ELSEVIER

International Journal of Pharmaceutics 164 (1998) 37–44

international journal of pharmaceutics

Lymphatic uptake of MK-386, a sterol 5α-reductase inhibitor, from aqueous and lipid formulations

Gloria Y. Kwei [a,*], Linda B. Novak [b], Lisa H. Hettrick [b], Elisabeth R. Reiss [b], Eva K. Fong [b], Timothy V. Olah [a], Alice E. Loper [b]

[a] *Department of Drug Metabolism, Merck Research Laboratories, RY 80-A9, Rahway, NJ 07065, USA*
[b] *Department of Pharmaceutical Research and Development, Merck Research Laboratories, West Point, PA, USA*

Received 7 May 1997; received in revised form 16 October 1997; accepted 17 November 1997

Abstract

4,7-β-Dimethyl-4-aza-5α-cholestan-3-one (MK-386) is a specific inhibitor of type-1 5α-reductase, with over 10^4-fold greater solubility in lipid-type vehicles than in water. The absorption of orally administered MK-386 was investigated with three formulations to test the possibility that formulation can influence the absorption by altering the relative lymph/blood partitioning of MK-386. Drug concentrations in mesenteric lymph or in portal plasma were determined after administration of a 5 mg/kg dose in aqueous suspension, a mono-diglyceride/polysorbate 80 (MDG/PS80) vehicle or a soybean oil solution to conscious rats. Lymph volume collected over a 6 h period was in the order aqueous suspension > MDG/PS 80 > soybean oil-dosed animals. Total mass of MK-386 collected in lymph also followed this trend. MK-386 radioactivity equivalents from all formulations were as much as 80-fold greater in lymph compared with portal plasma, and radioactivity was exclusively associated with parent drug in lymph. Traces of metabolites, but virtually no MK-386, were detected in portal plasma. Distribution of MK-386 into lymph was insignificant after intravenous administration. The results demonstrate that systemic availability of orally administered MK-386 was due to lymphatic, not portal blood transport, and the rate of transport determined in this model was influenced by formulation.

Keywords: Lymphatic absorption; Lipid formulations; Sterol 5α-reductase inhibitors

1. Introduction

Administration of poorly water-soluble drugs in lipids or emulsions with a significant long chain fatty acid composition has been reported to improve oral bioavailability by stimulating

Abbreviations: MDG, mono-di-glycerides of medium chain fatty acids; SDS, sodium dodecyl sulfate; PEG 400, polyethylene glycol 400; BSA, bovine serum albumin, MTBE, methyl-*t*-butylether; TFA, trifluoroacetic acid.

* Corresponding author. Tel.: +1 732 5945302; fax: +1 732 5941416.

0378-5173/98/$19.00
PII S0378-5173(97)00392-X

lymphatic absorption (Palin and Wilson, 1984; De Nijs, 1987; Barnwell et al., 1992; Ichihashi et al., 1992a). While there are potential advantages in targeting lymphatic transport, such as bypassing hepatic first-pass extraction, the kinetics and dynamics of intestinal lymph flow relative to portal blood flow (1:500–1000 ratio) suggest that promoting lymphatic absorption may alter drug exposure in mesenteric lymphatics or influence systemic rate and/or extent of absorption. Lipid-containing formulations can also alter systemic distribution, as demonstrated recently with an oil-in-water emulsion formulation that increased accumulation of a lipid modulator drug in the adipose tissue of rats (Hauss et al., 1994).

It is desirable in early stages of drug evaluation to define a formulation that provides the maximal physico-chemical stability, greatest bioavailability and the least variability in animals. 4,7-β-Dimethyl-4-aza-5α-cholestan-3-one (MK-386) (Fig. 1) is a specific inhibitor of type-1, skin-specific 5α-reductase, the enzyme responsible for converting testosterone to dihydrotestosterone in skin and hair follicles (Ellsworth et al., 1996). The solubility of MK-386 is less than 1 μg/ml in water, greater than 140 mg/ml in mono-diglyceride/polysorbate 80 (MDG/PS80) solution and greater than 80 mg/ml in soybean oil and organic solvents such as acetone and ethanol. Not only the compound's lipophilicity, but structural and physico-chemical similarities between this 4-aza-cholestan analog of cholesterol and cholesterol suggested the possibility of intestinal absorption through the mesenteric lymphatics. The objective of this study was to determine the extent to which lymphatic transport contributed to orally administered MK-386, and whether lymph/blood distribution of MK-386 could be altered by three formulations differing in aqueous/lipid composition.

