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Report

In Vitro 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 vitro*. The effect of the intestinal lipid digestion was considered on the partition of the drug from olive oil or middle-chain triglyceride (MCT) into phases of simulated intestinal content. The phases obtained after ultracentrifugation were analyzed for their Ciclosporin content and characterized for their lipid classes. For both lipid vehicles the presence of lipolysis products did not promote the partition of the drug into the aqueous phase. The absorption *in vivo* was not related to the drug amount in the aqueous phase and in the oil phase. Therefore, phase quantification *in vitro* cannot simulate the dynamics of *in vivo* absorption events following application of a poorly water-soluble drug in a lipid vehicle.

KEYWORDS: Ciclosporin; intestinal absorption; lipid vehicle; *in vitro* model; lipid digestion.

INTRODUCTION

The bioavailability of poorly water-soluble drugs can be improved by formulation with lipid vehicles (1). In addition, the absorption appears to be dependent on the nature of the lipid vehicle, as DDT and Probucol absorption is significantly greater following administration in arachis oil [triglyceride (TG) of long-chain unsaturated fatty acids (FA)] compared to Miglyol 812® [synthetic TG of middle-chain saturated FA (MCT)](2,3). Ciclosporin (Sandimmune®), a recently introduced immunosuppressant, is sparingly soluble in water (6 μg/g at 37°C) (4). During preformulation work, some preliminary tests have shown that Ciclosporin has an immunosuppressive effect when given as a solution in olive oil (5). On the other hand, results obtained with MCT were not satisfactory. Unsaturated fatty acids might also play an important role in absorption of this drug. Our objectives are (a) to determine the cause of this phenomenon and (b) to develop an *in vitro* model in order to differentiate lipid vehicles in their effect on *in vivo* absorption of poorly water-soluble drugs. The most important step in gastrointestinal lipid digestion is enzymatic hydrolysis in the small intestine (6,7). The presence of lipolysis products as monoglycerides and fatty acids incorporated in bile salt micelles should increase the solubility of poorly water-soluble drugs. This should then lead to the partition of these drugs in the aqueous phase of intestinal content and to the further enhancement of their absorption. Accordingly, the present study reports on an *in vitro* model that takes into account the influence of the intestinal lipid digestion on the partition of Ciclosporin from olive oil or MCT into various phases of simulated intestinal content.

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, sodium taurodeoxycholate, tributyrin puriss, and *p*-bromophenylboronic acid pur. 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.

In Vitro Lipid Digestion

Reactions were 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 a total volume of 9.0 ml containing final concentrations of 2 m*M* Tris–maleate, 8 m*M* sodium taurodeoxycholate, 150 m*M* NaCl, 10m*M* $CaCl_2$, 24 tributyrin units /ml of pancreatic lipase in the form of the clear supernatant of a 10% (w/v) centrifuged (Beckman L 8-70 centrifuge, rotor 70 ITi, 4°C, 6000 rpm, and 15 min.) pancreatic suspension [lipase activity was determined according to Patton *et al.* (8)], and 450 μl of an oil (olive oil or MCT)–gum arabic emulsion [oil and 10% gum arabic (1:2, by volume)] emulsified with a Polytron homogenizer [Type PT 20 (Kinematic Ltd., CH-Lucerne)]. The droplet size distribution of the emulsions was determined using an optical microscope, and the arithmetic mean diameter and the surface area were then calculated. A 2% (w/v) [^{3}H]Ciclosporin

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concentration in oil was used. In the control the same conditions were employed but without lipase.

Phase Quantification and Subsequent Analysis

Lipid digestion was terminated at 12 min with the addition of 25 μl of 0.01 m*M* *p*-bromophenylboronic acid (9) dissolved in methanol; the dispersions were then transferred into Polyalomer tubes (Beckman) and centrifuged (Beckman L 8-70 centrifuge, rotor 70 ITi, w^2t = 9.14 × 10^9 radians^2/sec, 37°C, slow accelerator selected). Portions of the separated phases were analyzed (a) in a high-performance liquid chromatographic (HPLC) system with a radioactivity detector (10) for labeling and Ciclosporin stability; (b) in a liquid scintillation spectrometer (Tri-Carb Model 3375, Packard Instruments) using Lumagel as scintillator (Lumac AG, CH-Basel) for total radioactivity; and (c) following extraction three times with diethyl ether after acidification with HCl, spotted on silica gel 60 F-254 TLC plate (Merck), developed with diethyl ether–NH_4OH (99 + 1), and visualized by iodine vapor for their respective lipid classes. For comparison purposes the solubility of Ciclosporin at 37°C was assayed after shaking for 24 hr in the same formulation (see *In Vitro* Lipid Digestion) but without oil.

