Intestinal alkaline phosphatase release is not associated with chylomicron formation

Andromeda M. Nauli, Shuqin Zheng, Qing Yang, Ronggui Li, Ronald Jandacek, and Patrick Tso

Department of Pathology and Laboratory Medicine, University of Cincinnati College of Medicine, Cincinnati, Ohio 45267-0529

Submitted 7 November 2002; accepted in final form 1 December 2002

Nauli, Andromeda M., Shuqin Zheng, Qing Yang, Ronggui Li, Ronald Jandacek, and Patrick Tso. Intestinal alkaline phosphatase release is not associated with chylomicron formation. Am J Physiol Gastrointest Liver Physiol 284: G583–G587, 2003. First published December 4, 2002; 10.1152/ajpgi.00482.2002.—Intestinal alkaline phosphatase (IAP) is one of the major sources of alkaline phosphatase in circulation. It is secreted into the intestinal lumen, serum, and lymph. After the ingestion of lipid, lymphatic alkaline phosphatase secretion increases significantly. We have found that the nonabsorbable fat olestra is unable to stimulate lymphatic alkaline phosphatase secretion. We also found that the hydrophobic surfactant Pluronic L-81, which blocks chylomicron formation, fails to inhibit this increase in lymphatic alkaline phosphatase secretion. These results suggest that it is the lipid uptake into the mucosa and/or reesterification to form triacylglycerols, but not the formation of chylomicrons, that is necessary for the stimulation of the secretion of alkaline phosphatase into the lymph.

material and METHODS

Materials. Pluronic L-81 was kindly donated by BASF (Parsippany, NJ). The alkaline phosphatase assay kit, triolein, egg phosphatidylcholine (PC), and sodium taurocholate were purchased from Sigma (St. Louis, MO). The triglycerides (triacylglycerols) assay kit was purchased from Randox Laboratories (Crumlin, Antrim, UK). Olestra was a kind gift from Procter & Gamble (Cincinnati, OH).

Animals. Adult male Sprague-Dawley rats weighing 230–360 g were fasted overnight before surgery. After the rats were anesthetized with halothane, their intestinal lymph ducts were cannulated with soft vinyl tubing as discussed by Bollman et al. (4) with slight modification. Instead of using suture to secure the lymph cannula, we used a drop of cyanoacrylate glue (Krazy Glue) to secure the lymph can-

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
sonicated until they appeared homogeneous.

Brie

fl

measures the released glycerols from the hydrolysis of tria-

lina. A silicone tube (1.6 mm OD) was inserted ~2 cm into

the duodenum via a fundal incision of the stomach. Tubing

was secured with a transmural suture (for the duodenum),

and the fundal incision was closed by a purse-string suture.

Immediately after surgery, rats were infused with 5% glucose

in saline (145 mM NaCl, 4 mM KCl, 0.28 M glucose) over-
night at a rate of 3 ml/h until the following day, when the
glucose/saline solution was replaced with the prepared nu-

trient infusate described below. The fasting lymph was col-
clected for 1 h before nutrient infusion. The nutrient infusion

continued for 6 h, and lymph was collected hourly. Animals

were killed at the end of the 6-h infusion. Six animals in each

of the four infusion groups were studied.

Nutrient infusate preparation. Four different infusates

were used: lipid, lipid/L-81, olestra, and vehicle. 0 h. Fasting lymph.

No significant difference between groups is found (P > 0.05). Values

are means ± SE.

nm. Background was eliminated by adding two drops of

concentrated HCl. The difference between the initial and

the background absorbance corresponded to the measured

concentration. Appropriate standards were provided by

the manufacturer.

Statistical analysis. All values are expressed as means ±

SE. Two-way repeated-measures ANOVA with Tukey’s as a

posttest analysis was used to compare all the groups

throughout the 6-h infusion. When comparing groups only at

a particular hour of the experiment (e.g., fasting lymph flow

rate), one-way ANOVA with Tukey’s as a posttest analysis

was performed. The difference was considered significant if

the \( P \) value was <0.05. All statistical analyses were carried

out by using the statistics program SigmaStats version

2.0 (SPSS).

RESULTS

Lymph flow during continuous intraduodenal infu-

sion. Figure 1 shows the hourly lymph flow rate of each
group during the course of a 6-h infusion. The volume

of the fasting lymph collected during the 1-h fasting

period before administering the test infusion was not

statistically different for any of the groups (\( P = 0.33 \)).

When the lymph flow was compared for the overall 6-h

infusion among the groups, there was no significant
difference. The lymph flow rate of ~3 ml/h obtained

from this experiment for all groups is comparable to

lymph flow observed in other infusion studies carried

out in this laboratory. The lymph flow rate during the

first hour was lower, probably due to the change of

infusate from glucose/saline infusion to the test infu-
sion solution.

