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Formulation assessment of MK-886, a poorly water-soluble drug, in the beagle dog

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Summary

MK-886 is an orally active leukotriene biosynthesis inhibitor which may have therapeutic benefits in inflammatory diseases. The compound is the sodium salt of an organic acid with low aqueous equilibrium solubility and potentially poor oral absorption characteristics. Since there is no adequate in vitro method of predicting oral bioavailability for such poorly water-soluble compounds, assessment of formulations was conducted in vivo. The beagle dog was chosen as an animal model to select a suitable dosage form with good absorption characteristics. Dosage form performance was evaluated by comparing the following pharmacokinetic parameters: area under the plasma concentration vs time curve (AUC), peak plasma concentration (C_{max}) and the time required to reach this peak (T_{max}). Since no intravenous formulation was available, oral absorption from the different solid and liquid soft gelatin capsule formulations was evaluated relative to the absorption from a reference oral solution of MK-886 in PEG 400. Oral absorption of MK-886 in PEG 400 solutions was independent of solution concentration within the 15-fold range of concentration tested and was not affected by encapsulating the solutions in a soft gelatin shell. Food or co-administration of an alkalizing agent had no effect on the absorption of MK-886 from the soft gelatin capsules. Solid oral dosage forms, both capsules and tablets, had similar absorption characteristics to those of the PEG 400 solutions when the drug was granulated with an aqueous solution of polyvinylpyrrolidone (PVP), a hydrophilic polymer. PVP-granulated solid oral dosage forms had superior absorption characteristics when compared with a dry filled capsule of ungranulated drug.

Introduction

MK-886 (Fig. 1) is a leukotriene biosynthesis inhibitor with potential therapeutic benefits in the treatment of respiratory (Gillard et al.; 1989) and inflammatory bowel disease (Samuelsson, 1983; Ford-Hutchinson, 1985, 1989). The sodium salt monohydrate, used in these studies, had good physicochemical properties as a potential drug candidate. The compound was crystalline by X-ray powder diffractometry. The monohydrate was stable over a range of relative humidities from 12 to 76%. The drug substance was chemically stable with no loss of intact drug or observable degradation products by HPLC analysis following storage

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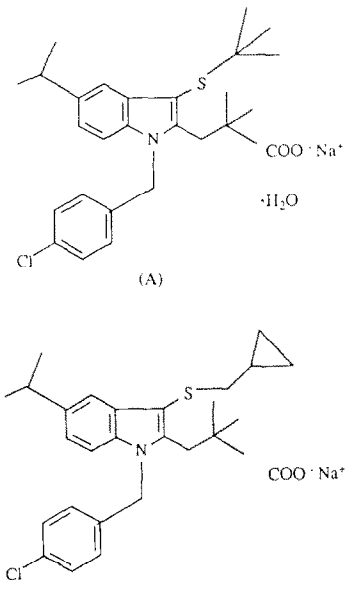

Fig. 1. The molecular structures of (a) MK- 886 (3-[1-(4-chlorobenzyl)-3-*t*-butylthio-5-isopropylindol-2-yl]-2,2-dimethylpropanic acid sodium salt monohydrate), and (b) the internal standard, L-669,572.

at 60°C/3 months or 50°C/6 months, respectively. The compound was not photosensitive and, when exposed to 4 klux fluorescent light at 30°C for 2 weeks, showed no indication of degradation by HPLC analysis. Because of the low equilibrium aqueous solubility of the compound (< 8 μg/ml), it was not possible to formulate a conventional intravenous dosage form. Maximizing oral bioavailability was an issue that needed to be addressed. This prompted the development and biological assessment of several experimental formulations.

