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Report

Enhanced Intestinal Absorption of Cyclosporine in Rats Through the Reduction of Emulsion Droplet Size

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The intestinal absorption of cyclosporine was measured *in situ* in rats using an olive oil emulsion prepared by either stirring or homogenization. The surface area of the homogenized dosage form was twice that of the stirred dosage form. The apparent permeability of cyclosporine from the homogenized emulsion was about twice that of the emulsion prepared by stirring. The examination of absorption in different intestinal segment lengths suggested the presence of an "absorption window." The absorption of cyclosporine appeared to be concentration independent and, therefore, non-carrier mediated. The dependence of absorption upon the intestinal perfusion rate suggested that the stagnant aqueous layer is the rate-limiting barrier in cyclosporine absorption. These results indicate that the bioavailability of cyclosporine administered in an emulsion can possibly be increased by enhancing its rate of absorption through the reduction of droplet size.

KEY WORDS: cyclosporine; intestinal absorption; emulsion dosage form.

INTRODUCTION

Cyclosporine is a potent immunosuppressant that lacks myelotoxicity (1). It is an important component of the recently improved survival time in organ-transplanted patients. Cyclosporine is available in both oral and intravenous dosage forms. Its oral absorption is slow and incomplete (2,3), leading to a poor and erratic bioavailability. The current oral dosage form vehicle consists of olive oil, alcohol, and polyoxyethylated oleic glycerides (Labrafil) (40:18:42) with a cyclosporine concentration of 100 mg/ml (3). Prior to administration this form of the drug (Sandimmune) is stirred with several milliliters of a noncarbonated liquid beverage, which forms a coarse dispersion.

Emulsion droplet size can be a determining factor in the rate and extent of drug absorption (4,5). This may be explained by the fact that pancreatic lipase acts only at the interface of the oil droplets. The smaller the oil droplets, the more rapid the breakdown of the triglycerides that carry the drug and the more rapidly the drug is released from the vehicle. Since the solubility of the drug in the oil vehicle may be 1000 to 10,000 times greater than its water solubility, the partitioning of drug into the surrounding aqueous solution can be considered negligible. This has been shown by administering drugs in nonabsorbable vehicles and finding poor drug absorption characteristics (6,7). Therefore, the hydrolysis of the oil by lipase is an important factor in drug release from the vehicle. Lipid digestion is also important in the formation of micelles, which aid in solubilizing the drug in the aqueous environment of the intestinal lumen. The more rapid the hydrolysis of triglyceride, the more rapid and the greater the quantity of micelles that are formed. The fatty acid and monoglyceride components of micelles are thought to aid in the transport of fat-soluble drugs into the microvillar spaces, which greatly enhances the surface area of absorption (8).

The purpose of this study was to investigate the effect that dispersion droplet size might have on cyclosporine absorption. The results provide further insight as to why cyclosporine is poorly absorbed.

MATERIALS AND METHODS

Preparation of Rats

Male Sprague-Dawley rats (270–350 g) were used in these studies. Animals were anesthetized with 1.5 mg urethane/kg body weight. Each animal was fasted overnight, then anesthetized, and the peritoneal cavity was opened by a midline incision. A segment of the small intestine was cannulated proximally and distally so that perfusate entering the proximal cannula traversed the intestinal segment and left via the distal cannula. The proximal and distal cannulas were polyethylene tubing, PE 160 and PE 260, respectively, that had the luminal end flared by heating. The cannula was tied in place with a loop of silk suture placed tightly about the intestine, forming a seal that prevents perfusate leaking from the system. The distal cannula had a relatively large internal diameter to allow a relatively high rate of perfusion with minimal pressure in the lumen. The cannulated intestinal segment was arranged in the peritoneal cavity so that it was

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not kinked or twisted and the abdominal incision was closed with wound clips. All segments that were infused started 3 cm distal to the stomach and perfused intestinal lengths of either 8, 16, or 32 cm distal from that point. The bile duct was ligated so that any drug eliminated in the bile could not return via the bile to the perfusate.

After cannulation of the intestine, the anesthetized animal was placed on a temperature-controlled heating pad to maintain the body temperature at 37.3°C. The intestine was rinsed with perfusion fluid that was free of the drug and oils until the perfusate was clear and clean.

