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Pharmaceutical Nanotechnology

The role of biopharmaceutics in the development of a clinical nanoparticle formulation of MK-0869: a Beagle dog model predicts improved bioavailability and diminished food effect on absorption in human

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Abstract

MK-0869 (aprepitant), a potent substance P antagonist, is the active ingredient of EMEND® which has recently been approved by the FDA for the prevention of chemotherapy-induced nausea and vomiting. Early clinical tablet formulations of MK-0869 showed significant food effects on absorption, suggesting that formulation could have a significant role in improving bioavailability. A Beagle dog model was developed in an effort to guide novel formulation development. Using the suspension of the micronized bulk drug used for the tablet formulations, the food effect on absorption was confirmed in the dog at a similar magnitude to that observed in humans. Further dog studies demonstrated a clear correlation between particle size and in vivo exposures, with the nanoparticle (NanoCrystal®) colloidal dispersion formulation providing the highest exposure, suggesting dissolution-limited absorption. The NanoCrystal® dispersion also eliminated the food effect on oral absorption in the dog at a dose of 2 mg/kg. Regional absorption studies using triport dogs indicated that the absorption of MK-0869 was limited to the upper gastrointestinal tract. These results provided strong evidence that the large increase in surface areas of the drug nanoparticles

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could overcome the narrow absorption window and lead to rapid in vivo dissolution, fast absorption, and increased bioavailability. In addition, the dog model was used for optimizing formulation processes in which the nanoparticles were incorporated into solid dosage forms, and for selecting excipients to effectively re-disperse the nanoparticles from the dosage units. The human pharmacokinetic data using the nanoparticle formulation showed excellent correlations with those generated in the dog.
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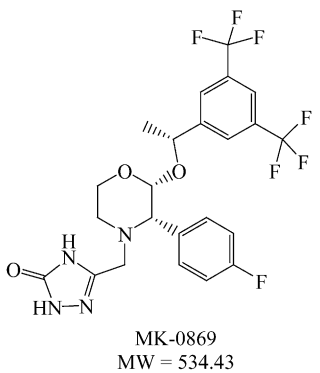
Keywords: MK-0869; Nanoparticles; Beagle dog; Food effect; Particle size effect; Site-specific absorption; Formulations

1. Introduction

MK-0869 (Fig. 1), a selective high-affinity antagonist of human substance P/neurokinin 1 (NK_1) receptors, is the active ingredient of EMEND® which has recently been approved by the FDA for the prevention of chemotherapy-induced nausea and vomiting (CINV). It has little or no affinity for serotonin, dopamine, and corticosteroid receptors, the targets of existing therapies for CINV. It is the first new treatment for CINV in more than a decade. Clinical data showed that MK-0869 prevents delayed emesis after treatment with cisplatin and other chemotherapeutic agents. Moreover, combining MK-0869 with a 5-HT_3 antagonist (e.g. granisetron or ondansetron) plus dexamethasone improves the prevention of acute emesis (Nanvari et al., 1999; Hesketh et al., 2003; Poli-Bigelli et al., 2003). MK-0869 undergoes extensive metabolism, primarily via CYP3A4 mediated oxidation. It is eliminated primarily by metabolism and is not renally excreted. The apparent terminal half-life in human ranged from 9 to 13 h.

Early preclinical studies showed less than dose proportional increases in systemic exposure in Beagle dogs at oral doses of 2 and 32 mg/kg with suspensions of conventional micronized MK-0869. A similar dose–exposure relationship was observed in early clinical studies using tablet formulations made of micronized bulk drug. In addition, a significant positive food effect on absorption was seen in healthy young male volunteers who had been given a high fat breakfast prior to dosing. As the projected efficacious human dose would be relatively high based on the exposures from the tablet formulations, development of a more bioavailable formulation could potentially reduce the dose. In addition, the requirement of a high fat meal for maximum exposure would be problematic for the development of MK-0869 due to the specific target patient population. Hence, formulation efforts to develop a more bioavailable formulation that would not exhibit a food effect on absorption were crucial for the future of the program.



MK-0869
MW = 534.43

Fig. 1. Chemical structure of MK-0869.

Low oral bioavailability of drugs can be attributed to one or a combination of the following factors: slow dissolution rate, poor solubility, first-pass metabolism by the gut and liver, chemical instability in the gastrointestinal (GI) tract, efflux transport, and poor permeability across the intestinal mucosa (Aungst, 1993; Hörter and Dressman, 2001; Martinez and Amidon, 2002). Many of these issues have been addressed during the drug discovery and development candidate selection processes. Compounds with poor aqueous solubilities often result in low and erratic bioavailability and less than linear dose proportionality. The in vivo performance would become even less desirable if a drug is primarily or only absorbed in the upper GI since the transit time of dosage forms in this region is normally around 2–4 h (Davis et al., 1986). Effective absorption in this narrow and limited window requires rapid disintegration of the dosage form and dissolution of the drug particles. It is also common to observe positive food effects on absorption for poorly water-soluble but highly permeable drugs, i.e. class II compounds according to the Biopharmaceutics Classification System (BCS) (Fleisher et al., 1999; Sun et al.,

