Cyclosporin Absorption Is Impaired by the Fat Substitutes, Sucrose Polyester and Tricarballylate Triester, in the Rat

Kamel Benmoussa,[1,2] Alain Sabouraud,[1] Jean-Michel Scherrmann,[1] and Jean-Marie Bourre[1]

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The effect of non-absorbable fat substitutes (sucrose polyester (SPE) and tricarballylate triester (TCTE)) on cyclosporin A (CsA) intestinal absorption was studied in the rat using *in situ* perfusion and gastric intubation techniques. A first experiment using the recirculating intestinal perfusion model showed that emulsions of either 5% SPE or TCTE significantly reduced ($p < 0.0008$) CsA absorption, whereas no difference was found between results for saline and 5% olive oil emulsion. In single-pass intestinal perfusion experiments SPE dose-dependently inhibited CsA absorption at SPE concentrations of 0.31% ($p<0.0004$) and higher. Using gastric intubation, whole blood CsA concentrations significantly decreased when administered with SPE and TCTE in comparison with olive oil ($p < 0.04$). These results confirm that the CsA fraction dissolved in the undigested oil phase, constituted by the undigested and non-absorbed fat substitute, is unavailable for intestinal absorption.

KEY WORDS: fat substitutes; sucrose polyester; tricarballylate triester; cyclosporin; intestinal absorption; rat.

INTRODUCTION

Cyclosporin A (CsA) is a highly lipid-soluble cyclic endecapeptide (log P = 2.92 (1)) usually administered with an olive oil vehicle which significantly promotes CsA absorption when digested (2). When administered orally, CsA is mainly distributed in lipid droplets which are dispersed by bile salts to release CsA for absorption (2). The effect of concomitant food intake, and specially the fat quality and content of meals, on CsA bioavailability was studied to establish the mechanisms of CsA absorption and its clinical relevance (3).

Since nonabsorbable lipids provide a persistent oil phase in the lumen of the intestinal tract and fat soluble materials that are retained in this phase are unavailable for absorption (4), it was of interest to examine their effect on CsA absorption. The nonabsorbable fat substitutes, sucrose polyester (SPE) and tricarballylate triester (TCTE), were shown to reduce digitoxin absorption in the rat (5). The present study investigates the effect of these two fat substitutes on the intestinal absorption of CsA, with a focus on the dose-related effect of SPE. The animal models used are the *in situ* perfused rat intestine technique and *in vivo* gastric intubation.

[1] INSERM U26, Hôpital Fernand Widal, 200 rue du Faubourg Saint-Denis, 75475 Paris Cedex 10, France.

[2] To whom correspondence should be addressed.

MATERIALS AND METHODS

Materials

Cyclosporin A was a gift from Laboratoires Sandoz (Rueil Malmaison, France). Cyclo-Trac(R) SP-whole blood radioimmunoassay for cyclosporin was from Incstar Corporation (Stillwater, MN, USA). Polyethylene glycol (PEG 4000) was from Merck Clevenot (France). Radiolabelled Cyclosporin ([*mebmt*-β-^{3}H] Cyclosporin A, specific activity: 8.8 Ci/mmol; purity: 98.2%) was from Amersham (Les Ulis, France) and polyethylene glycol ([1,2^{14}C]PEG, specific activity: 13.0 mCi/g) was obtained from New England Nuclear-Du pont de Nemours (Paris, France). Olive oil was of the cold first-press variety (Sidi-Aich, Algeria). Sucrose polyester consisted of hepta- and octaesters of sucrose prepared with sunflower oil (Lipochim, Marseille, France). Tricarballylate triester was prepared with tricarballylic acid and sunflower fatty alcohols (Lipochim, Marseille, France). Egg lecithin (lipoid E80) was from Seppic (Paris, France). Lipase and sodium taurocholate were from Sigma (Saint-Quentin-Fallavier, France). All other chemicals were of analytical grade.