The perfusion fluid was distilled water buffered to pH 5.5 with 10 m*M* Mes [2-(*N*-morpholino)ethanesulfonic acid] and made isotonic with NaCl. The pH of 5.5 was used, as previous experiments have shown that the perfusate pH gravitates to a pH of 5.5 if the starting pH is above or below this value. The perfusate also contained 6 m*M* sodium taurodeoxycholate.

The distal cannula flowed into a collection flask. The proximal cannula withdrew perfusate from a reservoir placed on a thermostatically controlled stirrer-hot plate (maintained at 39°C) with a Harvard Model 1210 peristatic pump (Harvard Apparatus, South Natick, Mass.). A magnetic stir bar was used to keep the contents of the reservoir well mixed. Possible changes in perfusate volumes upon passage through the intestine were monitored through the use of radiolabeled PEG 4000 (Sigma Chemical Co., St. Louis, Mo.). The changes in PEG 4000 between the proximal and the distal tubules was determined through scintillation counting.

Preparation of Emulsions

Concentrated cyclosporine stock emulsions were made by adding 1 ml of Sandimmune to 25 ml of distilled water. These emulsions were made either by stirring, which is in accordance with the manufacturers directions or by homogenization with a Polytron (Brinkmann Instruments, Westbury, N.Y.). Droplet size distribution was performed using a Model TAII Coulter counter (Coulter Electronics Inc., Hialeah, Fla.) equipped with an aperture of 70 μm. Stock emulsion (0.2 ml) was added to 25 ml of normal saline for immediate droplet size analysis. Surface area was calculated using the median diameter. The stock solutions were then added in the appropriate amount to 50 ml of perfusate to obtain concentrations ranging from 0.04 to 0.18 mg/ml.

Administration of Emulsions to Rats

These drug solutions were then infused at a rate of either 0.24, 0.42, or 1.05 ml/min. The perfusate entering the intestine was also analyzed for oil droplet size distribution by the use of a micrometer and a light microscope. The time at which the drug solution first entered the intestinal segment (t_0) was noted. The lag time was the difference in time between t_0 and the time at which the drug solution appeared in the distal end of the intestinal segment. To characterize the rate of disappearance of the drugs, small samples of perfusate were removed at 15-min intervals, accounting for lag time, for 2 hr from the proximal and distal cannulas. The concentration of the drugs was then determined by the methods mentioned elsewhere in the text. Blood samples (1 ml) were also drawn by cardiac puncture at the 2-hr termination point of the experiment and cyclosporine levels were determined. The possibility of intralumen metabolism was monitored by measuring the loss of cyclosporine added to the perfusate leaving the intestine. This was monitored at room temperature over a 3-day period.

Pharmacokinetic Analysis

The determination of the apparent permeability coefficient was accomplished by a method similar to that of Sawchuk and Awni (9). The fraction of drug remaining to be absorbed at steady state within a specified intestinal length is described by the equation of Ho and Higuchi (10):

$$C(1)/C(0) = e^{-2\pi rlPe/Q}$$

where $C(1)/C(0)$ is the fraction of drug concentration leaving the intestinal segment, l is the intestinal segment length (cm), r is the effective radius of the intestinal lumen (cm), Q is the bulk fluid flow rate (cm^3/min), and Pe is the apparent permeability coefficient (cm/min).

The average concentrations of drug solution entering [$C(0)$] and leaving [$C(1)$] the intestine at steady state were calculated by averaging the $C(0)$ and $C(1)$ for each rat between 30 and 105 min.

The effective radius of the small intestine was measured by removing a segment of intestine and filling it with saline. The radius was calculated both volumetrically and gravimetrically assuming the intestine lumen to be a right cylinder.

Analysis of Cyclosporine

Cyclosporine A analysis in blood was measured by the HPLC method of Takada *et al.* (11) using a Hewlett Packard 1084A high-performance liquid chromatograph equipped with an ultraviolet spectrophotometer set at a wavelength of 235 nm. Perfusate samples were analyzed by dilution (1:2) with a 30% acetone solution prior to injection into the HPLC.