1994). It is known that the nature and extent of food effect on absorption is a function of the physicochemical properties of the drug, the dose, the composition of the formulation, the quantity and ingredients of the food, the physical conditions of the tested subjects, and the timing of food ingestion in related to drug administration (Welling, 1989; Charman et al., 1997). For poorly water-soluble drugs, a positive food effect can be attributed to (1) increased secretion of digestive juices, especially bile salts, which could increase the rate of dissolution via enhanced wetting of drug particles and increase the overall solubilization capability of the GI tract via the formation of mixed micelles; (2) increased GI motility; and (3) increased visceral blood and lymph flow (Charman et al., 1997). In the case of high dose, poorly water-soluble drugs, alterations in drug dissolution under fed and fasted conditions have been considered as a likely source influencing the variability in drug absorption (Dressman et al., 1984; Dressman and Fleisher, 1986). For poorly soluble acid-stable drugs, the absorption may be increased with delayed gastric emptying under the fed conditions, providing a greater residence time for drug dissolution or release and a greater amount of drug in solution available for intestinal absorption (Fleisher et al., 1990).

Among the reported formulation approaches to improve the bioavailability of poorly water-soluble drugs, the use of alternative salt forms (for greater aqueous solubility), particle size reduction and amorphous dispersion (for increasing rate of dissolution), specific complexation with cyclodextrins, the addition of surfactants, and the use of lipid-based excipients (for increasing saturation solubility) have been frequently employed (Bastin et al., 2000; González et al., 2002; Hauss, 2002; Müller et al., 2001; Redenti et al., 2000; Wasan, 2001). A prerequisite for utilizing these approaches is that the drug has to be physically and chemically stable in the presence of excipients under stressed conditions. Reduction of the drug particle size from microns to submicrons or nanometers has provided significant increases in bioavailability in several reported examples (Kondo et al., 1993; Liversidge and Conzentino, 1995a; Liversidge and Cundy, 1995b; Loper et al., 1999). Furthermore, nanoparticles of various configurations, namely drugs loaded inside the polymer matrix of the particles, drugs encapsulated inside the particles, and drugs physically adsorbed or chemically coupled onto the particle surfaces, have been employed (Labhasetwar, 1997).

NanoCrystal® is an enabling technology licensed to Merck from Elan/Nanosystems (King of Prussia, PA, USA) to mill drug particles to few hundred nanometers or less (Merisko-Liversidge et al., 2003). NanoCrystal® technology generates physically stable dispersions that can be administered through oral, injectable (i.v., s.c., and i.m.), and topical routes. In addition, aqueous nanoparticle dispersions can be processed into solid dosage forms. MK-0869 was one of the first Merck development compounds that employed this media-milling technology to reduce the particle size in an effort to improve the performance of clinical formulations. In this study, a Beagle dog model was developed for rapid evaluation of the in vivo performance of the proto-type NanoCrystal® formulations. Several effects including food, drug particle size, and formulation composition and process on absorption were investigated. In order to understand the underlying reason for the elimination of food effect on absorption in the dog using a NanoCrystal® dispersion formulation, regional absorption studies using triport dogs were also conducted to pinpoint the site of drug absorption.

2. Materials and methods

2.1. Materials

The alpine-milled, jet-milled (dry-milled), and wet-milled (Dynomil) MK-0869 were provided by Chemical Engineering R&D of Merck Research Laboratories (Rahway, NJ, USA). The NanoCrystal® colloidal dispersion of MK-0869 was prepared at Nanosystems (King of Prussia, PA, USA).

2.2. Preparation of formulations

The alpine-milled, jet-milled, and wet-milled materials were suspended in 0.5% methylcellulose in water with 0.02% sodium dodecyl sulfate (SDS) at a concentration of 0.8 mg/ml. The colloidal dispersion was prepared using a ball milling process (Liversidge and Cundy, 1995b) with 4% hydroxypropyl cellulose, 0.08% SDS, and 20% sucrose at a concentration of 50 mg/ml in water. Particle size analysis was performed on a Horiba LA-910 laser scattering particle

size distribution analyzer (Horiba Instruments, Inc., Irvine, CA). The instrument uses a 632.8 nm He–Ne laser and a tungsten halogen lamp. Water was used as the diluting medium. A refractive index of 1.52 was assumed. Suspensions or nanosuspensions were diluted to 0.02–0.10 mg/ml for each measurement.

The soft gelatin capsules were hand-filled with a mixture containing 7.5 mg of MK-0869, 555 mg of mono- and diglycerides of medium chain fatty acids (MDG; IMWITOR 742®), 180 mg of polysorbate 80, and 7.5 mg of ascorbyl palmitate (as an anti-oxidant), with a total weight of 750 mg per capsule.