Lymphatic output of triacylglycerols. The fasting

lymph triacylglycerol outputs varied between 3.45 and

8.06 mg/h in the four groups of rats. As shown in Fig. 2,

the lymphatic triacylglycerol level of the lipid group

was higher than that of the other three groups (\( P \)
values < 0.001) during the 2–6 h infusion period. Although

the outputs of the lipid/L-81 and the olestra

groups were not significantly different from each other,

they were higher than that of the vehicle group and the

differences were significant (\( P = 0.010 \) and \( P = 0.006, \)

respectively) after the first hour of infusion. The fast-

Fig. 1. The lymph flow rate during the continuous intraduodenal

infusion of lipid, lipid/L-81, olestra, and vehicle. 0 h. Fasting lymph.

No significant difference between groups is found (\( P > 0.05 \)). Values

are means ± SE.

Fig. 2. Triacylglycerol contents in lymph collected hourly during

continuous intraduodenal infusion of lipid, lipid/L-81, olestra, and

vehicle. Groups not having common letters are signiﬁcantly different

(\( P < 0.05 \)). Values are means ± SE.
major contributor of lymphatic alkaline phosphatase from cytosolic IAP, i.e., IAP II (19). Hence, IAP II is the main source of lymphatic alkaline phosphatase (6, 8, 22, 30). Additionally, it has been determined that the major IAP regulated by lipid feeding is IAP II (20).

Air-water interface tension. It has been demonstrated that triacylglycerol level of the vehicle group was notably lower than that of the olestra group \( (P < 0.01) \).

**Lymphatic alkaline phosphatase secretion.** Lymphatic alkaline phosphatase secretion is calculated as a product of lymph flow (ml/h) and lymph alkaline phosphatase concentration (mU/ml). Figure 3 depicts hourly lymphatic alkaline phosphatase secretion of the lipid, lipid/L-81, olestra, and vehicle groups. Lymphatic alkaline phosphatase secretion of the lipid group was not significantly different from that of the lipid/L-81 group \( (P = 0.708) \). However, the secretion by the lipid group was significantly greater than that of both the olestra \( (P = 0.001) \) and the vehicle groups \( (P = 0.001) \) during the 2–6 h infusion period. Similarly, after the second hour, the secretion by the lipid/L-81 group was significantly greater than both the olestra \( (P = 0.003) \) and vehicle groups \( (P = 0.001) \). On the other hand, secretion by the olestra group was not different from that of the vehicle group \( (P = 0.273) \).

A similar trend is also seen in a comparison of the cumulative lymphatic alkaline phosphatase secretions (Fig. 4). These cumulative values were obtained by summing the total alkaline phosphatase secretion during the 6 h of infusion of the different test solutions. Lipid infusion increased lymphatic alkaline phosphatase secretion, and this increase was not blocked by the administration of the chylomicron inhibitor Pluronic L-81.

**DISCUSSION**

There are two IAP isoforms in rats. IAP I is associated with the apical membrane of intestinal epithelial cells and is probably the predominant source of luminal alkaline phosphatase (19). In contrast, IAP II is currently believed to be associated with surfactant-like particles (SLP) (1, 2, 7, 31, 33). SLP, which consist of PC and surfactant proteins A, B, and D, decrease the air-water interface tension. It has been demonstrated that the major IAP regulated by lipid feeding is IAP II (6, 8, 22, 30). Additionally, it has been determined that the main source of lymphatic alkaline phosphatase is from cytosolic IAP, i.e., IAP II (19). Hence, IAP II is a major contributor of lymphatic alkaline phosphatase activity measured in our current studies, and measurement of lymphatic alkaline phosphatase activity monitors the secretion of alkaline phosphatase by the intestinal epithelial cells.

We sought to determine whether or not the increased secretion of alkaline phosphatase into lymph during active fat absorption is caused by 1) the exposure of the intestinal mucosal surface to lipid-like molecules in the small intestinal lumen, 2) the uptake and/or reesterification of the lipid digestion products to form triacylglycerols, or 3) the formation and secretion of chylomicrons. As shown in Figs. 3 and 4, it is apparent that the vehicle alone was not capable of stimulating IAP release to lymph. Thus the stimulation of lymphatic alkaline phosphatase secretion by lipid absorption is not mediated by the presence of vehicle in the intestinal lumen. To answer the first question of whether the stimulation of lymphatic alkaline phosphatase secretion by fat absorption is caused by exposure of intestinal mucosa to lipid-like molecules, we used olestra, a unique nondigestible lipid. Although olestra is not hydrolyzed by pancreatic lipase and is not absorbed by the intestinal epithelia (16–18), it is emulsified by bile salts to form lipid particles. Our study demonstrated that the intraduodenal infusion of olestra did not result in an upregulation of lymphatic alkaline phosphatase secretion. Because the physical properties of olestra are similar to those of undigested triacylglycerols, it is unlikely that nonspecific interaction of lipid with intestinal brush borders is capable of stimulating IAP.