Emulsion Preparation

First experiment. Four perfusion solutions were prepared in (i) physiological saline : 0.9% NaCl, (ii) 5% (v/v) olive oil, (iii) 5% SPE (v/v) and (iv) 5% (v/v) TCTE.

An aqueous solution was prepared by adding CsA (50 μg/ml), [^{3}H]CsA (1.17 mCi/ml), PEG (5 mg/ml), [^{14}C]PEG (22.5 μCi/ml), lipase (0.6%, w/v), and taurocholic acid (10 mM) to physiological saline. Each oil was mixed with the aqueous solution and controlled as previously described (5).

Second experiment. Seven perfusion solutions with the same concentrations of CsA, PEG, lipase, and taurocholate as above were prepared with 0.15, 0.31, 0.62, 1.25, 2.5, 5, and 10 % (v/v) SPE.

Third experiment. Three CsA solutions (3.5 mg/ml) were prepared in emulsion with 5% olive oil, SPE and TCTE, without addition of PEG, lipase, or taurocholate.

Partition Coefficient Determination

The CsA partition coefficient between the lipid (olive oil, SPE or TCTE) and aqueous (physiological saline) phases was determined as previously described (5).

Cyclosporin Absorption

Sprague Dawley male rats (Iffa Credo, Lyon, France) weighing 280-300 g were fasted with free access to water 16 to 20 hours before experimentation.

In situ intestinal perfusion. Rats were anesthetized with an i.p. injection of sodium pentobarbital (55 mg/kg). All experiments were performed between 10 a.m. and 6 p.m.

For the first experiment, the *in situ* perfused rat intestine technique was as previously described (5). The selected duodeno-jejunal segment was 38-42 cm in length, and is the segment in which maximum absorption of CsA occurs (2).

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The outflow cannula ensured perfusate recirculation in the reservoir: the recirculating model allowed us to determine the kinetics of CsA by measuring levels in the reservoir (PEG was used as a non-absorbable marker to verify any transfer of water into or out of the intestinal lumen (6)). Fifty μL samples (in duplicate) were drawn from the reservoir after 0, 10, 20, 30, 45, 60, 90, and 120 minutes of perfusion. After 120 minutes, the intestinal segment length was measured. Each group consisted of 6 rats.

In the second experiment, groups of 6 rats were prepared as above except that the outflow cannula was positioned to drain into collecting tubes. The perfusion time was divided into 3 periods of 10 min for each SPE concentration. The average residence time of the perfusion solution in the segment was 19 min. Fifty μL samples (in duplicate) were drawn in the tubes corresponding to the 2 latter perfusion periods for each concentration.

Gastric intubation. After an overnight fast, the caudal artery of ether-anaesthetized rats was cannulated with polyethylene tubing (i.d. 0.38 mm, Biotrol, Paris, France) for blood sampling, and thereafter the rat was given 10 mg/kg (≈ 1 mL) of CsA in emulsions containing 5% olive oil, SPE, or TCTE, administered by gastric intubation. Thereafter, the rats were kept in restraining cages with free access to food and water. Blood samples (0.4 mL) were collected at 0.5, 1, 2, 4, 6, 8, 10 and 24 h after CsA administration in tubes containing one drop of half-diluted edetic acid (Sigma, France) as an anticoagulant agent. After each blood sampling, an equivalent volume of saline was administered, and the caudal cannula was filled with saline. Samples were frozen until analysis. Each group consisted of 5 rats.

Analytical Methods

In situ intestinal perfusion. Samples were mixed with 3 ml Pico-Fluor 40(R) scintillation liquid (Packard, Rungis, France) in a minivial. Radioactivity was measured by double-label ($^{14}C/^{3}H$) liquid scintillation counting with a beta-counter (Tri-Carb 4530, Packard, les Ulis, France).

