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Report

In Vivo Model for Ciclosporin Intestinal Absorption in Lipid Vehicles

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The influence of lipid vehicles on the intestinal absorption of Ciclosporin was studied *in vivo*. The model takes into account the effect of the intestinal lipid digestion on the absorption after intraduodenal administration of [^{3}H]Ciclosporin in olive oil or middle-chain triglyceride (MCT) to the bile duct-cannulated rat. Digested vehicles significantly promoted the absorption compared to nondigested vehicles. In the nondigested state, olive oil was a significantly better vehicle than MCT, whereas the difference between both lipids was only a trend in the digested state. Further studies with variants of this *in vivo* model should determine the influence of abnormalities of fat digestion and absorption on the pharmacokinetics and pharmacodynamics of a drug with a low therapeutical index.

KEY WORDS: Ciclosporin; intestinal absorption; lipid vehicle; *in vivo* model; lipid digestion.

INTRODUCTION

Ciclosporin (Sandimmune®), a recently introduced immunosuppressant, has a low and highly variable bioavailability (1). Studies in humans and animals have previously demonstrated that the first-pass effect by the liver is low (2,3), suggesting that the low and variable bioavailability of this drug is a consequence of its poor absorption from the gut into the portal blood. During preformulation work, some preliminary tests have shown that Ciclosporin has an immunosuppressive effect when given as a solution in olive oil [triglyceride (TG) of long-chain unsaturated fatty acids (FA)] (4). On the other hand, results obtained with Miglyol 812® [synthetic TG of middle-chain saturated FA (MCT)] were not satisfactory. Gastrointestinal lipid digestion is one of the main factors that influences the absorption of poorly water-soluble drugs administered as a lipid-containing dosage form (5). The most important step is enzymatic hydrolysis in the small intestine (6,7). This study involves the influence of intestinal lipid digestion on the absorption of Ciclosporin after intraduodenal administration of [^{3}H]Ciclosporin in olive oil or MCT to bile duct-cannulated rats.

MATERIALS AND METHODS

Materials

Ciclosporin (Sandoz, CH-Basel), olive oil Ph.H.VI, Miglyol 812 (MCT) B.P., pancreatin (42,400 FIP units/g) (Biochemie, A-Kundl), gum arabic Ph.H.VI, and sodium taurodeoxycholate were used as received. [^{3}H]Ciclosporin labeled in the aminobutyric acid moiety (577 μCi/mg) was synthesized by the Sandoz radiochemical laboratory. The radiochemical and chemical purity of the drug was checked by thin-layer chromatography (TLC) and amounted to at least 99%. All other reagents and chemicals were of analytical grade.

Drug Preparations

In vitro lipid digestion was run for 12 min in a pH stat (Metrohm, CH-Herisau) at pH 6.5, 37°C, under continuous agitation obtained by magnetic stirring. In the nondigested state, the same conditions were employed but without lipase. Composition of the administered dispersions was 170 μg [^{3}H]Ciclosporin + 8.3 μl olive oil or MCT + 1.7 mg gum arabic + 150 m*M* NaCl + 10 m*M* $CaCl_2$ + 8 m*M* sodium taurodeoxycholate + 2 m*M* Tris–maleate (pH 6.5) ± pancreatin (24 U lipase/ml) + 0.5 ml bidistilled water.

Procedure

Male Wistar rats (Ifacredo, F-Lyon) weighing about 300 g were used. The method for surgical preparation of bile duct-cannulated rats was that described by Weis and Dietschy (8). The rats were anesthetized by intraperitoneal injection of penthotal. 0.5 ml of the dispersions was administered intraduodenally by means of a polyethylene tube, just after the bile duct cannulation; for this purpose, the tube was introduced orally into the stomach and pushed forward under visual control into the upper part of the duodenum, where the dispersion was released. After the abdominal incision was closed, the rats were placed in a cage with free access to Ringer solution and food. Bile and urine were collected over 72 hr. The bile and urine samples were assayed for total radioactivity in a liquid scintillation spectrometer (Tri-Carb Model 3375, Packard Instruments) using Lumagel® as scintillator (Lumac AG, CH-Basel).

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Data Analysis

In order to make statistical comparisons between lipid dispersions, the data were tested for normal distribution and variance homogeneity. Depending on the outcome of that analysis, either a *t* test or a Wilcoxon test was used to test for equality between the mean values.

RESULTS

The cumulative excretions of the total radioactivity in bile and urine after administration of the different lipidic dispersions are shown in Table I. 32.1% was excreted through bile and urine over 72 hr after administration of nondigested olive oil, and 47.9% after administration of digested olive oil. This difference was highly significant. A similar difference was detected for MCT, where the excretion was increased from 23.6% (nondigested) to 39% (digested). Both urine and bile excretions followed the same tendency as the overall values. The digested state exhibits a larger intersubject variability than the nondigested state. For example, the variability coefficients for olive oil were 3.8 and 24% for nondigested and digested, respectively. Comparing olive oil and MCT, a significant difference was detected between the two lipid vehicles in the nondigested state. However, in the digested state no significant difference, only a trend, was detected ($P = 0.2$). As shown in Fig. 1, the profiles of the biliary excretion vs time curves of the total radioactivity are similar for olive oil and MCT, digested or nondigested; the excretion rate decreases with first-order kinetics and a half-life of about 8 hr.