RESULTS AND DISCUSSION

A plot of the fraction of the concentration of drug leaving the intestinal segment [$C(1)/C(0)$] versus time is shown in Fig. 1. The plot shows data from the mean of five rats, where one group was perfused with the Sandimmune dosage form prepared by the usual stirring method and the other group was perfused with the Sandimmune dosage form prepared by homogenization. It is apparent from this plot that the homogenized dosage form showed a greater fraction absorbed. No loss of cyclosporine was seen when added to the perfusate leaving the intestine, suggesting no intralumenal metabolism. The particle size distribution differences between the stirred and the homogenized dosage forms are presented in Fig. 2. Microscopic examination of the perfusate entering the intestine showed similar droplet size distributions indicating no appreciable change in droplet size upon dilution. The calculated surface area was 1.65×10^6 cm^2/g for the stirred dosage form, versus 3.3×10^6 cm^2/g for the homogenized dosage form. The apparent permeability coefficients (Pe) are presented in Table I.

No significant difference between the concentration of

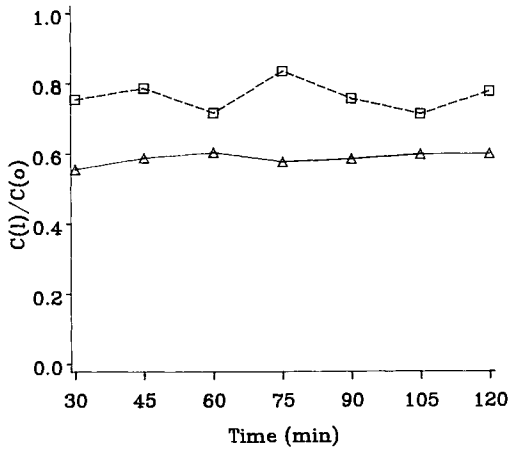

Fig. 1. Plot of the fraction of cyclosporine remaining [$C(1)/C(0)$] versus time for five rats with an intestinal perfusional length of 16 cm. Both the homogenized (△) and the stirred (□) emulsions contained a cyclosporine concentration of 0.08 mg/ml and were infused at a rate of 0.42 ml/min.

PEG 4000 leaving and that entering the perfused intestinal segment was noted, suggesting no appreciable water flux.

To test whether the enhanced permeability coefficient noted in the homogenized dosage form actually led to greater plasma concentrations of cyclosporine, blood samples were taken at 2 hr after gut perfusion was initiated. Cyclosporine blood levels in rats given the homogenized Sandimmune dosage form were about 1.7 times greater than blood levels in the stirred formulation (Table I). This suggests that blood levels and bioavailability increase with increasing permeability coefficients.

The fraction of drug absorbed did not vary among the doses. The higher doses showed the same fraction of cyclosporine absorbed and hence the same apparent permeability coefficient as the lower doses (Table II). These dose-independent absorption rates suggest a nonsaturable absorption process. This was also noted by Ueda *et al.* (3) in a cyclosporine bioavailability study in rats. In their study an increase in doses from 6 to 22 mg/kg did not show a decreased bioavailability, indicating nonsaturable and noncarrier-mediated absorption.

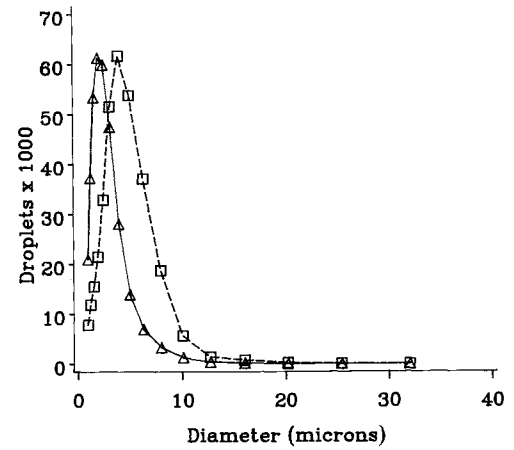

Fig. 2. Plot of the number of oil droplets versus diameter. Emulsions were prepared by stirring (□) and homogenization (△). The plot was normalized so that the same number of oil droplets was present in each emulsion.

Table I. Differences in the Median Droplet Sizes, the Apparent Permeability Coefficient (*Pe*), and the Whole-Blood Concentration of Cyclosporine Between the Stirred and the Homogenized Sandimmune Dosage Form (0.08 mg/ml)*

Test group	Median droplet Size (μm)	*Pe* (cm/min)	Blood conc. (mg/liter)
Stirred	4.0	6.19×10^{-3}	0.906 (0.13)
Homogenized	2.0	1.28×10^{-2}	1.51 (0.12)

* Both the *Pe* and the blood concentrations of cyclosporine were significantly different ($P < 0.05$) between the two test groups.