2. Materials and methods

2.1. Reagents

Unlabeled and tritiated [1,2-^{3}H]MK-386 (Fig. 1), molecular weight 415.7, and its cholanic acid metabolite, L-751,697, were synthesized in the Department of Medicinal Chemistry, Merck Research Laboratories. MK-386 has a relatively low melting point (crystalline material melts at about 60°C). [1α,2α-^{3}H]cholesterol was purchased from Amersham Life Science Products (Arlington Heights, IL). Unlabeled cholesterol, bovine serum albumin (BSA) (type V, fatty acid free) and polysorbate 80 (polyoxyethylene sorbitan monooleate) were products of Sigma (St. Louis, MO). Mono-diglycerides of capric and caprylic fatty acids (MDG, Imwitor 742™) was a product of Hüls America (Piscataway, NJ). Super-refined soybean oil was obtained from Croda (Parsippany, NJ).

2.2. Formulations

An ethanolic solution of labeled and unlabeled MK-386 was evaporated under N_2 and triturated in 0.5% aqueous methylcellulose (Methocel) with 0.02% sodium docecyl sulfate (SDS). Examination of this formulation under the light microscope showed non-birefringent droplets of approximately 10 μm, indicating that the compound did not recrystallize under these conditions. The two solution formulations were prepared by addition of super-refined soybean oil or 1/1 (v/v) MDG/PS80 to the evaporated material to obtain concentrations of 1 mg/ml for administration. Specific activity of [^{3}H]MK-386 in these formulations was approximately 100 μCi/mg.

A toluene/ethanol solution of labeled and unlabeled cholesterol was evaporated under N_2 and suspended in 1% BSA for a final concentration of

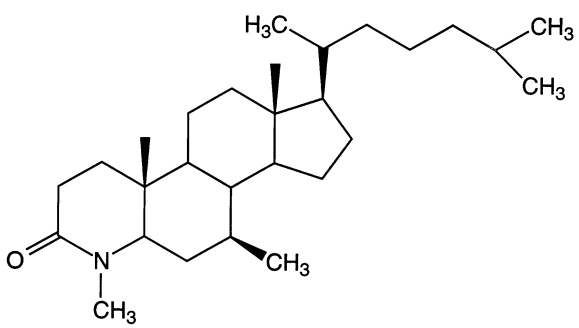

Fig. 1. Structure of MK-386.

100 mM, then 1 ml of this suspension (100 μmol or 38 mg) was administered to rats as a reference compound known to be transported into mesenteric lymphatics. All formulations were sonicated for 10 min before oral gavage.

2.3. In vivo studies

Animal studies were reviewed and approved by the Institutional Animal Care and Use Committee. Experiments were conducted in a mesenteric lymph collection model modified from Tso et al., 1981. The mesenteric lymph duct and the portal vein of male, Sprague–Dawley rats, 350–400 g (Charles River, MA) were catheterized with vinyl and silastic tubing, respectively. Animals were restrained in Bollman cages after surgery and allowed to recover overnight with 2 ml/h lactated Ringer's solution infusion into the mesenteric vein. Drug was administered to animals at 5 mg/kg, 5 ml/kg the following morning and only those with both patent lymph and portal vein cannulas were used. Ringer's solution was infused at 6 ml/h to replace fluids collected during study. At each time-point, 0.5 ml of blood was collected from the portal vein or the jugular vein and centrifuged immediately to obtain plasma. Lymph was collected continuously at 0.5-h intervals from the mesenteric cannula. An intravenous dose (5 mg/kg in ethanol/saline/polyethylene glycol 400 (PEG 400), 20/40/40, v/v/v) was administered to an additional group of animals via a jugular vein cannula.

2.4. Analytical

Total radioactivity in lymph and plasma after administration of MK-386 or cholesterol was determined by scintillation counting (LS-6000IC Liquid Scintillation Counter, Beckman Instruments, Fullerton, CA) of lymph or plasma samples, reported as μg equivalents/ml (MK-386) or μM equivalents (cholesterol).