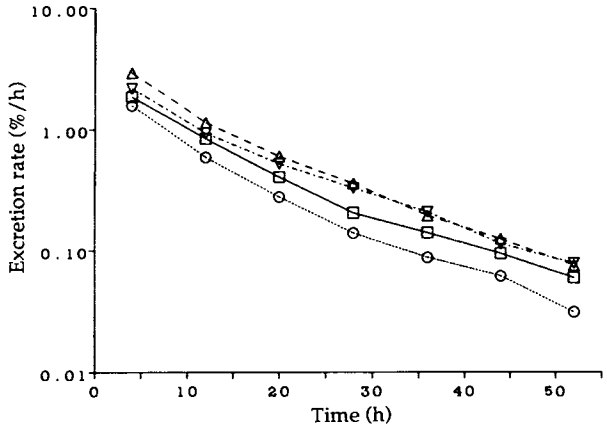

Fig. 1. Excretion kinetics of total radioactivity in bile after intraduodenal administration of 170 μg [^{3}H]Ciclosporin in 0.5 ml different lipid vehicles to bile duct-cannulated rats. Each point represents the mean of four to six rats. (□) Olive oil, nondigested; (△) olive oil, digested; (○) MCT, nondigested; (▽) MCT, digested.

DISCUSSION

The promoting effect of predigested lipid vehicles on the absorption of a poorly water-soluble drug compared to a nondigested vehicle confirms the results of Yamahira *et al.* (9), who used the antiinflammatory agent SL-512 as a model drug in the rat. However, with nondigested lipids, Ciclosporin is still absorbed. It cannot be ruled out that nondigested formulations may be partially digested in the small intestine of the bile duct-cannulated rat. On the one hand, lingual lipase [optimum range, pH 2–pH 8 (10)] may be active in the small intestine. On the other hand, small pancreatic ducts open sometimes directly into the duodenum and not into the hepatic duct as is the case for the main pancreatic duct (11). This may produce a partial lipid digestion. As a clinical consequence, one can consider that the bioavailability of Ciclosporin may decrease during the postoperative phase after a pancreatic transplantation because of the weak pancreatic secretions into the gut. However, as far as we are aware, such trends have not yet been described. This is, at least partly, a consequence of the large intra- and intersubject variabilities observed in the pharmacokinetics of Ciclosporin in such patients. In animals, the influence of pancreatectomy on blood and lymphatic absorption in dog is controversial (12,13).

By comparing olive oil with MCT, our findings confirm those of Palin *et al.* with DDT and Probucol (14,15). Considering the mechanisms that may differentiate the two lipids in relation to their effect on the absorption of Ciclosporin, one can exclude an influence on gastric emptying or on bile or pancreatic secretions (16), since they were administered intraduodenally to bile duct-cannulated rats. Differences in the digestibility of the two vehicles can be ruled out: on the contrary, we demonstrated in the preceding study (17) that after 12 min of lipid digestion, the percentage of saponification of olive oil was 41% and that of MCT 71%. An enhanced lymphatic absorption, which has been mentioned for DDT (14), cannot be applied to Ciclosporin since lymphatic absorption of this drug is minimal compared with blood absorption (3). Thus, the most probable explanation is the different effect of the two lipids on the permeability of the intestinal mucosa as demonstrated by Muranishi (18). Long-chain unsaturated fatty acids disorganize the membrane structure more than medium-chain saturated fatty

Table I. Cumulative Excretion of the Total Radioactivity over 72 hr in Bile, Urine, and Bile + Urine (Total) After Intraduodenal Administration of 170 μg [^{3}H]Ciclosporin in 0.5 ml Different Lipid Vehicles to the Bile Duct-Cannulated Rat[a]

	Olive oil				MCT					
	N	Bile	Urine	Total	*N*	Bile	Urine	Total	*R*	*P* (total)
Nondigested	4[b]	29.28 ± 0.87	2.79 ± 0.43	32.07 ± 0.62	6	22.28 ± 1.33	1.30 ± 0.39	23.57 ± 1.67	1.35	<0.05
Digested	6	43.71 ± 4.65	4.19 ± 0.52	47.90 ± 4.69	6	35.36 ± 4.85	3.61 ± 0.77	38.97 ± 5.44	1.23	NS (=0.2)
P		<0.05	<0.05	<0.05		<0.05	<0.05	<0.05		

[a] Percentages of the dose are given as means ± SE. *R* = fraction for total excretion of olive oil over MCT.
[b] Two rats died after 24 hr.

acids. Therefore, the membrane permeability to the drug should be higher with long-chain unsaturated fatty acids.

In vivo, where the actual situation is the digested state, differences between the two lipids were found only as a trend. The lack of significance is due partly to the high inter-subject variability and especially to the unavoidably small number of animals. This trend is consistent with preliminary tests during the galenical development of this drug (4). Furthermore, the fraction of olive oil over MCT for cumulative excretion of the total radioactivity in bile and urine is comparable in both nondigested and digested states (1.35 to 1.23).

In conclusion, further validation of the *in vivo* model we used is required to determine the differences between olive oil and MCT and between digested and nondigested mixtures. Furthermore, variants of this *in vivo* model could allow peroral and intraduodenal administration to be differentiated. This should determine the influence of abnormalities of fat digestion and absorption on the pharmacokinetics and pharmacodynamics of a drug with a low therapeutical index.

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