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Relationship of phosphatidylcholine to hydrophobic surfactant on rat intestinal chylomicron secretion

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Abstract. Hydrophobic surfactants such as Poloxalene inhibit triglyceride secretion into lymph by enterocytes. The inhibitory effect of these agents on triglyceride secretion is reversed when lipid presented for absorption is exclusively in the form of phosphatidylcholine (PC) and not triglyceride. The present investigation performed in conscious mesenteric lymph fistula rats was designed to determine whether various mixtures of triglyceride and PC given intraduodenally with Poloxalene would also reverse the inhibitory effect of Poloxalene on triglyceride secretion into lymph. A 50–50 mixture of triolein (TO) and PC resulted in normal triglyceride secretion into lymph. However, when the mixture of lipids was 75–25, TO to PC, results for triglyceride recovery in lymph were considerably reduced. The transport rate for triglyceride into lymph was not as depressed, however, as observed for Poloxalene treated rats given lipid for absorption basically in the triglyceride form. Substitution of phosphatidylethanolamine for PC had no beneficial effect on triglyceride secretion in Poloxalene treated rats.
It is concluded that PC can reverse the inhibitory effect of Poloxalene on triglyceride secretion into lymph even when considerable amounts of triglyceride along with PC are presented for absorption.
Key words. Hydrophobic surfactant; phosphatidycholine; triglyceride; small intestine; lipid absorption.

Block copolymers of polyethylene oxide (hydrophilic component) and polypropylene oxide (hydrophobic component) have been shown to cause delayed secretion of chylomicrons from the enterocytes into intestinal lymph[1–3] and malabsorption of lipids[4–7]. Most of these observations have been made with the hydrophobic surfactants Pluronic L-81 (90% polypropylene oxide)[1–3,5] and Poloxalene 2970 (70% polypropylene oxide)[4,6,7]. The mechanism of action of these surfactants on chylomicron secretion is not known presently but morphologic studies indicate these agents produce an interruption in intracellular transport of absorbed lipids in the enterocytes[2]. Despite the inhibition of chylomicron secretion it was observed that enterocytes continued to secrete the smaller very low density sized lipoproteins (VLDL)[1,2].

In subsequent experiments it was observed that when lipid was presented to the intestine for absorption in the form of phospholipid and not triglyceride (TG) hydrophobic surfactants had no major inhibitory effect on secretion of triglyceride rich lipoproteins by the enterocytes[8]. This observation suggested that there may be competition between phospholipids and hydrophobic surfactants at the intracellular level that affects intracellular lipid transport and the subsequent secretion of chylomicrons into lymph. The present study was performed to determine how much phospholipid is required to successfully reverse the inhibitory effects of hydrophobic surfactant on chylomicron secretion and whether this effect was specific for phosphatidylcholine (PC), the phospholipid used previously[8].

Materials and methods

Male Sprague-Dawley rats (200–250 g) were used. Rats were fed rat chow *ad libitum* prior to study and were fasted 18 to 24 h prior to surgical placement of lymphatic and duodenal cannulas. Rats were operated upon under pentobarbital anesthesia, 40 mg/kg I.P. A cannula was placed in the mesenteric lymphatic vessel draining the proximal small bowel and another cannula was placed via the stomach and into the duodenum as described[1]. Postoperatively animals were placed in restraining cages and infused with a glucose-electrolyte solution, pH 6.4, previously described[1] at a rate of 1.68 ml/h via the duodenal cannula and allowed to recover overnight.