Gastric intubation. CsA concentrations in whole blood were determined by specific radioimmunoassay (Cyclo-Trac(R)) with a monoclonal antibody and ^{125}I-labelled CsA (7). The quality control showed cross-reactivity of CsA metabolites less than 1.07%. Intraassay coefficient of variation was 3.1-3.2% for high (625 ng/mL) and medium (186 ng/mL) concentrations and 10.7% at 46 ng/mL. Interassay coefficient of variation was 4.6-10%. Limit of quantification was 8.7 ng/mL. Radioactivity was measured with an auto Gamma counter equiped with a radioimmunoassay software (Minaxi-Gamma 5000, Packard, Les Ulis, France).

Control of CsA stability and adsorption on materials. CsA radioactivity was measured before and after the emulsion preparation. We verified that the total initial radioactivity was recovered following the counting of oil and aqueous phases in the partition coefficient determination. Perfusate was circulated in a closed-loop of catheters, and radioactivity was measured in the reservoir at the beginning and the ending (120 min) of the perfusion. Stability of CsA in perfusate solutions was checked by thin-layer chromatography on Silica Gel (Merck, France) with cyclohexan-aceton (1:1) as solvent system.

Data Analysis

In situ intestinal perfusion. ^{3}H radioactivity results were converted to CsA concentrations and corrected for water movements measured by [^{14}C]PEG as previously described (5). With the recirculating model, the percentage of initial concentration remaining in the reservoir was plotted against time. Pharmacokinetic parameters of CsA elimination from the reservoir were calculated as previously described (5).

With the single-pass model, the extraction coefficient E was calculated using the equation: $E = (C_{in}-C_{out})/C_{in}/L$, where C_{in} and C_{out} are the inflow and the outflow concentrations, respectively. The percentage of the control (0% SPE) extraction coefficient was plotted against SPE concentrations in a sigmoidal model using GraphPAD Inplot 4.03 software (GraphPAD software, San Diego, CA, USA).

Gastric intubation. The pharmacokinetic parameters were calculated using the Siphar(R) program. Experimental area under the curve (AUC_{0-24}) was determined by the trapezoidal method. Maximum CsA concentration (C_{max}) and the time to achieve C_{max} (i.e T_{max}) were observed.

Results are expressed as mean ± SEM. Statistical analysis was performed using analysis of variance, and significance was set at $p < 0.05$.

RESULTS

The CsA partition coefficient with olive oil was 2.5 ± 0.6 (mean ± SEM, n = 3) and did not differ significantly from TCTE (3.4 ± 0.26, p = 0.07), or SPE (1.5 ± 0.15, p = 0.1). During *in situ* intestinal perfusion, [^{14}C]PEG measurements were constant (PEG ratio equal to 1.006 ± 0.003 (mean ± SEM, n = 168)), indicating that water transfers were minor. CsA adsorption to apparatus was less than 2 %. Consequently, the decrease in ^{3}H radioactivity in the reservoir was considered to be mainly due to intestinal absorption of CsA plus its intestinal metabolites and appeared as a first-order process (Fig. 1). After 120 minutes of perfusion, the 5% olive

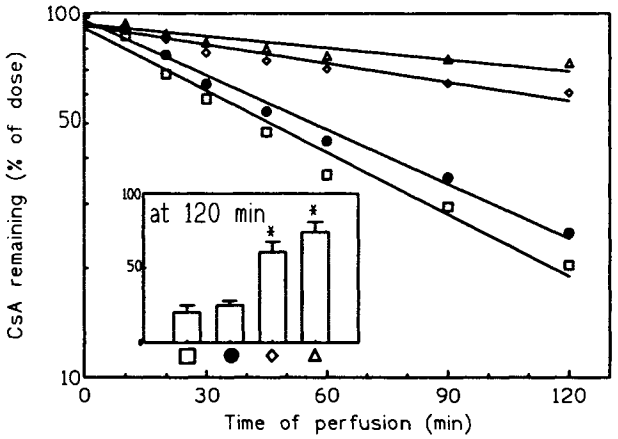

Figure 1: Experiment 1. Percentage of [^{3}H]CsA remaining in the reservoir during recirculating perfusion in saline (●), or in emulsion with either olive oil (□), tricarballylate triester TCTE (◇), or sucrose polyester SPE (△). Results are expressed as mean for 6 rats. The histograms show the results at 120 minutes. Vertical lines show SEM. Statistical key: *: $p < 0.05$ compared to saline- and olive oil-treated groups.

