In Situ Intestinal Absorption of a Poorly Water-Soluble Drug From Mixed Micellar Solutions of Bile Salt and Lipolysis Products in Rats[1]

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Received: October 11, 1984; accepted: April 2, 1985.

Abstract: The role of a bile salt (sodium taurocholate) and lipolysis products (monoglyceride and fatty acid) in the intestinal absorption of a poorly water-soluble drug, diazepam, was investigated. Absorption rates and bioavailabilities were determined with the *in situ* rat gut technique of Doluisio et al. and analyzing the diazepam concentrations in the luminal solution, intestinal membrane, blood and lymph. The absorption rate constant of diazepam decreased with the increase in bile salt concentration. Thus, although the bile salt increased the solubility of diazepam, the net effect was a decrease in apparent diazepam absorption rate. On the other hand, the lipolysis products incorporated in the bile salt micelles increased the solubility of diazepam without affecting the absorption rate constant, and as a result the apparent absorption rate of diazepam increased. In addition, the solubilized diazepam was absorbed almost uniformly throughout the small intestine. The drug solubilized in mixed micelles of bile salt-lipolysis products was not absorbed through the lymphatic system along with the lipolysis products, rather it was absorbed directly into the blood. The possible mechanism of the effect of dietary fat in the absorption of drug is discussed.

The bioavailability of poorly water-soluble drugs was reported to increase *in vivo* because of the presence of lipids in the dosage forms (1–4). However, the mechanism of this increased bioavailability has not been well characterized (5). Possible reasons for the increased bioavailability are the inhibitory effect of lipid on proximal small intestinal motility and increased dissolution rate of the drug which is due to higher bile salt and lysolecithin concentrations resulting from lipid-stimulated bile secretions (2, 3). Yamahira et al. (6) observed that the digestibility of lipid is a major factor in the absorption of a poorly soluble drug administered as a lipid-containing dosage form. It was suggested that the hydrolysis of fat to form micellar lipolysis products might contribute to the increased absorption of drugs (1). Using triolein digestion mixture, Grisafe and Hayton (7) concluded that the increased oral absorption of a poorly water soluble drug is due solely to enhanced dissolution and not to its absorption from micellar and oily phases.

There is a relatively greater consensus on the effect of fat in the absorption of cholesterol. The presence of lipolysis products such as monoglycerides and fatty acids incorporated in bile salt micelles increased the solubility and thus the partitioning of cholesterol in the micellar phase of the intestinal solution (8). Cholesterol is readily absorbed from such micellar solutions (9, 10) and the provision of a necessary amount of lipolysis products is the basis of the well-documented enhancement of cholesterol absorption by dietary fat (11, 12).

In the present investigation, the influence of micelles composed of bile salt-lipolysis products on the absorption of a poorly water-soluble drug, diazepam, was studied, and a mechanism of the effect of dietary fat in the absorption of drug is described.

Materials and Methods

Materials

Diazepam (Hoffmann-La Roche), 1-monoolein, oleic acid, sodium oleate, and sodium taurocholate (K & K Labs) were used as received. All other reagents and chemicals were of analytical grade or better.

In situ Drug Absorption

Drug absorption from the whole small intestine was studied by the *in situ* rat gut technique of Doluisio et al. (13). Two L-shaped glass cannulae were inserted into the exposed small intestine of a female Sprague-Dawley albino rat (Camm Research, N. J.), 240–275 g, through two small slits – one at about 2 cm distal to the common bile duct junction and the other proximal to the cecal end – and tied with surgical sutures. After washing the intestine, two 20 ml hypodermic syringes were connected to the cannulae, and 10 ml of a test solution was administered into the intestine through the duodenal syringe. This technique was modified for the purpose of perfusion of different segments of the small intestine. The average rat small intestine was approximately 100 cm long. Either 40 cm of the upper small intestine starting downward from 2 cm distal to the common bile duct junction or 40 cm of lower small intestine starting upward from the cecal end was measured carefully with a 40 cm thread by placing it alongside the intestine. The cannulae were then inserted and tied, and 4 ml of the test solution was introduced into the intestinal segment after washing. In the case of the whole intestine, 5 ml of the luminal solution was pumped alternately into either of the syringes at 5 min intervals, and a 0.1 ml aliquot was collected with a syringe (Hamilton) as the solution started flowing back to the lumen on release of pumping pressure. When a segment of intestine was used, the volume of the aliquot was the same, but only 2 ml of solution was pumped into the syringe.