2.3. Animal experimental protocol

Purpose-bred Beagle dogs from Marshall Farms (North Rose, NY, USA) were housed in a USDA-approved facility in accordance with AAALAC guidelines. The dog weights ranged from 11.3 to 15.0 kg at the beginning of this series of studies, and they were weighed prior to each period of the study. The dogs were fasted overnight without water prior to each study day. Dogs designated to be fed ate a 374-g can of Alpo "Chunky with Beef for Dogs," which has 55% of total calories from fat, approximately 30 min prior to dosing. For both fed and fasted groups, the dosing volumes including formulations and water rinse were maintained the same as indicated in Sections 2.3.1–2.3.3. Standard laboratory chow and water were offered ad libitum 4 h after dosing. To eliminate the variability of absorption among the dogs, all studies were conducted in a randomized crossover design. Five milliliters of blood samples were withdrawn with a 21G needle and syringe through a heparin lock on a 21G indwelling catheter in the cephalic vein at predose, 15, 30 min, and 1, 2, 4, 6, and 8 h. The 12, 24, 48, and 72 h samples were obtained by venipuncture. Blood was immediately transferred to a heparinized blood collection tube (VACUTAINER, Becton Dickinson, Franklin Lakes, NJ, USA) and held on ice until samples were centrifuged at 2500 rpm, 5 °C for 15 min. The plasma was transferred to polypropylene tubes, and samples were stored at −70 °C until analyzed by LC/MS/MS.

2.3.1. Study of particle size and food effect

MK-0869 suspensions were administered orally to five fasted, male Beagle dogs at a dose of 2 mg/kg in a randomized crossover design. Suspension (0.8 mg/ml) of alpine-milled (mean particle size of 5.49 μm), jet-milled (mean particle size of 1.80 μm), and wet-milled material (mean particle size of 0.48 μm) were dosed orally at 2.5 ml/kg followed immediately with 2.5 ml/kg water by gavage tubes. For the colloidal dispersion (50 mg/ml; mean particle size of 0.12 μm), dogs were dosed at 0.04 ml/kg orally based on the study day weight followed by 5 ml/kg water orally.

2.3.2. Regional absorption study

Six male Beagle dogs with chronically implanted jejunal, colonic and portal vein ports (Marshall Farms, North Rose, NY, USA) were used in a randomized three-period full-crossover study (Kwei et al., 1995). In addition to oral dosing, only the jejunal and colonic ports were used for dosing in this particular study. The colloidal dispersion (50 mg/ml; mean particle size of 0.12 μm) was administered as a bolus dose at 0.04 ml/kg via oral gavage or jejunal/colonic catheter followed by 5 ml/kg water for each route.

2.3.3. Study with soft gelatin capsules

Two 7.5-mg potency soft gelatin capsules filled with 75% (w/w) MDG and 25% (w/w) polysorbate 80 were dosed orally to five dogs under fed and fasted conditions as described above, followed by 5 ml/kg water orally.

2.4. Bioanalytical methods

A validated method using liquid chromatography/atmospheric pressure chemical ionization tandem mass spectrometry (LC/APCI-MS/MS) for the determination of MK-0869 in dog plasma was used for the analysis of the unknown samples. The method employed a liquid–liquid extraction procedure with methyl-*t*-butyl ether to isolate MK-0869 from the biological matrix. The organic extracts were collected and the extraction solvents were removed under a gentle flow of nitrogen. The reconstituted extracts were analyzed by LC/APCI-MS/MS on an Applied-Biosystems API300 triple quadrupole mass spectrometer (Applied Biosystems, Foster City, CA, USA). Chromatography was performed on a 150 mm × 4.6 mm Betasil C18 column (Thermo Hypersil-Keystone, Bellefonte, PA, USA) using isocratic elution with 80:20 aqueous 10 mM ammonium acetate (pH 5.0) and acetonitrile. A chemical analogue of MK-0869, L-000743955,

was used as the internal standard. Under these conditions, no interference was observed for both MK-0869 and L-000743955 from the endogenous components of the plasma. The assay had a lower limit of quantitation (LOQ) of 1 ng/ml for MK-0869 based on 0.5 ml aliquots of dog plasma. The standard curve was linear from 1 ng/ml to 2000 ng/ml. The analysis time was 3.7 min per sample.

2.5. pK data processing

WinNonlin v. 3.1 (Scientific Consulting, Inc., Apex, NC) was used for noncompartmental analysis of the data. Area under the plasma concentration–time curve ($AUC_{0–72\,h}$) was calculated from observed plasma concentrations from 0 to 72 h. Any plasma concentrations below the limit of quantitation were set equal to zero. Geometric and arithmetic mean and geometric standard error of the mean (S.E.M.) of AUC, observed maximum plasma concentration (C_{max}), and time of C_{max} (T_{max}) were calculated with Microsoft Excel v. 97 SR-2(f). SigmaPlot 2001 for Windows v.7.0 was used for plotting the arithmetic mean plasma concentration–time curves. Treatment and animal effects on the AUC values and observed C_{max} were determined with the General Linear Models (GLM) procedure of the statistical programs of SAS (SAS Institute, Inc., Cary, NC, USA; Release 6.07). Also, an interaction model with dog, formulation, and fed/fasted state was examined to confirm the interaction of food with formulation. The AUC and C_{max} values were log-transformed to normalize the distributions. The Wilcoxon matched-pairs signed ranks test was used to evaluate differences in T_{max} values between groups. Differences were only considered significant at $p \leq 0.05$.