In dosage form design it is often difficult to predict drug release and absorption characteristics in man prior to introduction of formulated material into the clinic. This is particularly the case for poorly water-soluble drugs where in vitro dissolution in aqueous media do not adequately simulate in vivo processes. With drug candidates where absorption processes are dissolution rate-limited, an animal model is probably most predictive of absorption in man assuming that: (1) plasma drug concentrations are a good monitor of the absorption process and (2) a rank order of bioavailability similar to that in man can be obtained from a comparison of plasma concentration vs time profiles for differently formulated materials. The beagle dog was chosen as the model because of the well-documented similarity of its gastrointestinal physiology to man (Hamilton, 1957; Anderson, 1970; Dressman, 1986; Lui et al., 1986) and the added flexibility of being able to administer formulations of the same size, shape and composition as those proposed for clinical use. From initial trials the plasma drug concentrations obtained indicated that the first assumption probably was reasonable. The second assumption cannot be tested prior to initiating the clinical program. Nevertheless, an initial clinical formulation with satisfactory drug release characteristics must be available to assess safety and tolerability of the compound in man. Therefore, as a primary objective of this study, drug absorption from experimental formulations was evaluated in the dog to ensure that a dosage form was selected with drug release characteristics comparable to an oral solution.

Materials and Methods

Dosage form preparation (see Table 1)

(1) PEG 400 solutions: PEG 400 solutions were prepared by dissolving 1 g of drug in 100 ml polyethylene glycol with constant stirring to obtain a final clear solution of 10 mg/ml. These solutions were administered by gavage. The weight of each animal was determined and the volume of solution administered was adjusted to an equivalent of 10 mg/kg.

(2) Soft gelatin capsules: Soft gelatin capsules containing drug dissolved in PEG 400 were prepared at concentrations of 75, 100 and 150 mg/ml. The 150 mg/ml formulation was filled into capsules using a rotary die process at R.P. Scherer Co., Windsor, Ontario, Canada. The remaining capsule strengths were filled extemporaneously into air-filled soft gelatin shells (R.P. Scherer Co.).

(3) Enteric-coated soft gelatin capsules: A batch of the 150 mg/ml capsules was also en-

teric-coated with a cellulose acetate phthalate film, plasticized with diethyl phthalate and applied from a methanol/acetone solution in a 4-inch diameter air suspension Wurster-type column.

(4) Methocel suspensions: Suspensions (1%) were prepared by adding a weighed amount of Methocel 400 cps (grade E) as powder to a measured volume of water with constant stirring. Drug was ground to a fine powder in a mortar. The (1%) Methocel suspension was added to the pulverized drug with trituration. The final concentration of drug in the suspension was 10 mg/ml. This suspension was dosed by gavage at 1 ml/kg to give a final drug dose of 10 mg/kg in experiments with both fed and fasted animals.

(5) Dry-filled capsules: Dry-filled capsules were prepared by blending drug and lactose (1:4) with 0.25% magnesium stearate (Witco Chemical, Montreal, Quebec, Canada) in a mortar. This powder blend was hand filled into hard gelatin capsules (Capsugel, Greenwood, SC).

(6) Dry-filled capsules-PVP granulation: Drug was granulated with 4% by wt polyvinylpyrrolidone, PVP, (BASF Corp., Parsippany, NJ). An aqueous PVP solution was added to drug and thoroughly mixed in a mortar. After drying and sizing, the granulation was blended with lactose and microcrystalline cellulose (FMC Corp., Philadelphia, PA) (1.04:1:1) with the addition of 1.9% croscarmellose sodium type A (FMC Corp.) and 0.25% magnesium stearate. Capsules were prepared from this granulation by hand filling into hard gelatin capsules.

(7) Compressed tablets-PVP granulation: Tablets also were prepared from this granulation on an eccentric press (Stokes model E) using standard concave tooling. The resulting tablets were

TABLE 1

C_{max}, T_{max}, bioavailability (AUC) and degree of variability (% C.V.) of MK-886 following administration of different dosage form (mean ± SD)