Collection of Blood

A complete blood curve could not be obtained from a single animal because of the small blood volume in a rat. Only two

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samples, 1 ml each, were collected from each rat with a 1 ml heparinized syringe. The first sample was collected from the dorsal aorta, and the second sample was collected directly from the heart. The blood was frozen immediately and kept as such prior to analysis. No aliquot was collected from the luminal solution during this study; however, for the maintenance of proper stirring condition, 5 ml of the solution was pumped alternately into one of the attached syringes at 5 min intervals and then sent back to the lumen.

Collection of Intestinal Lymph

Intestinal lymph was collected by modifying the method of Bollman et al. (14). The rat was anesthetized with ether and a polyethylene tube (Intramedic PE-50, Clay-Adams, New York) filled with a dilute solution of heparin, was threaded 3 mm into the lymphatic duct and ligated. Drops of tissue cement, methyl cyanoacrylate (Eastman), were applied according to Warshaw (15), to hold the tubing in line with the direction of the lymphatic. The lymph began to flow immediately through the tube. A midline incision was then made, and the drug solution was introduced into the entire small intestine as described above for *in situ* drug absorption. Both the duodenal and the ileal ends were then ligated with silk sutures and the syringes removed. With the cannula led out at the lateral end of the incision, the wound was closed with a running suture to all layers of the abdominal wall except the skin, which was closed with metal clips. The drug solution in the intestine remained unstirred in this procedure.

Determination of Membrane-Bound Drug

The small intestine was isolated and hung, and air was blown through the intestine from the upper end for 5 min to drain all the solution. It was then cut into pieces and homogenized with 50 ml ethanol for 30 min by a homogenizer (Osterizer) fitted with an 8 oz minicontainer. The homogenate was centrifuged, the ethanolic layer was collected, and the solid was reextracted with another 50 ml ethanol. The two ethanolic extracts were combined, and a 2 ml aliquot of the mixture was dried *in vacuo* before analysis.

Test Solutions

The compositions of the test solutions are given in Table I. The solutions were made approximately isotonic by adding sodium chloride, and preliminary experiments showed that the change in volume of each test solution at the end of a perfusion study was < 0.5 ml. The concentrations of diazepam shown are equilibrium solubilities at 30°C. After shaking at this temperature for 4 h with excess of diazepam, the test solutions were filtered through a 0.22 μm Millipore filter, the concentrations of diazepam were determined, and then the solutions were transferred to a water bath at 37°C. Solution No. 3 looked slightly turbid which did not change on filtration or aging. The solutions warmed up to 37°C were used for the perfusion studies. Warming up to 37°C made the solution sufficiently unsaturated to prevent any precipitation of diazepam from the solution after its introduction to the lumen.

Table I. The Compositions of Test Solutions and the Rate Constants of *In Situ* Absorption of Diazepam from the Test Solutions.

	Solution 1	Solution 2 Compositions	Solution 3
Sodium taurocholate	70 mM	40 mM	40 mM
Oleic acid	–	–	10 mM
Sodium oleate	–	–	10 mM
Monoolein	–	–	10 mM
Sodium chloride	130 mM	130 mM	120 mM
Diazepam	0.56 mg/ml[a]	0.32 mg/ml[a]	0.60 mg/ml[a]
	Absorption Rate Constants (min^{-1})		
	0.012	0.031	0.028

[a] Equilibrium solubility at 30°C.