3. Results and discussion

MK-0869 is a basic compound with a pK_a value of 9.7 within the pH range from 2 to 12. It is a white to off-white crystalline non-hygroscopic solid with a melting point at 254 °C. Early salt form screening was conducted but all salts tested showed rapid disproportionation in water and poor chemical stability. The free base form of the molecule was chosen for development based on the overall physicochemical properties. The free base has two polymorphs (Forms I and II) which were identified in the course of early clinical formulation development. The thermodynamically more stable free base Form I has a solubility of 7 μg/ml in water (at native pH of 8.0) and was used for this study and for formulation development. Its aqueous solubility (3–7 μg/ml) is very low in the pH range of 2–10, and increases to 0.13 mg/ml at pH 1.0. The compound has a log P value of 4.8 at pH 7.0, suggesting a relatively high lipophilicity, the potential for dietary fat and bile to solubilize the drug in vivo, and potentially reasonable permeability.

3.1. Food effect on absorption in the dog using micronized suspensions

Phase I and IIa clinical studies using tablet formulations made with micronized drug particles showed a significant positive food effect on absorption at a dose of 100 mg of MK-0869 with exposures of about three-fold increase under fed conditions. The food effect was more pronounced at a higher dose of 300 mg. In addition, the exposures achieved with the tablet formulations suggested that efficacious doses from tablets would be high. To evaluate whether Beagle dogs were a suitable model for assessing food effect on absorption, a suspension of the micronized bulk drug (mean particle size of 5.49 μm) was orally dosed to dogs at 2 mg/kg under both fed and fasted conditions. Fig. 2 shows the comparison of the mean plasma concentrations of MK-0869 following oral administrations of a conventional suspension under fed and fasted conditions. In the dog, feeding a diet with 55% of calories from fat 30 min prior to dosing significantly increased the area under the plasma-concentration curve ($AUC_{0–72\,h}$) and the peak plasma concentrations (C_{max}). A 3.2-fold increase in AUC and a 2.3-fold increase in C_{max} were observed in the fed state (Table 1). The difference in T_{max} was not significant due to the high variability in the fasted state. The dog data were quantitatively similar to the human results with ratios of AUC_{fed}/AUC_{fasted} of about 3 ($N = 6$ for humans and $N = 5$ for dogs). These results suggested that at the respective doses dogs could serve as useful surrogates for evaluating food effects with proto-type formulations.

Liquid-filled capsule formulations were not a viable commercial formulation because of limited solubility of MK-0869 in non-aqueous solvent systems and the projected dose for adequate exposure in humans.

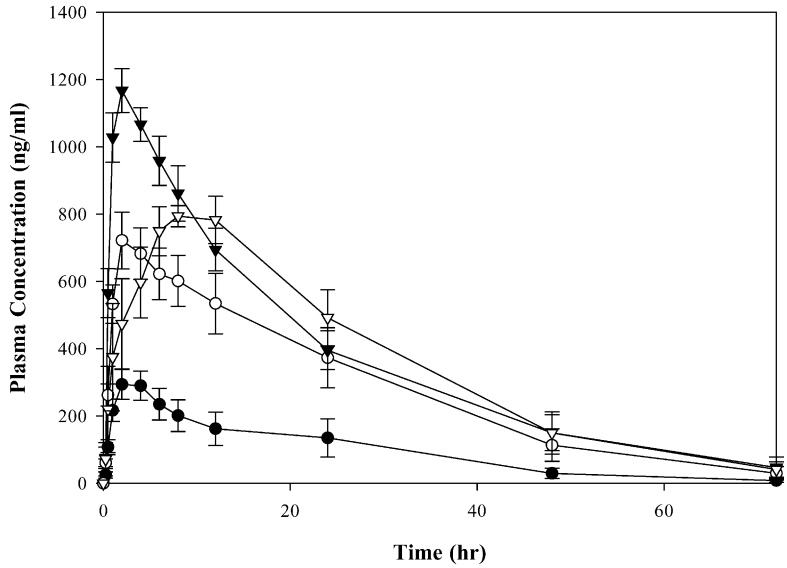


Fig. 2. Comparison of mean (±S.E.) plasma concentrations of MK-0869 following oral administrations in Beagle dogs ($N = 5$) of a conventional suspension (●, fasted; ○, fed) and a NanoCrystal® dispersion formulation (▼, fasted; ▽, fed) of MK-0869 at a dose of 2 mg/kg.

Nevertheless, to test our hypothesis that maximum exposures could be achieved if the entire dose was solubilized and in vivo re-precipitation of the drug would not occur or would be minimum, a solution of MK-0869 in a self-emulsifying vehicle, MDG-polysorbate 80 (75:25), was hand-filled into soft gelatin capsules. The solubility of MK-0869 in MDG-Tween 80 (75:25) was 10 mg/ml which was significantly higher than the aqueous solubility.