HPLC analysis was conducted to determine the fraction of radioactivity which was associated with unmetabolized compound in lymph versus plasma. Extraction and chromatographic analysis were conducted by a procedure which separated MK-386 from the cholanic acid metabolite, L-751,697, and other polar metabolites. Plasma or lymph from individual animals dosed with the same formulation were pooled, since the objective of the analysis was to characterize any formulation effect on mechanism of uptake of MK-386 in rats. Standard curves were linear from 57 to 5700 ng-eq [^{3}H]MK-386 in 200 μl of lymph or plasma. Samples were acidified by addition of 100 μl of 1.0 N sulfuric acid, then extracted with 2 ml methyl-*t*-butylether (MTBE). The organic layer was removed after centrifugation and evaporated, then 1 ml of methanol and 2 ml of n-heptane were added to the remaining aqueous layer and reextracted. The n-heptane layer was transferred and combined with the residue from the MTBE extract and evaporated to dryness. To each sample, 350 ng of unlabeled MK-386 in ethanol was added, the sample then evaporated and reconstituted in the HPLC mobile phase. In selected standards, L-751,697 was added. Recovery of radioactivity in the combined organic extracts was greater than 99% from [^{3}H]MK-386 standards. Compared with standards, larger fractions of non-extractable radioactivity found in lymph (0.3–23%) and plasma (2–38%) were assumed to be due to highly polar metabolites.

Extracts were analyzed by HPLC (ISS-200 Autosampler with Series 410 LC Pump, Perkin Elmer, Norwalk, CT) with a UV detector (783A Programmable Absorbance Detector, Applied Biosystems, Foster City, CA) and radioactivity detector in series (Packard Radiomatic Flo-One Beta with Series A-5000 Data System, Packard Instruments, Meriden, CT). A C-8 reverse phase column, 4.6 × 100 mm (SynChropak RP-8, SynChrom, Lafayette, IN) was held at ambient temperature. The mobile phase was 90% solution A and 10% solution B, where solution A was 90% acetonitrile/10% water with 0.1% trifluoroacetic acid (TFA), and solution B was 90% water/10% methanol with 0.1% TFA. Flow rate was 1 ml/min. Wavelength for UV detection was 210 nm. The scintillation cocktail (Ultima-Flo, Packard Instruments, Meriden, CT) was pumped into the effluent from the UV detector at a rate of 3 ml/min. Radioactivity above background associated with unknown peaks was designated 'trace

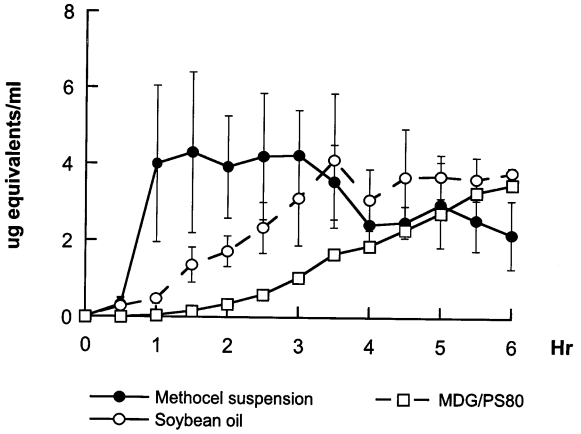

Fig. 2. Radioactivity in mesenteric lymph of rats after oral administration of [^{3}H]MK-386. MK-386 equivalents in mesenteric lymph of rats following oral administration of 5 mg/kg in Methocel suspension (solid circles), MDG/PS80 (open squares) or soybean oil solution (open circles). Results are the mean ± S.E.M. of four rats per group.