The digestion of fat in the small intestine is due to its enzymatic hydrolysis to fatty acids and 2-monoglycerides which form mixed micelles with bile salt molecules (16). 1-Monoolein was, however, used in the present study in place of 2-monoolein because of its greater commercial availability and to avoid transesterification of 2-monoolein; solubility studies showed it to be identical to 2-monoolein (17).

Analysis of Diazepam

Aliquots were analyzed spectrophotometrically by the method of de Silva et al. (18).

Results

Solubility of Diazepam

The solubilities of diazepam in bile salt solutions were reported earlier (19). Table I shows that 0.56 and 0.32 mg of diazepam were solubilized per ml of 70 and 40 mM solutions of sodium taurocholate, respectively, at 30°C. On incorporation of 30 mM concentration of lipolysis products, which is equivalent to an undigested lipid concentration of 10 mM, to the latter solution, the solubility of diazepam almost doubled.

Absorption of Diazepam

The absorption of diazepam from sodium taurocholate and mixed micellar solutions of sodium taurocholate-lipolysis products is shown in Fig. 1. Because of the very poor solubility of diazepam in water (0.04 mg/ml at 30°C), no perfusion study was conducted with water alone as the solvent. Since there is an equilibration period because of initial membrane accumulation of drug (13), the luminal diazepam concentrations starting from 10 min of perfusion were used in calculating the rate constants of drug absorption. The results are tabulated in Table I. Although the solubility of diazepam increased by a factor of 1.75 with the increase in the bile salt concentration from 40 mM to 70 mM, there was a decrease by a factor of more than 2.5 in the absorption rate constant of diazepam. On the other hand, the effect of lipolysis products on the absorption rate constant was very small; the absorption rate constant changed only from 0.031 min^{-1} to 0.028 min^{-1} in the presence of 30 mM lipolysis products in a 40 mM bile salt solution.

Table II gives the amounts of diazepam bound to the membrane at 15 min and 40 min of perfusion of Solution No. 3. The total amounts of diazepam absorbed into the body compartment from this solution in 0–15 min and 0–40 min are also shown in Table II. Total drug reaching the body compartment during these two time periods were 33 and 66%, respectively.

The blood concentrations of diazepam were determined in three rats from 25 min to 180 min of perfusion with Solution No. 3. The concentrations varied between 2.5 and 4.2 μg/ml. Also, with Solution No. 3, the intestinal lymph was collected from three rats for 5 to 7 h. An average lymph flow of 0.3 ml/min was obtained from each rat. The lymph turned viscous and

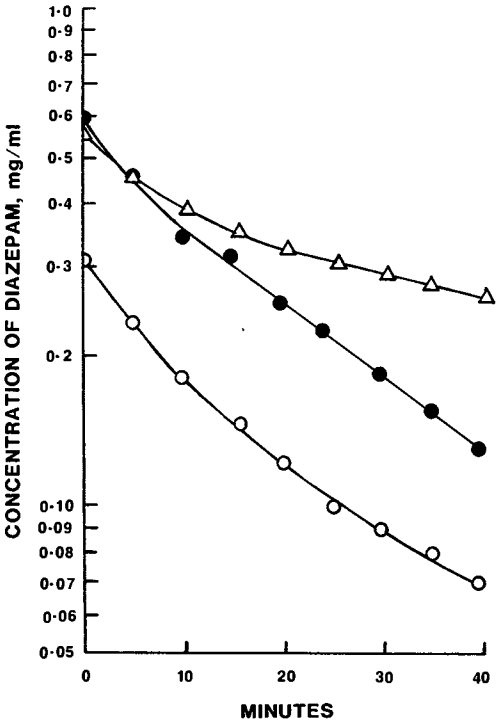

Fig. 1 *In situ* intestinal absorption of diazepam from micellar solutions of sodium taurocholate and mixed micellar solution of sodium taurocholate-lipolysis products in rats. Each point represents the average values from two rats. Key: △, Solution No. 1; ○, Solution No. 2; and ●, Solution No. 3.