Table I. Pharmacokinetic Parameters of [^{3}H]CsA after Recirculating Perfusion in Saline, or in Emulsion with Olive Oil, SPE, or TCTE. The Results are Expressed as mean ± SEM (n = 6)

Parameters	Saline	Olive oil	TCTE	SPE
Remaining (% of dose)	25 ± 3	20 ± 5	61 ± 7*	73 ± 7.4*
t½ (min)	62 ± 6	55 ± 9	230 ± 70*	640 ± 300*
CL (μl · min^{-1} · cm^{-1})	6.4 ± 0.7	7.3 ± 1	2.1 ± 0.4*	1.4 ± 0.4*

* $p < 0.05$ compared to saline- and olive oil-treated groups.

oil emulsion-treated group showed a higher CsA absorption but not significantly different from that in the saline-treated group ($p=0.42$). With 5% SPE emulsion, CsA absorption was reduced by 65 % ($p<0.0001$) compared to the saline-treated group. On the other hand, 5% TCTE emulsion reduced CsA absorption by 47 % ($p=0.0008$) compared to the saline-treated group. This result did not differ significantly from that of the SPE-treated group ($p=0.22$). Half-life and clearance were also reduced in the same way (table 1).

Figure 2 shows the SPE-dose effect on CsA absorption. CsA absorption decreased when the amount of SPE in the perfusate increased from 0 to 10% according to a Hill function with a slope coefficient equal to -1.97. A significant reduction in CsA absorption was attained when 0.31% of SPE was added ($p < 0.0004$). The ID_{50} for SPE was 0.36%.

Figure 3 shows the blood concentration-time curves of CsA after gastric intubation. The pharmacokinetic parameters of CsA in blood are listed in table 2 and show a significant reduction in CsA peak concentration (C_{max}) and AUC_{0-24} by both SPE ($p<0.0037$) and TCTE ($p<0.04$) compared to olive oil. The extent of the reduction of the C_{max} is 61.5% and 34.3% for SPE and TCTE, respectively.

DISCUSSION

The *in situ* perfusion model and the blood pharmacokinetic study demonstrate an inhibitory effect of SPE and TCTE on CsA absorption. The both models, similar extents in reduction of CsA absorption are observed. The decrease of ^{3}H radioactivity accounts for unchanged CsA and intestinal metabolites since the major metabolic pathway of CsA in the rat (8) do not affect the site of radio-labelling (hydrogen of β carbon of amino acid 1). This inhibition can be attributed to the resistance of SPE and TCTE to hydrolysis and their subsequent non-absorption (4). Therefore, the CsA dissolved in the nondigested oil phase of the fat substitutes is not available for absorption. The SPE-dose effect on CsA absorption follows a decreasing Hill function with ID_{50} at 0.36% SPE. Mattson and coworkers (9) showed a linear decrease in cholesterol absorption when SPE replaced 0 to 50% of the total dietary fat, the overall content of SPE varying from 0 to 15% of the emulsion administered to rats. Reymond and coworkers (2) found a highly significant reduction of biliary and urinary excretion of CsA administered intraduodenally to rats with non-digested olive oil compared to the digested form, possibly because of the non-digestion of the fatty vehicle.

Wile the mechanism of absorption of CsA remains unclear (10, 11), one pathway is thought to be via fat absorption (3). Lipid-lowering therapies generally involve agents interfering with absorption of fat soluble materials via complexation of bile salts or sequestration of the fatty materials. Cholestyramine is generally used in lipid-lowering therapy (12) and SPE is proposed as an alternative lipid-lowering agent without sacrificing the organoleptic properties of fat (13). TCTE is also a candidate low calorie replacement for edible fats in food use (14). Keogh and coworkers (15) found that cholestyramine did not alter CsA absorption in cardiac transplant recipients. On the other hand, Kahan (16) quoted an unpublished report of abolition of CsA immunosuppres-