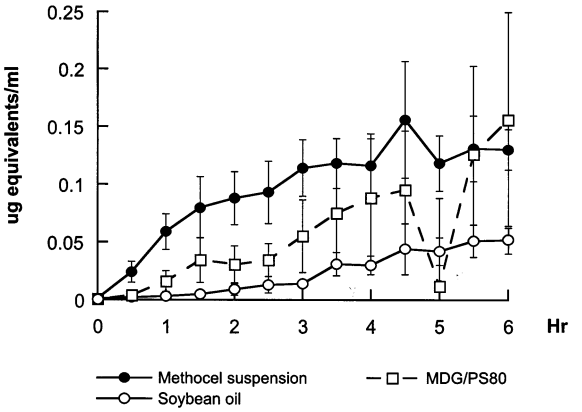

Fig. 3. Radioactivity in portal plasma of rats after oral administration of [^{3}H]MK-386. MK-386 equivalents in portal vein plasma of rats following oral administration of 5 mg/kg in Methocel suspension (solid circles), MDG/PS80 (open squares) or soybean oil solution (open circles). Results are the mean ± S.E.M. of four rats per group.

metabolite' if the area counts from the radiomonitor were below those of the lowest [^{3}H]MK-386 standard. Radioactivity associated with L-751,697 was established based on retention time with authentic standard.

3. Results and discussion

MK-386 radioactive equivalents in the mesenteric lymph and portal blood compartments after oral gavage of the Methocel suspension, MDG/PS80 solution and the soybean oil solution formulations are shown in Figs. 2 and 3, respectively. There were differences in the rate of appearance of radioactivity in lymph from these three formulations, likely due to delayed gastric emptying of the lipid MDG/PS80 and soybean oil formulations (Fig. 2). It is evident comparing the lymph versus portal plasma profile that radioactivity was overwhelmingly found in lymph rather than in portal plasma (Fig. 3) in all groups at all time points. The volume of lymph fluid collected over the 6 h period was greatest from the Methocel suspension-dosed rats (12.9 ± 1.73 ml versus 6.7 ± 1.6 ml in the MDG/PS80 group and 5.0 ± 0.8 ml in the soybean oil group); however, it was the least opaque and lipid-laden in appearance. The lymph was milky white approximately 1 h after administration of soybean oil and coincided with significant increases in radioactivity in lymph samples. Absorption of MK-386 from all formulations was prolonged and was not complete at the termination of the studies. Total mass of MK-386 equivalents collected in lymph over 6 h was 101.3 ± 16.0, 9.85 ± 3.2 and 2.12 ± 0.97 μg from the Methocel suspension, MDG/PS80 and soybean oil groups, respectively, representing 4.8 ± 0.78, 0.45 ± 0.14 and 0.10 ± 0.04% of the administered dose in these groups.

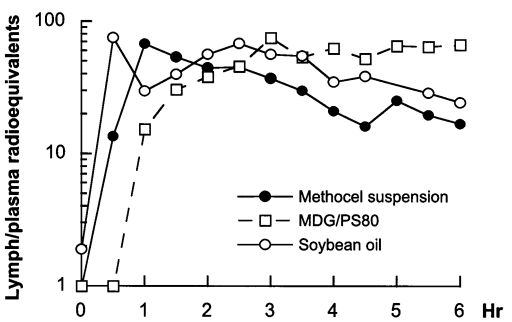

Fig. 4. Relative lymph/portal uptake of [^{3}H]MK-386 from aqueous or lipid formulations in rats.