Table II. The Extent of Membrane-Binding and Absorption of Diazepam from Solution No. 3 during *in situ* Perfusion of Whole Small Intestines of Rats.

	Amounts of diazepam (mg)	
	15 min	40 min
Membrane-bound diazepam	0.87 ± 0.19[a]	0.64 ± 0.16[a]
Diazepam content of luminal solution[b]	3.14 ± 0.21[a]	1.41 ± 0.23[a]
Diazepam in membrane + luminal solution	4.01	2.05
Diazepam absorbed into the body[c]	2.0	4.0

[a] Average of three determinations ± SD.
[b] Diazepam concentration x total volume of luminal solution drained out of the intestine.
[c] Difference between the initial amount of diazepam (6 mg) and the amount of diazepam in membrane and luminal solution.

milky because of the presence of chylomicrons after the introduction of the test solution in the intestine. The flow rate was considerably less than 1 ml/min observed in normal rats (14), possibly because the normal physiological condition of the intestine was disturbed. The average concentration of diazepam in the lymph was 5.2 ± 0.4 μg/ml which was somewhat higher than the concentration in the blood. However, since the lymph flow was very small, the total amount of diazepam absorbed through the lymphatic system was insignificant in comparison with the total amount of diazepam absorbed. These results agree with the findings by Yoshikawa et al. (20) that the absorption of bleomycin in rats was remarkably increased by sodium taurocholate-monoolein mixed micelles but the concentration in blood and lymph was almost identical. Ueda et al. (21) also observed that when cyclosporin A, a lipophilic and poorly water soluble drug, was administered to rats as an olive oil solution, the intestinal lymphatics accounted for only 1 to 2 per cent of the total amount absorbed.

Fig. 2 shows the absorption of diazepam from Solution No. 3 in the upper and lower segments of the small intestine. The absorption rate constants were 0.027 and 0.028 min^{-1}, respectively, which showed that there was no significant difference in the absorption rate throughout the small intestine.

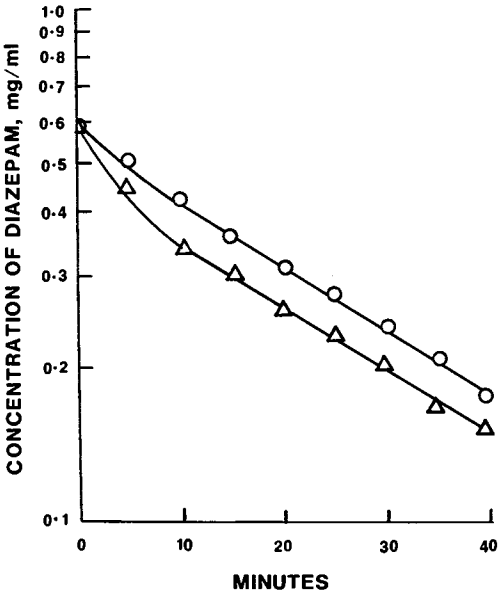

Fig. 2 *In situ* absorption of diazepam from micellar solution of sodium taurocholate-lipolysis products (Solution No. 3) through intestinal segments. Each point represents the average values from two rats. Key: △, upper 40 cm; and ○, lower 40 cm.

Discussion

Absorption of Diazepam

It was shown by Grisafe and Hayton (7) that

$$\frac{dC_T}{dt} = -K_{app} C_T \qquad \text{(Eq. 1)}$$

where dC_T/dt is the absorption rate of drug from the lumen, C_T is the total solubility of the drug in the micellar system and K_{app} is the apparent absorption rate constant. Since, in the present investigation, there was only a minor change in rate constant when lipolysis products were added to the bile salt solution, the increased flux of drug through the membrane was due primarily to increased C_T. On the other hand, an increase in C_T by an increase in bile salt concentration alone did not increase the absorption rate of drug because there was a simultaneous decrease in the rate constant. Rather, the rate decreased in the latter case because the extent of decrease in K_{app} was greater than the increase in C_T.