The in vivo data from the fasted dogs dosed with two 7.5-mg potency capsules yielded a mean AUC of 13,451 (ng/ml) h (after dose normalization to 2 mg/kg) which was significantly higher than that generated from the conventional suspension (5883 (ng/ml) h). More importantly, the mean AUC value (12,859 (ng/ml) h, after dose normalization to 2 mg/kg) generated from fed dogs dosed with the soft gelatin capsules was statistically not different from the one generated under fasted conditions. These data clearly indicated that solubilization of the drug in a self-emulsifying vehicle eliminated food effect on absorption. In addition, the lack of dependence of exposures on food-induced increases in solubility with the self-emulsifying formulation suggested that no or minimum re-precipitation of the drug occurred in the dog small intestine. These data further confirmed that (1) the drug has good permeability in the GI; and (2) the absorption of the drug molecules was rapid as long as they were solubilized. Although the soft gel capsule formulation was feasible for a dog study at 2 mg/kg (i.e. ∼20 mg dose), it would not be suitable as a clinical formulation because a human dose of >80 mg would require at least 10 capsules

Table 1
Pharmacokinetic parameters following oral administrations in Beagle dogs ($N = 5$) of conventional suspension and NanoCrystal® dispersion formulations of MK-0869 under fed and fasted conditions

Formulations	Dose (mg/kg)	Feeding conditions	$AUC_{0-72\,h}$ ((ng/ml) h)[a]	C_{max} (ng/ml)[a]	T_{max} (h)[b]
Conventional suspension	2	Fasted	5,883 ± 1,862	312 ± 36.7	6.8 ± 4.3
Conventional suspension	2	Fed	18,715 ± 3,240	723 ± 71.4	2.8 ± 0.49
NanoCrystal dispersion	2	Fasted	25,287 ± 3,290	1,159 ± 65.2	2.0 ± 0
NanoCrystal dispersion	2	Fed	24,385 ± 3,261	879 ± 50.0	8.0 ± 1.9

[a] Geometric means and geometric standard errors of the means.
[b] Arithmetic means.

as a single dose, considering the maximum amount of solubilized drug that could be filled into a size zero soft gel capsule was 7.5 mg.

3.2. Elimination of food effect in the dog with NanoCrystal® colloidal dispersion

Feeding-induced increases in exposure often complicate clinical trial design, since the type and timing of the meal varies widely. The data generated with the liquid-filled capsules clearly demonstrated that formulations providing solubilization of the drug could result in significant increases in bioavailability and minimum impact by food. If absorption was actually dissolution rate, rather than solubility-limited in the fasted state, drug particle size reduction, leading to fast dissolution, could be as successful as a fully solubilized drug formulation.

The aqueous solubility of MK-0869 in the intestine is expected to be very low based on the solubility–pH profile. The food effect noted with a standard drug suspension and tablets suggested that there might be insufficient mixed micelles of bile salts in the fasted state to fully solubilize clinically relevant doses. To test where dissolution rate or solubility was limiting fasted-state bioavailability, the bulk drug materials with mean particle sizes of 5.49, 1.85, 0.48, and 0.12 μm were generated by alpine, air-jet, wet (without polymeric surfactants), and wet-media milling (with polymeric surfactants) processes, respectively. Suspensions made with these four different particles were dosed to dogs orally at a dose of 2 mg/kg. Fig. 3 shows the mean drug plasma concentration–time profiles from the four suspensions. It is very clear that the reduction of drug particle size from 5.5 μm to 120 nm correlated well with incremental improvements in oral absorption of MK-0869, up to four-fold increase in AUC (Table 2). Compared with the conventional micronized particles, the mean particle size of the nanoparticles was 45-fold smaller. In comparison, the particle surface areas were increased by 42-fold. These data provided strong confirmation that the absorption of MK-0869 is dissolution rate-limited. Although no permeability data were available at the time this work was done, the above data indicated the absorption would be optimal as long as the drug dissolves quickly after oral administration (Fig. 3).

Dogs were dosed under both fed and fasted conditions using a NanoCrystal® dispersion at a dose of 2 mg/kg. The mean AUC and C_{max} values for the fasted nanoparticle formulation are significantly greater than