Dosage forms [a]	T_{max} (h)	C_{max} (μg/ml)	AUC (μg h ml^{-1})	% C.V.
(1) PEG 400 Solution, 10 mg/ml (used as a reference for comparison)	1.1 ± 0.6	3.51 ± 1.39	16.4 ± 4.6	28.0
(2) Soft gelatin capsules, 75 mg/ml	1.3 ± 0.5	4.47 ± 2.12	13.7 ± 3.1	22.6
Soft gelatin capsules, 100 mg/ml	1.0 ± 0	6.27 ± 3.00	18.5 ± 3.7	20.0
Soft gelatin capsules, 150 mg/ml	1.5 ± 0.6	2.89 ± 1.18	15.3 ± 3.5	22.9
Soft gelatin capsules, 150 mg/ml (administered with food)	1.8 + 0.5	3.14 ± 0.76	14.2 ± 3.7	26.1
Soft gelatin capsules, 150 mg/ml (administered with $NaHCO_3$)	1.3 ± 0.5	4.58 ± 2.71	17.4 + 7.8	44.8
(3) Enteric-coated soft gelatin capsules, 150 mg/ml	4.0 ± 0 [b]	1.45 ± 1.07 [b]	6.2 ± 3.5 [b]	56.5
(4) Methocel suspension, 10 mg/ml	0.3 ± 0.5	4.87 ± 2.49	16.8 ± 5.9	35.0
Methocel suspension, 10 mg/ml (administered with food)	3.0 ± 2.7	1.50 ± 1.01 [b]	8.5 ± 2.6 [b]	30.6
(5) Dry-filled capsule, 100 mg	1.5 ± 0.6	1.96 ± 1.06 [b]	8.7 ± 3.8 [b]	42.7
(6) PVP granulation (capsule), 100 mg	1.3 ± 0.5	4.50 ± 2.80	19.1 ± 6.5	34.0
(7) PVP granulation (tablet), 100 mg	2.0 ± 1.4	4.50 ± 4.68	17.9 ± 12.2	68.2

[a] In all cases, animals were dosed to give 10 mg/kg. Dose strengths in the formulations were prepare to correct for individual body weight of each animal. Dose strengths indicated in the table are approximate.

[b] Significantly differenent from PEG 400 reference solution ($p < 0.1$) using two-tailed unpaired Student's t-test.

film-coated with a hydroxypropyl methylcellulose/hydroxypropyl cellulose (Shinetsu, Tokyo, Japan) coating from an aqueous base.

Animal study protocol

Four female beagle dogs weighing approx. 10 kg each, were given a single oral dose of 10 mg/kg using the formulations described above and summarized in Table 1. The same four animals were used throughout the study. Each animal served as its own control to reduce experimental variability for comparison among formulations. Conscious animals were used throughout the study protocol. A washout period of at least 2 weeks preceded administration of each formulation. In the fasted studies, food was withheld for 16 h before and 6 h after dosing. Water was available ad libitum. For studies in the fed state, the animals were given 50 g of Purina Dog Chow approx. 30 min prior to administration of the dosage form. Serial blood samples (1 ml each) were taken from the jugular vein at 0, 0.25, 0.5, 1, 2, 4, 7, 10 and 24 h after drug administration. Plasma was immediately separated by centrifugation at 4000 rpm for 10 min and stored at −15°C until analysis.

Analytical materials

MK-886 and L-669,572, used as an internal standard (Fig. 1), were supplied by the Merck Frosst Centre for Therapeutic Research (Montreal, Quebec, Canada). HPLC grade acetonitrile was obtained from BDH Chemicals (Toronto, Ontario, Canada). All other reagents were of analytical grade and were used as received.

HPLC conditions

The HPLC was a Hewlett Packard 1090 Series Model M (Palo Alto, CA) equipped with a binary solvent delivery system, auto sampler and a photodiode array detector. The separation was carried out on a CSC Hypersil-ODS 3 μm 100 mm × 4.6 mm column (Chromatography Sciences Co. Inc., Saint-Laurent, Quebec, Canada) at 50°C using a mobile phase consisting of 0.1% aqueous phosphoric acid/acetonitrile (30:70, v/v) at a flow rate of 1 ml/min. Detection was carried out at 226 mm.

Standard solutions

Concentrated stock solutions of MK-886 (1 mg/ml) and L-669,572, the internal standard, (1 mg/ml) were prepared in acetonitrile. Standard solutions at various concentrations of MK-886 were prepared by diluting the stock solution in acetonitrile to obtain working standards ranging from 0.1 to 100 μg/ml of MK-886. A final internal standard concentration of 1 μg/ml was used in the study. All solutions were stored at room temperature. Standard solutions, shown to be stable for at least 3 months, were replaced monthly.

Plasma sample preparation

A 100 μl sample of plasma was added to 100 μl of acetonitrile containing the internal standard and vortexed for approx. 15 s then centrifuged at 14000 rpm for approx. 20 min. The supernatant was transferred to an HPLC microvial and 25 μl of the solution injected into the HPLC column. Plasma drug concentrations were calculated using peak area ratios of MK-886 and the internal standard.