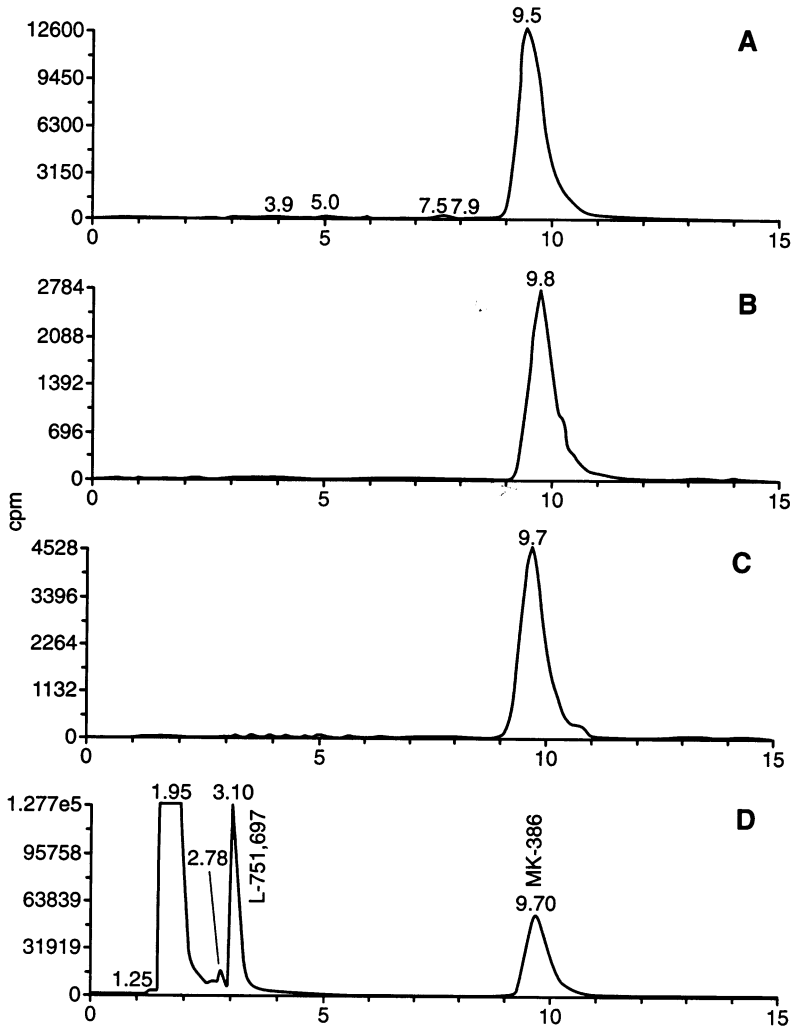

Fig. 5. Radiochromatograms of rat mesenteric lymph following oral administration of [^{3}H]MK-386. Representative radiochromatograms of lymph samples of rats given MK-386 in Methocel suspension (A), MDG/PS80 (B), or soybean oil (C). Panel D is the UV trace of standard compounds to indicate retention times.

A log ratio plot of the total radioactivity in lymph versus portal plasma indicated that drug preferred to partition into lymphatics, and the relative lymph/blood distribution of orally administered [3H]MK-386 was not affected by the formulation (Fig. 4). The results from this model showed differences in rate and extent of lymphatic absorption during the 6 h period after oral administration of these formulations. Previous pharmacokinetic studies in rats, however, indicated no effect in overall systemic bioavailability of MK-386 from aqueous crystalline suspension or lipid formulations. Only 6% of the oral dose was absorbed in 9 h, and the plasma profile indicated a late time to peak systemic plasma concentration (9–15 h), suggesting prolonged absorption in vivo (Merck, internal report). Since digestion and absorption of lipids and associated xenobiotics via the lymphatics occurs over longer time periods compared with absorption via the portal blood, the in vivo results are consistent with lymphatic absorption as the major route of uptake of MK-386.