Doubling the intestinal length from 16 to 32 cm produced no significant increase in the fraction of cyclosporine absorbed, suggesting the presence of an "absorption window." Such a window had been hypothesized by several investigators (12,13). The absorption window seemed to be quite narrow in the rat, since the maximum fraction of cyclosporine was absorbed in the proximal 8- to 16-cm section of the intestine. With the presence of this narrow absorption area, cyclosporine absorption and bioavailability should then be dependent on its rate of absorption. Ueda *et al.* (3) also noted the interdependence of the rate of absorption and the extent of bioavailability of cyclosporine.

Slowing the intestinal perfusion (Q) from 0.42 to 0.24 ml/min decreased the *Pe*, whereas, increasing the flow from 0.42 to 1.05 ml/min increased the *Pe*. The fraction absorbed was also decreased by about 30% when the flow was reduced from 0.42 to 0.24 ml/min. However, there was no significant difference in the fraction absorbed between the 0.42- and the 1.05-ml/min perfusion rates. This may be explained by the nonlinear decrease in the thickness of the aqueous boundary layer at increased flow rates (14). Apparently, the aqueous boundary layer is dramatically decreased (708 to 476 μm) when increasing the flow from 0.247 to 0.494 ml/min but is only slightly decreased (476 to 408 μm) when increasing the flow from 0.494 to 1.28 ml/min. This may explain why there is a decrease in the fraction of cyclosporine absorbed at lower flow rates. This also suggests that the aqueous layer may be the rate-limiting barrier in cyclosporine absorption.

Table II. Fraction of Cyclosporine Remaining [$C(1)/C(0)$] After Passage Through 16 cm of Intestine of Different Concentrations of Cyclosporine*

Concentration (mg/ml)	$C(1)/C(0)$ Stirred	$C(1)/C(0)$ Homogenized
0.04	0.693	0.520
0.06	0.704	0.536
0.08	0.714	0.572
0.12	—[a]	0.522
0.18	—[a]	0.572

[a] These studies were not conducted.

* No significant differences ($P < 0.05$) were noted among the various concentrations within the stirred and homogenized groups ($N = 5$). However, a difference was noted between the groups at each concentration.

The possibility that the absorption of cyclosporine is enhanced by reducing the size of the emulsion droplet is not supported by a recent paper by Nashan *et al.* (15). Nashan *et al.* placed cyclosporine in an oil vehicle which was encased by a soft gelatin capsule. This nondispersed dosage form was bioequivalent to the currently used dispersed oil dosage form. Assuming that smaller emulsion droplet sizes do enhance the intestinal absorption of cyclosporine as presented in this paper, then the bioequivalence between these two dosage forms either may be the result of the stomach's muscular contractions producing a coarse emulsion with the nondispersed oil formulation or may be due to the breakage of the dispersed oil formulation upon addition to gastric fluid. The latter appears to be the case, since preliminary studies in this laboratory indicate that emulsion breakage upon addition to simulated gastric fluid is relatively rapid for the coarser emulsion prepared by stirring. This tends to suggest that the only reason that cyclosporine is administered in the current self-emulsifying vehicle is to improve palatability, and not to enhance its absorption. Thus, the droplet size of the oil formulation may further influence the absorption of cyclosporine, since the homogenized emulsion would remain an emulsion upon entry into the duodenum.

One possible reason for the enhanced apparent permeability coefficients in the smaller-droplet-size emulsion is due to the greater surface area of the dosage form. With the greater surface area, the partitioning of drug into the aqueous environment and lipase activity are increased, causing the formation of more fatty acid and monoglyceride (8). The greater amount of fatty acid and monoglyceride leads to the formation of more micelles. Since cyclosporine is solubilized in micelles, which are contained in the aqueous phase, there is a higher aqueous solubility of cyclosporine. This higher aqueous solubility establishes a higher concentration gradient or driving force of cyclosporine into the stagnant aqueous layer of the intestinal mucosa (16). This is a typical Fick's law relationship. The higher aqueous cyclosporine concentration provides the more rapid absorption and greater apparent permeability coefficient seen in the homogenized dosage form.

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