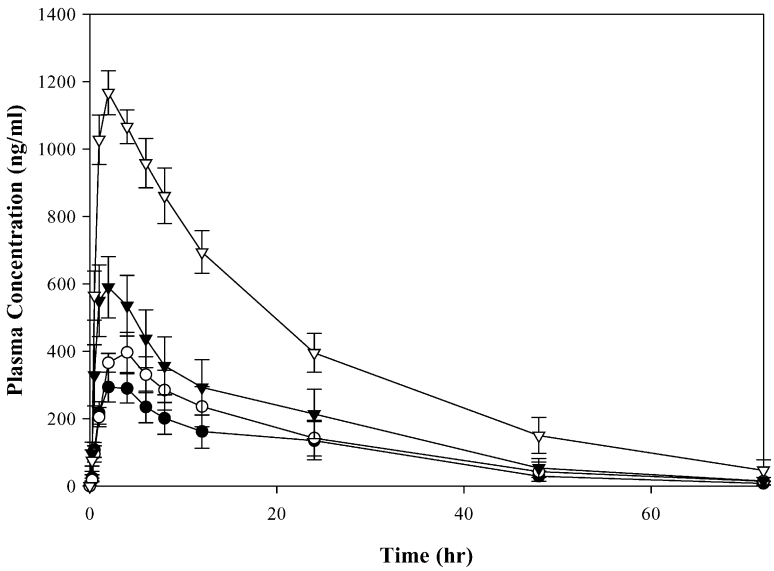


Fig. 3. Comparison of mean (±S.E.) plasma concentrations of MK-0869 following oral administrations in Beagle dogs ($N = 5$) of suspensions made of conventional (●, 5.5 μm), jet-milled (○, 1.8 μm), wet-milled (▼, 0.48 μm), and nano-milled (▽, 0.12 μm) MK-0869 at a dose of 2 mg/kg under fasted conditions.

Table 2
Pharmacokinetic parameters following oral administrations in Beagle dogs ($N = 5$) of suspensions of conventional (5.5 μm), jet-milled (1.8 μm), wet-milled (0.48 μm), and nano-milled (0.12 μm) MK-0869 at a dose of 2 mg/kg under fasted conditions

Formulations	$AUC_{0-72\,h}$ ((ng/ml) h)[a]	C_{max} (ng/ml)[a]	T_{max} (h)[b]	Particle size (μm)	Surface area ratio[c]
Conventional suspension	5,883 ± 1,862	312 ± 36.7	6.8 ± 4.3	5.49	1
Jet-milled suspension	7,542 ± 1,862	399 ± 46.4	3.2 ± 0.49	1.85	3.4
Wet-milled suspension	10,483 ± 2,699	570 ± 75.4	1.6 ± 0.24	0.48	9.4
NanoCrystal dispersion	25,287 ± 3,290	1,159 ± 65.2	2.0 ± 0	0.12	41.5

[a] Geometric means and geometric standard errors of the means.
[b] Arithmetic means.
[c] Assuming all particles are spherical.

those of the conventional micronized suspension ($p < 0.001$). As with the soft gelatin capsule formulation, the NanoCrystal® dispersion formulation produced similar exposures under fasted and fed conditions, as indicated by mean AUC values of 25,287 (ng/ml)h and 24,385 (ng/ml)h, respectively (Table 1). Furthermore, the mean AUC values for the fasted and fed nanoparticle formulation are not significantly different at $p = 0.05$, but the mean C_{max} value from the fed nanoparticle formulation is significantly lower than the fasted C_{max} value ($p < 0.05$).

The lower C_{max} and the later T_{max} under fed conditions with the dispersion are thought primarily due to delayed gastric emptying and dilution effect by the ingested food (Fig. 2). It should also be noted that under fed conditions the mean T_{max} observed with the NanoCrystal® dispersion (8 h) was significantly longer than that with the conventional suspension (2.8 h). For MK-0869, this may imply a change in the site of dissolution of nanoparticles in the GI tract in the fasted and fed states. In the fasted state, rapid dissolution in mixed micelles of bile salts and endogenous lipid may be occurring in the upper small intestine. Rapid absorption produces a high C_{max} and shorter T_{max}. Under fed conditions, dissolution in dietary lipids in the stomach may be relatively more important, and changes in the profile of gastric emptying produced by the fats can lead to a delay in rate of absorption without reducing extent of absorption (Kosoglou et al., 1995; Mojaverian et al., 1985). A contributing factor to the delayed and extended absorption in the fed state may also be viscosity changes produced by the increased extent of

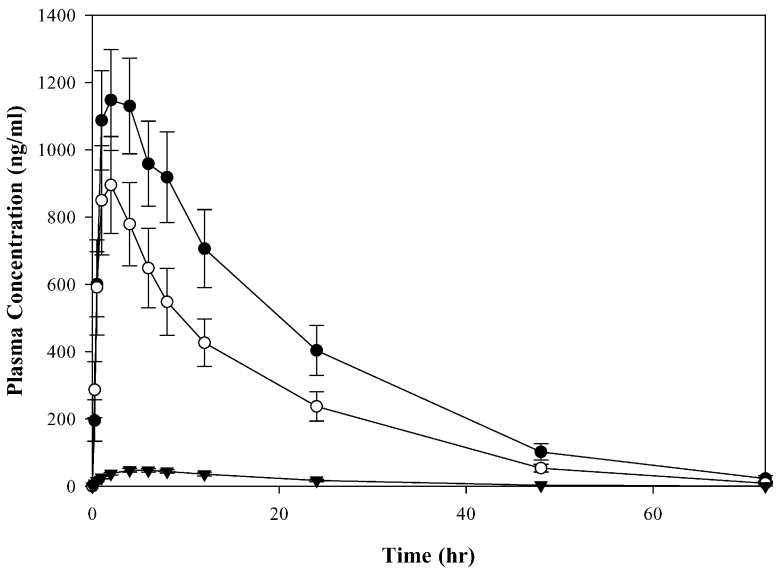


Fig. 4. Mean (± S.E.) plasma concentrations of MK-0869 following oral (●), jejunal (○), and colonic (▼) administrations in Beagle dogs ($N = 6$) at a dose of 2 mg/kg of a NanoCrystal® dispersion formulation of MK-0869 under fasted conditions.