HPLC method validation

Injection precision on 10 replicates with a 2.7% RSD was obtained for a 1 μg/ml solution of MK-886 and the internal standard.

A calibration curve for MK-886 was linear over the concentration range of 0.1–100 μg/ml with a regression coefficient of 0.999. The detection limit for the assay was estimated at 40 ng/ml at 2/1 signal-to-noise ratio.

The extraction efficiency of MK-886 from plasma was assessed by spiking plasma samples at six different levels over the concentration range of 0.1–10 μg/ml. The samples were analyzed in triplicate on three different days. Although the extraction efficiency of MK-886 from plasma was approx. 50% in the relevant concentration range, the absolute recovery for replicate extractions ($n = 9$) was reproducible with a coefficient of variation of 5.6% for a sample containing 1 μg/ml.

Sample stability was determined by processing and analyzing plasma samples: (i) immediately, (ii) after 24 h storage at room temperature, and (iii) after 24 h storage at −15°C. No degradation

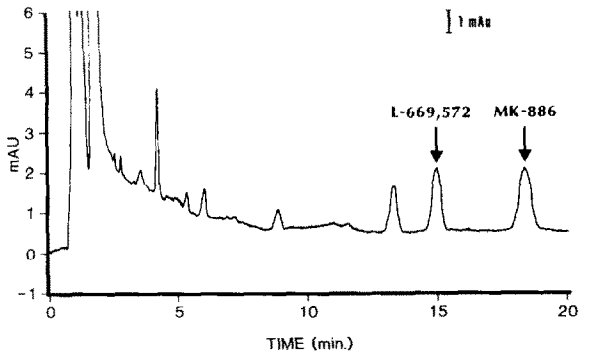

Fig. 2. An HPLC chromatogram of extracted dog plasma containing MK-886 (approx. 1 μg/ml) and internal standard obtained under conditions described in the text.

of MK-886 was observed. Fig. 2 shows a representative chromatogram obtained from a plasma sample following oral administration of MK-886.

Kinetic and statistical analysis

The area under the concentration vs time curve (AUC) was calculated, using the trapezoidal rule from 0 to the last point of detection which, generally, was 24 h. The peak plasma concentration of the drug (C_{max}) and the time to reach C_{max} (T_{max}) were obtained by visual inspection of the data. Statistical differences between the various parameters were evaluated using the two-tailed unpaired Student's *t*-test. Data are presented as mean standard deviation. An estimation of the relative variability for each parameter is defined by the coefficient of variation.

Results and Discussion

Oral absorption from PEG 400 solutions

The compound was soluble in PEG 400 (approx. 200 mg/ml). PEG 400 solutions administered orally appeared to give optimal absorption as would have been predicted if oral absorption of MK-886 was a dissolution rated-limited process. Because of a concern with nonlinear absorption kinetics, a dose proportionality study was carried out in PEG solutions to establish a suitable dose strength. An approximate linear correlation was obtained when the AUC was plotted against the MK-886 dose administered orally in the range of 5–20 mg/kg (Fig. 3). A 10 mg/kg dose was chosen to estimate the relative bioavailability of the various dosage forms investigated to ensure linear absorption kinetics. This formed the basis for comparisons with data documented in Table 1.

Soft gelatin capsule formulations

Drug plasma concentrations were significantly lowered when the compound was administered as a lactose/MK-886 powder blend in a dry-filled hard gelatin capsule (Table 1). As a consequence of this finding, soft gelatin capsules containing MK-886 in PEG 400 solutions were evaluated. The effect of the following factors on drug absorption were assessed as part of the formulation development process: (1) the effect of encapsulation; (2) the effect of drug concentration in PEG 400 solutions; (3) the effect of food; (4) the effect of co-administering an alkalizing agent; and (5) the effect of enteric coating the soft gelatin capsule.