On the following day all rats were infused with a standard lipid emulsion prepared in the same glucose-electrolyte solution containing sodium taurocholate, 19 mM, plant phosphatidylcholine (PC), 3 mM, and tri-

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olein (TO), 12 mM, infused at a rate of 3.34 ml/h for 6 h. Lymph samples were collected over ice at 2 h intervals. Based on prior observations rats were near steady-state conditions for lipid absorption and chylomicron secretion after 6 h of duodenal lipid infusion at this particular dose[1]. Only rats with a mesenteric lymph flow of 2 ml/h or greater were used for the second phase of the study that was conducted over the subsequent 6 h. Group (Gp) 1 was infused with the same lipid emulsion described above (controls). Gp 2 was infused with the same emulsion supplemented with Poloxalene 2930 (Pol), 0.5 mg/ml of infusate (Pol + TO, 100%). Gp 3 was infused with an emulsion containing no triolein but containing sodium taurocholate, 19 mM, plant PC, 3 mM, egg PC, 18 mM, and Pol, 0.5 mg/ml (Pol + PC, 100%). Gp 4 was infused with an emulsion of sodium taurocholate, 19 mM, and plant PC, 3 mM, plus TO, 6 mM, egg PC, 9 mM, and Pol, 0.5 mg/ml (Pol + TO, 50%, PC, 50%). Gp 5 was given a similar taurocholate-plant PC-Pol emulsion with TO, 9 mM, and egg PC, 4.5 mM, (Pol + TO, 75%, PC, 25%). Gp 6 was infused with the taurocholate-plant PC-Pol emulsion with TO, 6 mM, and phosphatidylethanolamine (PE), 9 mM (Pol + TO, 50%, PE, 50%). Duodenal infusion was continued with these emulsions at 3.34 ml/h over the next 6 h with lymph collections obtained at 2 hourly intervals. At the conclusion of the second infusion animals were sacrificed with exposure to CO_2–O_2, 50%, 50%.

Lymph samples were measured and a sample was taken for measurement of triglyceride content using kits as described[9]. A sample of lymph from each rat from the final 2 h of the first 6 h infusion and a sample from the final 2 h of the second 6 h infusion were extracted for lipid[10] and an aliquot of the lipid phase was taken for phospholipid assay[11].

Materials. Triolein was purchased from ICN Pharmaceutical, Cleveland, OH. Plant phosphatidylcholine was purified by silicic column chromatography and was found to be chromatographically pure using chloroform/methanol/water (65:25:4, vol/vol) on Silica gel G plates. Sodium taurocholate was supplied by Calbiochem-Behring, La Jolla, CA. Poloxalene 2930 was kindly donated by Smith Kline Beecham Pharmaceuticals, Philadelphia, PA. All reagents or solvents used were of analytical grade.

Statistics. Results were analyzed using Dunnett's Multiple Range Test[12] to compare results from multiple observations. As there was some variation from group to group regarding the rates of triglyceride secretion into lymph when steady state conditions were reached after 6 h of intraduodenal infusion of standard lipid emulsion it was decided to compare the results obtained for each group after the start of Poloxalene infusion with the results of lymph triglyceride recovery for the two hour period just before starting poloxalene treatment (baseline value) for each respective group. Comparisons of results among the various groups for triglyceride recoveries into lymph were not performed. Four animals were included in each group except for Gp 2 (POL + TO, 100%) which had five and Gp 5 (POL + TO, 75%, PC 25%) that included 6 rats.

Results

All rats in each group were infused the standard lipid emulsion containing sodium taurocholate, 19 mM, phosphatidylcholine (PC), 3 mM, and triolein (TO), 12 mM, at 3.34 ml/h for 6 h. By the end of this period lipid was being efficiently absorbed and transported into lymph by all animals. At this time the mean value for triglyceride (TG) secretion into mesenteric lymph for control rats was 21.9 ± 4.1 μmol/h. All of the other groups at this time were transporting lipid into lymph at similar or somewhat greater rates as shown in figures 1 and 2 (baseline values).

During the next 6 h lipid with Poloxalene (Pol) was infused into the duodenum in each group of rats except for the control group (Gp 1) which continued to receive the same standard lipid emulsion as infused during the first 6 h. The transport rate of TG into lymph of control rats increased somewhat over time reaching 27.5 ± 4.1 μmol/h by the end of the next 4 h of intraduodenal lipid infusion. The differences in triglyceride transport rates for the control group when results were compared to the baseline value for this group were not significantly different.