Triacylglycerols are hydrolyzed by pancreatic lipase, and their hydrolytic products, fatty acids and 2-monacylglycerols, are absorbed by intestinal cells. Once inside the intestinal cells, triacylglycerols are synthesized from the fatty acids and 2-monacylglycerols and transported to the lymph via chylomicrons. Because Pluronic L-81 blocks chylomicron formation and secretion, but does not interfere with the uptake and the reesterification of monoacylglycerols and fatty acids to form triacylglycerols (21, 24–27), we determined whether the stimulation of lymphatic alkaline phosphatase secretion by lipid absorption is mediated by the presence of intracellular lipid or by the formation of monoacylglycerols and fatty acids...
and secretion of chylomicrons. As shown in Fig. 2, the lymphatic triacylglycerol output increased dramatically during lipid absorption and the output was significantly different from the other three groups (P < 0.001). It is apparent from Fig. 2 that Pluronic L-81 was effective in inhibiting the formation of chylomicrons, because the lymphatic triacylglycerol output barely increased compared with the fasting level after the ingestion of lipid plus Pluronic L-81.

In Figs. 3 and 4, it is evident that Pluronic L-81 did not block IAP release into the lymph. Furthermore, these figures show that olestra and vehicle did not stimulate IAP release to lymph.

Chylomicron formation was inhibited by the compound Pluronic L-81 (21, 24–27), and yet, it is evident from Figs. 3 and 4 that Pluronic L-81 did not inhibit increase in lymphatic alkaline phosphatase secretion induced by active lipid absorption. This observation bears considerable resemblance to the observation on the regulation of diamine oxidase by fat absorption in a paper published by Wollin et al. (28). They observed that diamine oxidase secretion into lymph is stimulated by fat absorption, but inhibiting the formation of chylomicrons by Pluronic L-81 failed to block the increase in lymphatic diamine oxidase secretion stimulated by fat absorption.

The findings from our present study, however, contradict the findings of Alpers and colleagues (14, 33) who reported that Pluronic L-81 was capable of inhibiting serum (14) and duodenal alkaline phosphatase (33). However, the amount of Pluronic L-81 administered in those studies (~100 mg in a single dose) was markedly higher than the amount we administered (6 mg total over a 6-h period). The triacylglycerol profile of our lipid/L-81 group (Fig. 2) indicates that the amount of Pluronic L-81 used in our experiment was clearly sufficient to block the formation of chylomicrons and thus prevent the increase in lymphatic triacylglycerol output during fat absorption. These data support numerous other reports of the effects of Pluronic L-81 on chylomicron formation (21, 24–27).

We do not have a complete explanation for this apparent discrepancy between our observation and that of Alpers and colleagues (14, 33). However, it is possible that a high concentration of Pluronic L-81 inhibits SLP formation, hence inhibiting IAP II release from the intestine. Furthermore, our study more directly measured the secretion of alkaline phosphatase in lymph but not in the duodenum or serum. Serum alkaline phosphatase may not reflect IAP secretion from the intestine, because circulating alkaline phosphatase is rapidly cleared by the liver (13), giving the investigators only an indirect measurement of the secretion of IAP output from the intestine. The mesenteric lymph collected in our experiment had not entered the circulation, and therefore, the IAP in the lymph had not been metabolized by the liver. Plasma alkaline phosphatase activity is determined by both the net input into the circulation as well as the removal by the liver and the other organs. Consequently, a decrease in plasma alkaline phosphatase activity could be a result of decreased secretion, increased removal, or a combination of both.

We can make a number of conclusions from our studies regarding the stimulation of secretion of IAP into lymph by active lipid absorption. First, contact of the enterocytes with lipid-like molecules (olestra) in the small intestine did not induce the release of IAP by the small intestine. Second, the uptake and/or the reesterification of lipid digestion products to form triacylglycerols is/are responsible for stimulating IAP secretion into lymph by active fat absorption. We could not determine in our study whether it was the uptake step or the presence of reesterified triacylglycerols in the intestinal epithelial cells that was responsible for stimulating the secretion of IAP by the small intestinal epithelial cells. Third, the packaging of reesterified triacylglycerols in the intestinal epithelial cells to form chylomicrons appears to have little or no effect on the stimulation of intestinal alkaline phosphatase secretion.

This work was supported by the National Institute of Diabetes and Digestive and Kidney Diseases Grants DK-56910, DK-54504, and DK-56863.

REFERENCES