RESULTS

The mean diameters of the emulsions were 3.1 and 3.6 μm, corresponding to a surface area of 14,163 and 13,816 cm^2/ml for olive oil and MCT, respectively. With respect to this parameter the two emulsions were considered to be alike. After lipid digestion a three-phase system was observed macroscopically with both lipid vehicles: an oil phase, an aqueous phase, and a pellet, which confirms the finding of Patton and Carey (11). In the control situation only one oil phase and one aqueous phase were observed. Figure 1 presents the results after TLC analysis of the different phases for their lipid classes. Without digestion taking place, fatty acids were detectable in the aqueous phase in the case of olive oil (acid number, 2.5) but not in MCT (acid number, 0.1). The lipid phases were composed of triglycerides. After digestion of olive oil a well-defined oil layer was observed. The lipid classes identified in the individual phases agreed with the finding of Patton (11), who identified the pellet to be the calcium soap of fatty acids. For MCT, groups of oily droplets sticking to the upper part of the tube were observed. The oil phase contains mostly triglycerides, the aqueous-phase diglycerides, monoglycerides and fatty acids, and the pellet fatty acids. The HPLC analysis of the different phases showed that within the experimental conditions of the *in vitro* lipid digestion, the Ciclosporin labeling is stable and no metabolism occurs (4). Thus, the total radioactivity measured in the phase quantification is associated with Ciclosporin. Table I presents the results of the quantitative determination of the drug in each phase (as a percentage of the total amount). The values in the different aqueous phases in the presence of oil were compared statistically with the reference value without oil. The percentage of Ciclosporin in the oil phase in the case of olive oil was not significantly different with or without digestion having taken place. On the other hand, the Ciclosporin content of the aqueous phase was significantly lower after digestion, the value obtained without digestion being comparable to the reference value without oil. In the case of MCT virtually all the Ciclosporin was in the oil phase without digestion (95%). After digestion about 50% of the drug was in the aqueous phase and in the pellet whose content in Ciclosporin was significantly higher than that for olive oil under the same conditions.

DISCUSSION

The physichochemical stability of Ciclosporin under the conditions occuring in the GI tract has been previously demonstrated (12). Our HPLC analysis further demonstrates the biological stability of this compound in simulated intestinal content. Consequently, an intraluminal instability and/or metabolism might not account for the low bioavailability of the drug (13). The analysis of lipid classes shows that in the case of olive oil the diglycerides were in the oil phase, and in the case of MCT in the aqueous phase. This phase distribution of diglycerides of long- and medium-chain fatty acids is

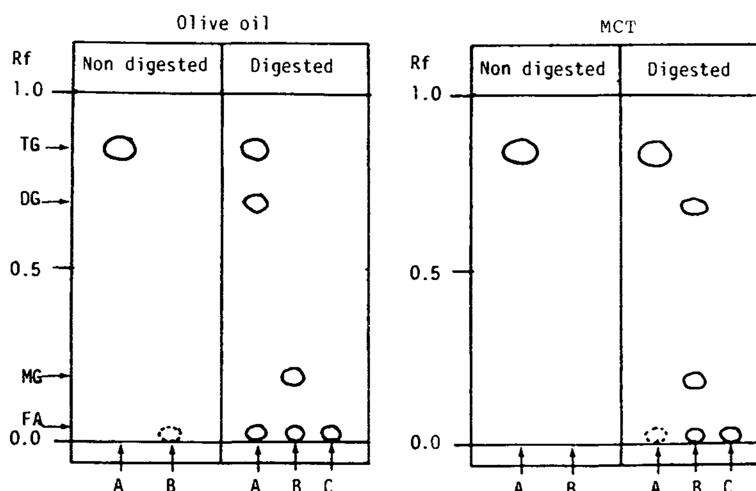

Fig. 1. Lipid classes of each phase separated after centrifugation: TLC chromatograms for olive oil and MCT. A, oil phase; B, aqueous phase; C, pellet.