Using dialysis cells Collett et al. (22) showed that salicylic acid from the micellar phase was practically unavailable for absorption in a reasonable length of time. On the basis of the equilibrium solubility in water, the maximum amount of diaze-

pam present in the aqueous phase of 10 ml of a test solution was 0.4 mg. Therefore, the percentages of diazepam present in the aqueous (nonmicellar) phases of Solution Nos. 1 to 3 were approximately 7, 13 and 7, respectively. However, it is apparent from Fig. 1 that the total drug absorbed from each solution in 40 minutes was much higher than the fraction initially present in the aqueous phase; in the case of Solution No. 3, this was 66 per cent of the total drug in the lumen (Table II). The analysis of diazepam in the membrane and the blood confirmed that the drug reached the body compartment.

Mechanism of Absorption

Westergaard and Dietschy (23) suggested a mechanism whereby bile salt micelles facilitate solute uptake into the intestinal mucosal cell. According to them, the principal role of bile salt micelles is to overcome the aqueous diffusion layer resistance and to maintain a maximum concentration of free solute at the membrane-water interface. The micelles are in equilibrium with free solutes, and the actual absorption is due to partitioning of free solute into the membrane. Amidon et al. (24) described this phenomenon on the basis of a physical model. According to this model, the aqueous diffusion layer resistance can be eliminated at high surfactant concentration because of the diffusion of micelles, and the activity of the solute at the membrane-water interface can approach the bulk solute activity. Therefore, if the concentration of surfactant is increased, the absorption rate constant would decrease because of a lower ratio of free solute to total solute concentration, i.e., a lower thermodynamic activity.

The decrease in the absorption rate constant of diazepam from 0.031 to 0.012 min^{-1} with an increase in bile salt concentration from 40 mM to 70 mM (Table I) is in general agreement with the above model. In contrast, only a minor decrease in rate constant (from 0.031 to 0.028 min^{-1}) was observed when a comparable decrease in thermodynamic activity was attained by solubilizing the drug in mixed micelles of bile salt-lipolysis products. The difference between the systems is that in the latter case, the increase in solubility of diazepam is due to the presence of lipolysis products which themselves are solutes within the bile salt micelles. Thus, it appears that the absorption rate constant depends primarily on the bile salt concentration and is unrelated to the influence of other solutes, e.g., lipolysis products.

One possible reason for the above deviation from the theoretical model may be that any interaction between the surfactant and the intestinal membrane was not considered in the model. Since the biological membrane is lipoidal in nature, it is possible that monomeric surfactant molecules, because of their distinct hydrophilic and lipophilic regions, are adsorbed to the membrane-water interface, possibly in the form of aggregates or hemimicelles. During this process, the surfactant micelles may break up to maintain an equilibrium between the monomeric and the micellar surfactant molecules, thus releasing excess free solute near the interface for partitioning into the membrane. Once the interfacial film is formed, there would be an equilibrium of solutes (diazepam and/or lipolysis products) between the adsorbed film and the bulk solution. At identical bile salt concentrations, the interfacial film would be the same. However, as lipolysis products from a mixed micellar system are absorbed from the interface, the critical micellar concentration at the aqueous-membrane interface should increase (23) and the solubilization capacity of the system should decrease. As a result, the thermodynamic activity of solute in the membrane-water interface of a bile salt-lipolysis product mixed micellar system would be higher than that in the bulk solution. Such a phenomenon may be responsible for the higher absorption rate constant of diazepam from Solution No. 3 as compared to Solution No. 1, although the initial thermodynamic activity of the dissolved drug was almost identical in the two solutions.

Effect of Intestinal Segments

Bile salts are absorbed primarily from the ileum, while the lipolysis products are absorbed throughout the small intestine (25). It was, therefore, of interest to examine the effects of these solubilizing agents on the segmental distribution of drug uptake. The uniform absorption rate throughout the small intestine showed that any decrease in absorption of drug in the lower segment because of a decreased surface area was probably compensated for by the release of excess drug by active absorption of bile salt itself in the ileum.

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