities for different treatments (i.e. food and volume effects) are not statistically significant at $P \leq 0.05$. However, the difference between bioavailabilities for fasted animals at 1 mL/kg dose and for fed animals at the 0.25 mL/kg dose was statistically marginally significant (P = 0.07). In addition, the increase in bioavailability in fed

Table 1
Pharmacokinetic parameters of L-683,453 (mean ± SEM) following single oral administration of 80 mg/kg formulated as a suspension in 0.5%, w/v, of methyl cellulose with or without 0.02%, w/v, of sodium dodecyl sulfate to fasted and fed Beagle dogs

Treatment	AUC (ng.h/mL)	C_{max} (ng/mL)	T_{max} (h)	Bioavailability (%)
Suspension in methylcellulose fasted dogs (n = 4)	74 ± 29	46 ± 8	1.5 ± 0.3	0.2
Suspension in methylcellulose containing SDS fasted dogs (n = 3)*	277 ± 97	75 ± 10	0.7 ± 0.2	0.6
Suspension in methylcellulose containing SDS fed dogs (n = 3)	820 ± 61	182 ± 28	2.3 ± 0.9	1.7

*One dog was eliminated due to inability to swallow the dose.

animals dosed with 0.25 mL/kg volume as compared to those dosed with 1 mL/kg volume was also statistically marginally significant (P = 0.09). It should be stressed however, that the bioavailability of L-683,453 from the SEDDS administered with food is significantly higher than that from suspension in methyl cellulose solution given with food. This confirms that the SEDDS itself enhances absorption other than through a simple food effect.

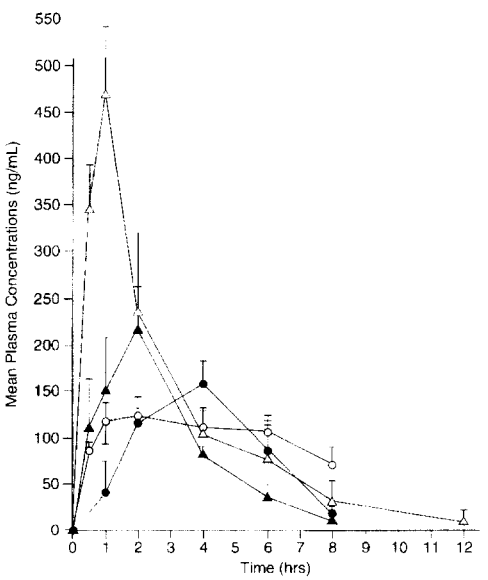

Fig. 5. Average plasma concentration vs. time profiles for L-683,453 (16 mg/kg) administered orally as SEDDS to four dogs: (●) fasted dogs, dosing volume 1 mL/kg; (○) fed dogs, dosing volume 1 mL/kg; (▲) fasted dogs dosing volume 0.25 mL/kg; (△) fed dogs, dosing volume 0.25 mL/kg.

At both respective dosing volumes, there was no statistically significant food effect on mean C_{max}'s. However, the volume affected C_{max} in fed (but not fasted) animals: 488 ± 64 ng/mL measured at the lower dosing volume was significantly higher (P = 0.02) than 134 ± 19 ng/mL measured at the higher dosing volume.

The data given in Table 2 and Fig. 5 indicate also that at the same dose of 16 mg/kg, the dosing volume markedly altered the absorption profile. Feeding animals shortly prior to dosing resulted in faster absorption at both dosing levels. For the larger volume, the decrease in T_{max} from 4.5 ± 0.5 h to 2.3 ± 0.6 h was significant (P < 0.01) while the decrease observed for the lower volume (from 1.8 ± 0.3 to 0.9 ± 0.7 h) was not significant.

The postulated mechanism of enhancement in oral absorption by SEDDS involves the facilitation of diffusion and subsequent absorption of the lipophilic compound from the GI tract from small droplets of oil with large interfacial area (Groves and de Galindez, 1976; Pouton, 1985a; Pouton, 1985b; Charman et al., 1992). The highest bioavailability was observed with the lower dosing volume (0.25 mL/kg) in fed animals. This may have been due to better emulsification of the smaller volume of SEDDS (2.25–3.5 mL) within the gastrointestinal tract coupled with stimulation of bile secretion.

The rate of drug absorption from the GI tract is probably limited by the rate of transport of the drug from the oil phase into the gut lumen. In vitro studies demonstrated a much more efficient emulsification as the ratio of SEDDS to water

Table 2
Pharmacokinetic parameters of L-683,453 (mean ± SEM) following single oral administration of 16 mg/kg as SEDDS to fasted and fed Beagle dogs (n = 4) at dosing volumes of 1 mL/kg or 0.25 mL/kg

Treatment	AUC (ng.hr/mL)	C_{max} (ng/mL)	T_{max} (h)	Bioavailability (%)
1mL/kg Fasted	764 ± 68	167 ± 22	4.5 ± 0.5	7.7
1 mL/kg Fed	986 ± 142	134 ± 19	2.3 ± 0.6	9.9
0.25 mL/kg Fasted	829 ± 325	231 ± 96	1.8 ± 0.3	8.3
0.25 mL/kg Fed	1365 ± 184	488 ± 64	0.9 ± 0.7	13.7

decreased. Additionally, the higher GI motility in the fed state may enhance emulsification and possibly increase the rate of drug release. It should be noted that possible contribution from lipid-based intestinal lymphatic transport was not investigated in this study. However, this would not be expected to play a significant role since the medium chain glycerides are not appreciably absorbed via the lymphatic system.

In summary, data presented in this paper demonstrate that the relatively simple SEDDS system may be effectively used to enhance the oral bioavailability of very poorly water soluble compounds.

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