Table I. Phase Distribution of Ciclosporin: Percentage Ciclosporin in Each Phase as the Mean Value ± SE ($N = 3$)

	Olive oil		MCT	
	Nondigested	Digested	Nondigested	Digested
Oil phase	81.80 ± 0.20	82.86 ± 0.71	95.54 ± 0.31	49.21 ± 2.09
Aqueous phase	18.20 ± 0.20	11.95 ± 0.53*	4.46 ± 0.31*	21.97 ± 1.26
Pellet	—	5.20 ± 0.82	—	28.53 ± 0.92

* Values in the aqueous phase significantly different ($P < 0.05$) from values measured without oil (19.60 ± 0.60).

a consequence of their different water solubility. The incorporation of lipolysis products, such as monoglycerides and fatty acids in bile salt micelles (giving so-called mixed micelles), is known to increase the partition of poorly water-soluble compounds into the water phase. This has been demonstrated for cholesterol and diazepam (14–16). Our results show, in fact, that for MCT the lipid digestion promotes the partition but does not significantly enhance the solubility of Ciclosporin in the water phase, whereas for olive oil the presence of the lipolysis products in the aqueous phase after digestion surprisingly decreases the distribution of Ciclosporin into this phase. Pellets contain Ciclosporin that has precipitated under the experimental conditions together with calcium soap. The percentage of drug associated with the calcium soap was significantly higher ($p < 0.001$) with MCT. This reflects the different reactivity (6) of the two substrates during lipid digestion. Under our experimental conditions 41% of olive oil and 71% of MCT were saponified; the amount of liberated fatty acids for MCT was 3.1 times higher than for olive oil. This explains the very small oil phase in the case of MCT.

In order to find an explanation of the surprisingly *a priori* finding of the phase distribution of the drug before and after digestion in the case of olive oil, the concentration in percentage (w/v) of Ciclosporin in the oil phase was calculated. The solubility of Ciclosporin in MCT is 10%. This value has not been achieved before or after digestion (1.95, 3.50%). On the other hand, for olive oil the solubility is 2%. This level is not achieved before saponification (1.67%). But the value of 2.9% after digestion is clearly higher. This is explainable only through the modification in lipid classes before and after digestion (Fig. 1). This has been demonstrated for Ciclosporin with Maisin®, an excipient of long-chain unsaturated diglycerides and monoglycerides of corn oil, compared to the original corn oil, where the solubility was 20 and 2%, respectively (17).

The working hypothesis of the development of our *in vitro model* was to correlate the amount of poorly water-soluble drug in the aqueous phase with its absorption *in vivo*. This is evidently not the case. Noguchi *et al.* (18), using the dye oil red XO, related the absorption of this model compound to the absorption of the oil phase. In fact, this does not explain our results, since the percentage of Ciclosporin in the oil phase was not related to the *in vivo* results (5). The significance of the fraction of the Ciclosporin in the calcium soap phase should be played down. *In vivo* the amount of calcium soap should be much lower than in *in vitro*, since lipid hydrolysis products are presumably absorbed quickly enough from the intestine to prevent insoluble calcium soap formation (19).

The reason for the unsuccessful predictability of the *in vivo* result through this *in vitro* model can be related to the static characteristics of the phase quantification. Figure 2 gives a schematic picture of the dynamic equilibrium of a lipophilic drug between the phases of a lipid digestion mixture. Absorption of the drug may occur from the oil, micellar, and monomer phases. In the case of our quantification the water phase represents the monomer and micellar phases. But recent research on the structure and composition of the aqueous phase during fat digestion (20) demonstrated that the physicochemical structure of lipid aggregates in the aqueous phase of intestinal content is a function of the relative concentrations of lipids and bile salts and will therefore change during the course of the digestion and absorption process. Figure 2 is therefore an oversimplification and the absorption may occur from several other phases.

In conclusion, the phase quantification as an instantenous picture cannot simulate the complexity and dynamics of *in vivo* absorption events following application of all lipophilic drugs in a lipid vehicle.

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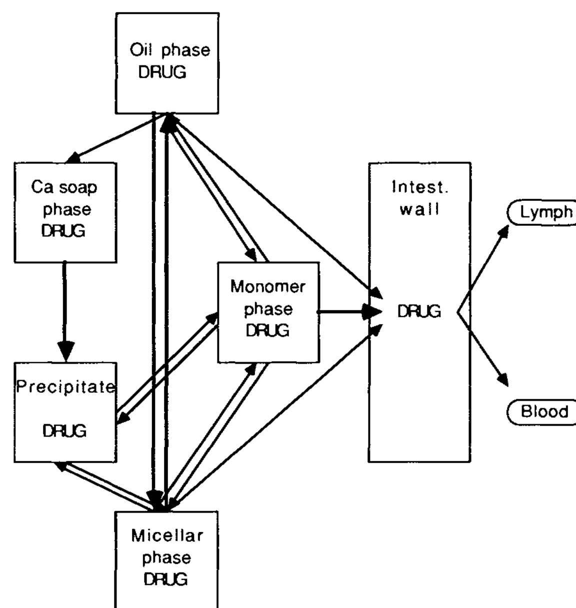

Fig. 2. Distribution of a lipophilic drug in the phases of a lipid digestion mixture during absorption.

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