The data in Table 1 compare the MK-886 plasma concentration vs time profiles for a series of encapsulated PEG 400 solutions at drug concentrations of 75, 100 and 150 mg/ml with that of the PEG 400 reference solution (10 mg/ml). From

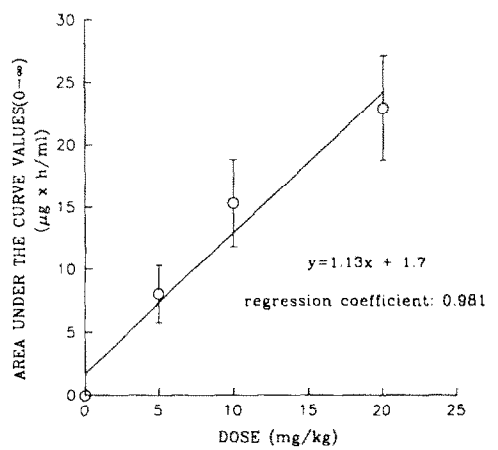

Fig. 3. AUC as a function of MK-886 dose. The dose was prepared as a PEG 400 solution. Data are displayed as mean ± S.D., $n = 4$. There was no significant departure from dose proportionality of AUC as detected by two-way ANOVA.

these data, it was concluded that drug absorption was not significantly affected by either encapsulating solutions as soft gelatin capsules or by a 15-fold change in the solution concentration.

From previous experiments the free acid was found to be less soluble in aqueous media. From the AUC data obtained, the free acid also was less bioavailable in the dog. As a result, it was postulated that administration of MK-886, the sodium salt of the free acid, may be better absorbed in the GI tract if neutralization of the conjugate base to free acid could be minimized by either co-administration of drug with food, co-administration with an alkalizing agent or administration as an enteric-coated formulation. In an effort to maximize drug absorption, these three alternatives were examined. When administered in the fed state or when co-administered with the alkalizing agent, $NaHCO_3$, absorption was not significantly affected (see Table 1). However, when soft gelatin capsules containing the drug solution were enteric-coated, total absorption decreased significantly. Substantial drug absorption in the upper GI tract may be one explanation for the observed results. However, because of the difficulty with obtaining samples at appropriate time intervals when investigating enteric-coated systems, critical samples may not have been taken and the AUC may have been underestimated.

Based on these observations, a soft gelatin capsule formulation containing drug in PEG 400 was selected as one potential candidate for drug development. The formulation had satisfactory absorption characteristics and good physical and chemical stability. Accelerated thermal stability studies with two batches of soft gelatin capsules containing 50 mg drug indicated no chemical degradation or change in the physical properties of the dosage form when samples were stressed at 50°C for 2 months or at 30°C for 12 months.

Solid oral dosage formulations

Efforts also were directed to the development of a solid oral dosage form with an absorption profile similar to that of the soft gelatin capsule. An initial comparison was carried out between the PEG 400 reference solution and a finely divided suspension of MK-886 in 1% Methocel. There appeared to be no significant difference in absorption between the PEG 400 solution and the Methocel suspension in the fasted state suggesting that a solid oral dosage form with a bioavailability profile similar to the soft gelatin capsule was possible if it could be designed with appropriate dissolution characteristics. However, the presence of food in the GI tract significantly decreased absorption when the compound was administered as a suspension.

A comparison of the AUC for solid oral dosage forms vs the suspension indicated that simple blending of the compound with excipients, the first prototype capsule formulation, reduced absorption. However, when the compound was granulated with PVP and filled into capsules or compressed into tablets absorption was equivalent to that obtained when the compound was administered as a solution or a suspension. It appeared that the wet granulation step with PVP, a hydrophilic polymer, may alter the wetting characteristics of the hydrophobic drug crystals and facilitate dissolution.

Table 1 summarizes the C_{max} and T_{max} values obtained for each administration of the oral dosage forms. It also includes the degree of variability in AUC values for different formulations. The tablet formulation showed the greatest variability which may be due to the disintegration, deaggregation, dissolution and transfer processes that must occur before the drug reaches the absorption site. Increased variability was also noted for MK-886 enteric coated capsules.

Conclusions

The dog model appeared to provide guidance for in vivo performance of different formulations. It was able to discriminate satisfactorily between formulations as a rank order of comparison. This was particularly of value when an in vitro dissolution test using an aqueous medium with satisfactory sink conditions and the potential to adequately reflect dissolution rate-limited absorption processes was not available. We have used this model to identify three formulations which closely approximate the optimal performance of a solu-

tion of MK-886 in PEG 400: a PEG 400 soft gelatin capsule, a PVP granulated hard gelatin capsule and a PVP granulated film-coated compressed tablet.

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