mixing between chyme and nanoparticles, leading to a highly viscous mixture. For some drugs exhibiting site-specific absorption (see Section 3.5), this meal-induced decrease in diffusivity may impede drug absorption as the drug moves past the absorption site (Pao et al., 1998; Reppas et al., 1991). This possible negative effect on diffusivity seemed to cancel some of the positive effects from the dietary fat and meal-induced bile salt secretion, leading to an insignificant change in the extent of absorption as indicated by a ratio of 0.9 for AUC_{fed}/AUC_{fasted}.

The food effect data obtained from the nanoparticle formulation were in sharp contrast to those generated with a suspension made of alpine-milled micronized particles, where a three-fold positive food effect was observed. With the suspension prepared from micronized particles, it is expected that feeding food containing high fat content will facilitate the rate of drug solubilization in the stomach and upper small intestine. In addition to the aid of fat in the meal, the dispersion and dissolution of the drug particles is further enhanced by feeding-induced secretion of digestive juice and longer gastric residence time. On the contrary, in the case of NanoCrystal® dispersion, as the surface areas of the particles increased significantly (42-fold), dissolution of the drug became rapid and extent of absorption became less dependent upon diet and physiological state.

Since our dog model showed similar extent of food effect at a dose of 2 mg/kg to that in the human at 100-mg dose (micronized particles were used in both studies), the data from food and particle size effect study in dogs using the nanoparticle formulation presented a very promising solution to the problems of low bioavailability and positive food effect on absorption in humans. Subsequently, the nanoparticle dispersion was tested in a Phase IIb study along with other solid dosage forms made of nanoparticles. The NanoCrystal® dispersion provided 3.5-fold increase in exposure in humans at a dose of 100 mg compared with a tablet formulation made of micronized particles.

It should be noted that doses need to be taken into consideration when evaluating food effect on absorption. Feeding conditions could produce more pronounced differences in exposure when the doses are high. Compared with a human dose of 100 mg (i.e. 1.4 mg/kg assuming an average body weight of 70 kg), 2 mg/kg in the dog is a reasonable dose to mimic the human experience. In fact, when the final capsule formulation containing spray-coated nanoparticles (see Section 3.4) was tested in healthy volunteers at a fixed dose of 125 mg (i.e. 1.8 mg/kg), it achieved a bioavailability of 59% with insignificant food effect on absorption (1.2-fold increase in mean AUC after feeding). However, when a higher dose (300 mg) of the spray-coated nanoparticle formulation was tested in humans, a 2.7-fold increase in mean AUC was observed in the fed state, compared with a 4.8-fold increase in mean AUC for the conventional tablet formulation. These data clearly illustrate the limitation of using particle size reduction for eliminating the food effect on the extent of absorption. At low doses, the absorption is dissolution rate-limited, and significant increases in particle surface areas and the rate of dissolution with the nanoparticles can compensate the narrow absorption window (see Section 3.3) and physiological changes under fed and fasted conditions. In contrast, the absorption becomes solubility-limited at high doses because of the limited solubilization capacity of the GI tract under fasted conditions. The significant increase in saturation solubility in the presence of dietary fat and delayed gastric emptying provide increased GI solubilization capacity and extended time for drug absorption to take place in the upper small intestinal region, leading to a significant increase in the extent of absorption.

3.3. Site specific absorption of MK-0869

A study of the site of drug absorption can often provide additional understanding of the impact of formulation changes on bioavailability. In general, for drugs that are evenly absorbed over the entire intestinal tract, rate of dissolution will not influence the extent of absorption. Food intake would only influence the extent of absorption if the dose/solubility ratio was unfavorable (Fleisher et al., 1999). However, drugs that possess narrow absorption windows often exhibit variable exposures when they are administered in the presence or absence of food due to changes in GI physiology (Pao et al., 1998; Reppas et al., 1991).