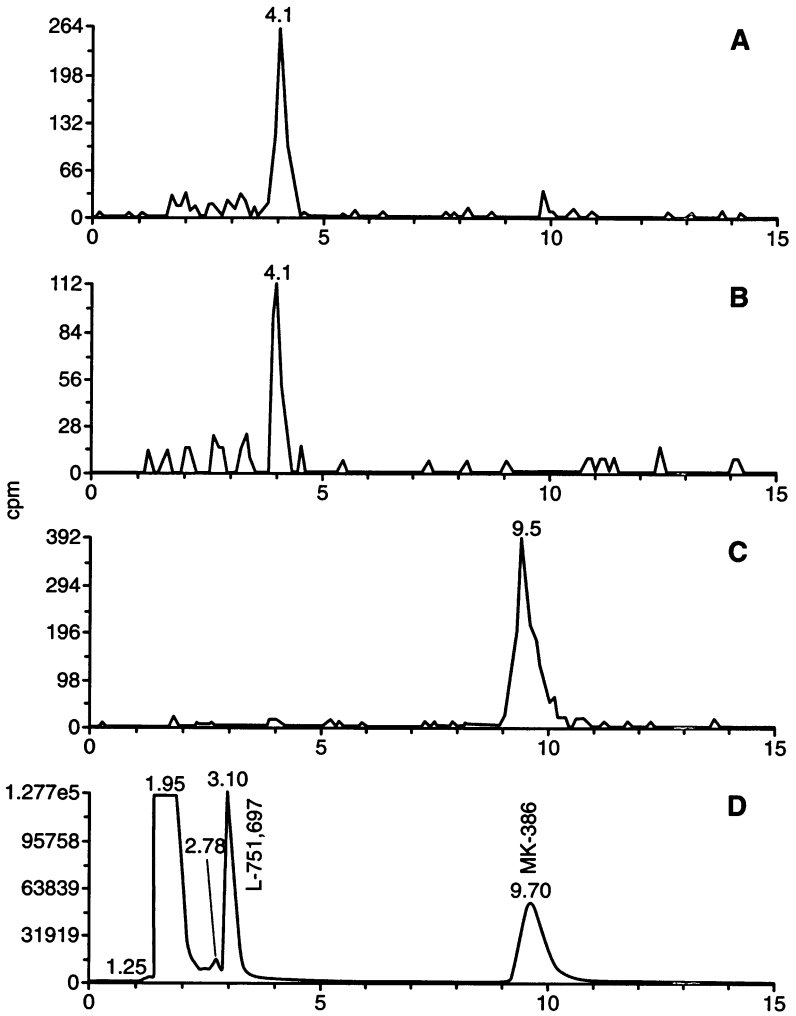

Fig. 6. Radiochromatograms of rat mesenteric plasma following oral administration of [^{3}H]MK-386. Representative chromatograms of portal plasma samples of rats given MK-396 in Methocel suspension (A), MDG/PS80 (B), or soybean oil (C). Panel D is the UV trace of standard compounds.

As a reference, $3.37 \pm 1.2\%$ (3.37 μmol or 1.3 mg) of an oral dose of cholesterol was recovered in lymph over 6 h when given as a suspension in 1% BSA. Mean lymph volume collection was 8.1 ± 0.9 ml in 6 h. Concentrations of cholesterol in lymph were 80–100-fold greater than that found in portal plasma (data not shown). Significant lymphatic uptake of cholesterol and MK-386 in aqueous suspension, in the absence of exogenously administered lipid, argues strongly that this is the normal route of absorption of these two extremely lipophilic compounds.

Representative radiochromatograms of lymph and plasma extracts from the oral MK-386 formulations are shown in three panels of Figs. 5 and 6, respectively. All radioactivity above background in lymph was MK-386 following oral administration for all formulations (Fig. 5). Extracts from pooled portal plasma from the Methocel suspension and MDG/PS80 formulations contained a small peak of radioactivity eluting at 4.1 min, which was completely resolved from L-751,697 under these chromatographic conditions, and no radioactivity was associated

with parent compound (Fig. 6, panels A and B) in these samples. Pooled portal plasma from the soybean oil group only showed traces of MK-386 (Fig. 6, panel C). MK-386 does not partition significantly into blood cells, thus systemic availability of MK-386 was not due to preferential distribution into portal blood. Since systemic concentrations of MK-386 (from previous in vivo studies) were greater than the trace concentrations detected in portal plasma, it is apparent that portal absorption could not contribute significantly to systemic exposure in spite of the much greater blood flow compared with lymph flow.

Following an intravenous dose of 5 mg/kg, $0.048 \pm 0.005\%$ of the dose was collected in mesenteric lymph, from a lymph output of 8.1 ± 0.9 ml. In contrast to the profile obtained after oral gavage, radioactivity was found predominantly in plasma (Fig. 7), with little distribution of radiolabel into mesenteric lymph. Most of the radioactivity in plasma and lymph eluted as MK-386, with small peaks of radioactivity eluting in the polar metabolite region of the chromatogram (not shown).

Collectively, these results showed that the normal route of uptake of orally administered MK-386 was via lymphatic transport, and relative chylo/portal partitioning in enterocytes was not altered by formulation. The aqueous/lipid composition of the formulation may, however, influence the rate of drug transport into the lymphatics. High solubility and retention of drugs in the lipid core of lipoproteins has been suggested as a prerequisite for significant lymph transport (Sieber et al., 1974; Charman and Stella, 1986; Ichihashi et al., 1992b), although other factors such as size, affinity for non-lipid components of lipoproteins or plasma, or partitioning with red blood cells could also influence lymph/blood distribution (Deak and Csaky, 1984; Porter and Charman, 1997). Similar to the fat-soluble nutrients cholesterol and sterol-derived vitamin D, the processing of lipophilic compounds within the enterocyte for secretion is complex and regulated, and is generally independent of the presentation to the enterocytes (i.e. cholesterol administered in aqueous suspension was still transported via lymphatics). Since drug transport into lymph is associated with intestinal lipoprotein formation and secretion, further studies examining the affinity of MK-386 and MK-386-like compounds with plasma lipoproteins would help in understanding the distribution of such compounds to target organs.