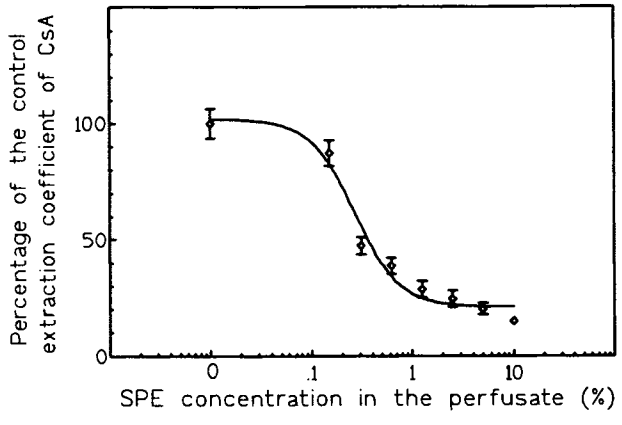

Figure 2: Experiment 2. Dose-related effect of SPE on [^{3}H]CsA concentration after single-pass perfusion. The results are expressed as percentage of control (0% SPE) extraction coefficient. Each point represents the mean for 6 rats with SEM bars. The curve followed a Hill function with a slope coefficient equal to -1.97.

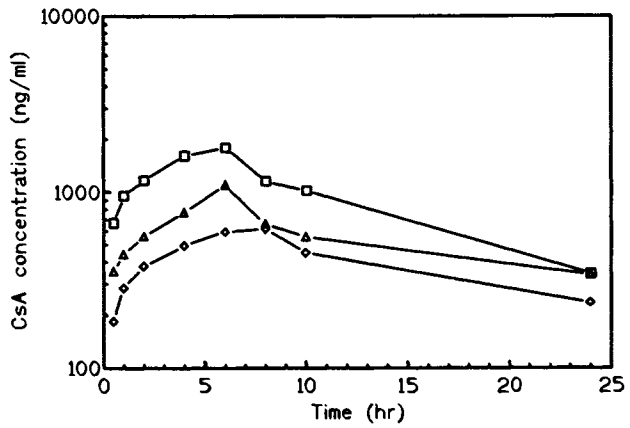

Figure 3: Experiment 3. Blood concentrations of CsA after oral administration in 5% olive oil (□), SPE 9 (◇), or TCTE (△) emulsions. Points represent mean for 5 rats.

Table II. Pharmacokinetic Parameters of CsA after Oral Administration in Emulsion with Olive Oil, SPE, or TCTE. The Results are Expressed as mean ± SEM (n = 5)

Parameters	Olive oil	TCTE	SPE
AUC_{0-24} (mg · hr^{-1} · liter^{-1})	23 ± 3.1	13 ± 3*	9.9 ± 0.5*
C_{max} (mg/liter)	1.8 ± 0.2	1.2 ± 0.1*	0.7 ± 0.1*
T_{max} (hr)	5.6 ± 0.4	5.6 ± 0.4	7.2 ± 0.5*

* $p < 0.05$ compared to olive oil-treated group.

sive activity after concomitant therapy with cholestyramine. The difference noted here can be related to the fact that cholestyramine interferes with bile salt solubilization, and therefore could indirectly influence CsA absorption. On the other hand, the fat substitutes interfere directly with CsA by sequestrating it in the undigested unabsorbed oil phase. Keogh and coworkers (17) reported that 33 to 50% of cardiac transplant patients demonstrate hypercholesterolaemia requiring lipid-lowering therapy. Since CsA is preferentially transported in the plasma on lipoproteins (18) and serum CsA levels are related to lipid levels (19), agents altering the lipid profile would be expected to affect the carriage of CsA.

In conclusion, this study demonstrates that the nonabsorbable fat-like materials, sucrose polyester and tricarballylate triester, reduce absorption and bioavailability of CsA, and therefore the digestibility of the oily vehicle is an important factor in the absorption of CsA.

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