To further investigate the site of absorption, a randomized three-period full-crossover dog regional absorption study was carried out using triported dogs. The NanoCrystal® dispersion was dosed at 2 mg/kg via oral gavage, jejunal bolus, and proximal colonic bolus doses (Fig. 4). Table 3 shows the systemic

Table 3
Pharmacokinetic parameters following oral, jejunal, and colonic administrations in Beagle dogs ($N = 6$) at a dose of 2 mg/kg of a NanoCrystal® dispersion formulation of MK-0869 under fasted conditions

Route of administration	$AUC_{0-72\,h}$ ((ng/ml) h)[a]	C_{max} (ng/ml)[a]	T_{max} (h)[b]	Relative to oral absorption (%)
Oral	23,687 ± 4,062	1,139 ± 158	2.3 ± 0.6	–
Jejunal	14,481 ± 2,906	855 ± 133	1.7 ± 0.2	61
Colonic	1,013 ± 144	48.4 ± 4.44	5.0 ± 0.7	4.3

[a] Geometric means and geometric standard errors of the means.
[b] Arithmetic means.

pharmacokinetic parameters from the three different routes of administration. It is very clear that the absorption of MK-0869 decreased rapidly as the drug moved down through the GI, indicated by ratios of 0.61 and 0.04 for $AUC_{jejunal}/AUC_{oral}$ and $AUC_{colonic}/AUC_{oral}$, respectively. The extremely poor absorption in the colon suggested that the absorption of MK-0869 almost exclusively occurred in the upper GI. These results are in an excellent agreement with our observation that the site-specific absorption is sensitive to feeding conditions due to changes in GI conditions and changes in formulation characteristics. Bile salts are reabsorbed in the small intestine, so micellar solubilization of MK-0869 in the proximal colon is negligible. Recently generated permeability data using Caco-2 cells suggested that MK-0869 has a relatively low permeability with a P_{app} value of 7.8×10^{-6} cm/s (Gibson, 2003). These factors can explain the low colonic absorption.

The low colonic absorption of MK-0869 with the NanoCrystal® formulation presented additional explanation for the low bioavailability of the micronized tablet formulations in the fasted state, since sufficient drug dissolution could not be achieved within the limited small intestinal transit time. On the contrary, rapid dissolution with the NanoCrystal® formulation resulted in effective absorption in this narrow window and minimum dependency on colonic absorption. Although the Caco-2 cell permeability and colonic absorption are low for MK-0869, good oral bioavailability with the NanoCrystal® dispersion suggested that the drug permeability in the small intestine would be substantially higher than that in the lower GI.

3.4. Effects of nanoparticle formulation composition and process on absorption

The NanoCrystal® dispersion of MK-0869 might be amenable in a clinical setting but would not be suitable as a marketed product because of the requirement for long-term physicochemical stability at ambient temperature. In order to incorporate the nanoparticles into a solid dosage form, spray-drying and spray-coating onto polymer beads were evaluated as potential scalable formulation processes. In vivo assessment of the two solid dosage forms derived from these two processes was conducted in dogs with hand-filled capsules at a fixed dose of 100 mg. The process of spray-coating onto beads yielded a formulation that provided slightly greater exposure with a mean AUC value of 1.2-fold of that from the spray-dried formulation. In addition, results from a separate in vitro dissolution test showed a faster dissolution rate for the spray-coated formulation (data not shown). The recommendation for selecting the spray-coated process was then made based on the formulation processibility, in vitro dissolution, and in vivo exposure data.

One of the key factors that could affect the rate of dissolution of the spray-coated formulation and bioavailability is the effectiveness of re-dispersion of nanoparticles from a solid dosage form into the GI fluid. Sucrose was used as a re-dispersant to facilitate the dispersion process. Formulations with different drug-sucrose ratios were prepared for optimizing the dispersion process. Once again, both in vivo and in vitro data (not shown) clearly indicated that a drug–sucrose ratio of 10:1 was superior to a ratio of 1:1 based on the extent of exposure and rate of dissolution.

4. Conclusions

Our in vivo regional absorption studies have shown that the absorption of MK-0869 is limited to the upper GI tract due to the combination of solubility and permeability. Oral and jejunal dosing provided significantly greater exposures than that from a colonic dosing. This

site-specific absorption, which is dictated by the relatively fixed small intestinal transit time, is considered to be the primary cause of low bioavailability and variable exposures using the early tablet formulations made from micronized MK-0869. Although the aqueous solubility of MK-0869 is very low at small intestinal pH and mixed micelles of bile salts are required for solubilization, our approach to overcome the limit of site-specific absorption through reducing the drug particle size, increasing particle surface areas, and increasing the rate of dissolution has been proven to be very successful. Compared with the conventional micronized formulation, the nanoparticle formulation not only enhanced bioavailability but also eliminated food effect on absorption. In fact, there is no restriction in the administration of EMEND® (doses at 80 and 125 mg) in regard to feeding conditions.

Our extensive in vivo studies have also demonstrated that the Beagle dog is an excellent model for evaluating the effects of particle size, feeding conditions, formulation process, and formulation composition on the absorption of MK-0869. More importantly, the Beagle dog data agree with human data, therefore, a preclinical model can be used for predicting effects of MK-0869 formulation changes on human pharmacokinetic profiles. It should be noted that using a specific animal model for humans is a compound specific issue. Although the dog is a useful and predictive model for humans in the case of MK-0869, there are GI physiological differences between the two species that may affect oral pharmacokinetic profiles for other test compounds (Paulson et al., 2001).

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