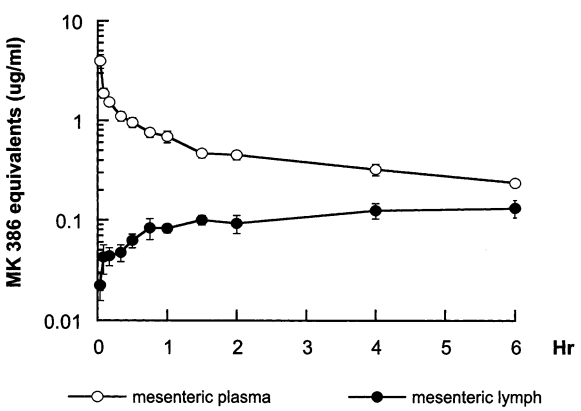

Fig. 7. Radioactivity in mesenteric lymph and portal plasma following intravenous administration of [^{3}H]MK-386. Radioactivity equivalents in mesenteric lymph (solid circles) or portal plasma (open circles) of rats given MK-386 by intravenous bolus administration.

References

Barnwell, S.G., Laudanski, T., Story, M.J., Mallinson, C.B., Harris, R.J., Cole, S.K., Keating, M., Atwood, D., 1992. Improved oral bioavailability of propranolol in healthy human volunteers using a liver bypass drug delivery system containing oleic acid. Int. J. Pharm. 88, 423–432.

Charman, W.N., Stella, V.J., 1986. Estimating the maximal potential for intestinal lymphatic transport of lipophilic drug molecules. Int. J. Pharm. 34, 175–178.

De Nijs, H., 1987. Targeting of drugs to the lymph. Acta Pharm. Technol. 33, 163–168.

Deak, S.T., Csaky, T.Z., 1984. Factors regulating the exchange of nutrients and drugs between lymph and blood in the small intestine. Microcirc. Endothel. Lymph. 1, 569–588.

Ellsworth, K., Azzolina, B., Baginsky, W., Bull, H., Chang, B., Cimis, G., Mitra, S., Toney, J., Bakshi, R.K., Rasmusson, G.R., Tolman, R.L., Harris, G.S., 1996. MK-386: a potent, selective inhibitor of the human type 1 5 alpha-reductase. J. Steroid Biochem. Mol. Biol. 58, 377–384.

Hauss, D.J., Mehta, S.C., Radebaugh, G.W., 1994. Targeted lymphatic transport and modified systemic distribution of CI-976, a lipophilic lipid-regulator drug, via a formulation approach. Int. J. Pharm. 108, 85–93.

Ichihashi, T., Kinoshita, H., Takagishi, Y., Yamada, H., 1992a. Effect of oily vehicles on absorption of mepitiostane by the lymphatic system in rats. J. Pharm. Pharmacol. 44, 560–564.

Ichihashi, T., Takagishi, Y., Yamada, H., 1992b. The factor determining intrinsic lymphatic partition rate of epitiostanol and mepitiostane. Pharm. Res. 9, 1617–1621.

Palin, K.J., Wilson, C.G., 1984. The effect of different oils on the absorption of probucol in the rat. J. Pharm. Pharmacol. 36, 641–643.

Porter, C.J.H., Charman, W.N., 1997. Uptake of drugs into the intestinal lymphatics after oral administration. Adv. Drug Deliv. Rev. 25, 71–89.

Sieber, S.M., Cohn, V.H., Wynn, W.T., 1974. The entry of foreign compounds into the thoracic duct lymph of the rat. Xenobiotica 4, 265–284.

Tso, P., Balint, J.A., Bishop, M.B., Rodgers, J.B., 1981. Acute inhibition of intestinal lipid transport by Pluronic L-81 in the rat. Am. J. Physiol. 241, G487–G497.

.