In Gp 2 the lipid presented for absorption after starting Pol treatment was in the form of triglyceride (TO). As shown in figure 1 the rate of TG secretion into lymph began to decline shortly after the start of Pol infusion and by the end of 4 h of Pol treatment TG secretion decreased to 11.4 ± 2.4 μmol/h ($p < 0.01$) compared to the baseline value for Gp 2 and further decreased to 9.9 ± 0.9 μmol/h ($p < 0.01$) after 6 h of Pol treatment. This was as expected confirming earlier observations on the inhibitory effects of hydrophobic surfactants on chylomicron secretion[1–3].

Gp 3 was given Pol plus PC containing an equivalent amounts of fatty acid component as infused as TO in Gp 2. Triglyceride transport rates into lymph also declined somewhat in this group after 2 and 4 h of Pol treatment but returned essentially to the pre-Pol basal level after 6 h of Pol treatment. At no time period was the triglyceride transport rate significantly less than the corresponding baseline value for this group.

Gp 4 was given 50% of the fatty acid component presented for absorption in the form of TO and 50% as PC along with Pol. A similar trend was noted in this group as with Gp 3 after Pol treatment was started with a slight reduction in TG transport rate observed 2 and 4 h after starting Pol treatment. By the end of 6 h of Pol therapy, however, triglyceride transport had re-

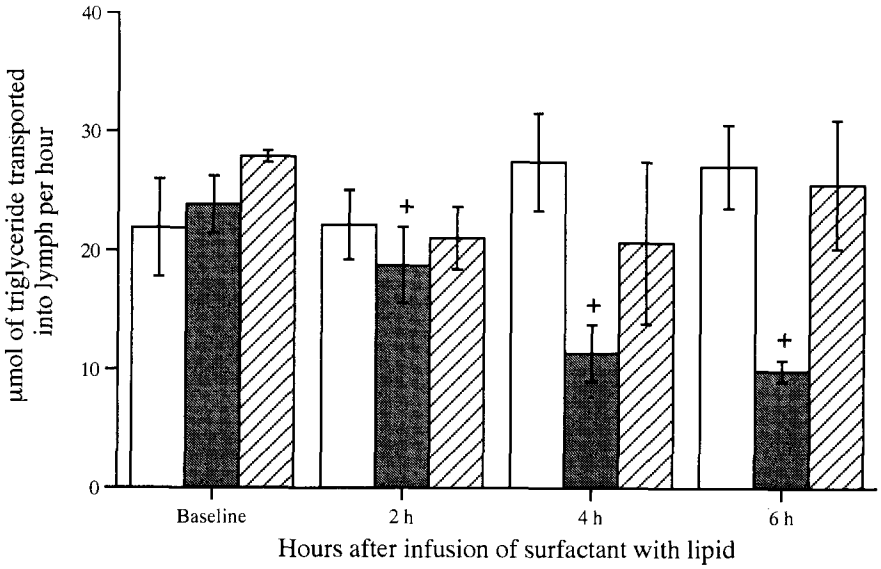

Figure 1. Secretion of triglyceride into lymph by Poloxalene treated rats given various lipids for absorption. Mesenteric lymph fistula rats were infused with a standard lipid emulsion into the duodenum containing triolein (TO), 12 mM, and no Poloxalene for 6 h. Over the next 6 h controls (Gp 1) were infused with the same mixture while one experiment group (Gp 2) was infused with TO, 12 mM, and Poloxalene and another experimental group (Gp 3) was infused with phosphatidylcholine, 18 mM, plus Poloxalene. Lymph samples were collected in 2 h periods and assayed for triglyceride. The height of the bars represents the mean ±SD. Data are presented for baseline or the results for the last 2 h of the 6 h infusion of the standard lipid emulsion and for each 2 h period after starting Poloxalene. The means of a given group for each period after starting Poloxalene is compared to the respective mean at the baseline for that group. *$p < 0.05$, $^{+}p < 0.01$. Open bar, Gp 1; solid bar, Gp 2; hatched bar, Gp 3.

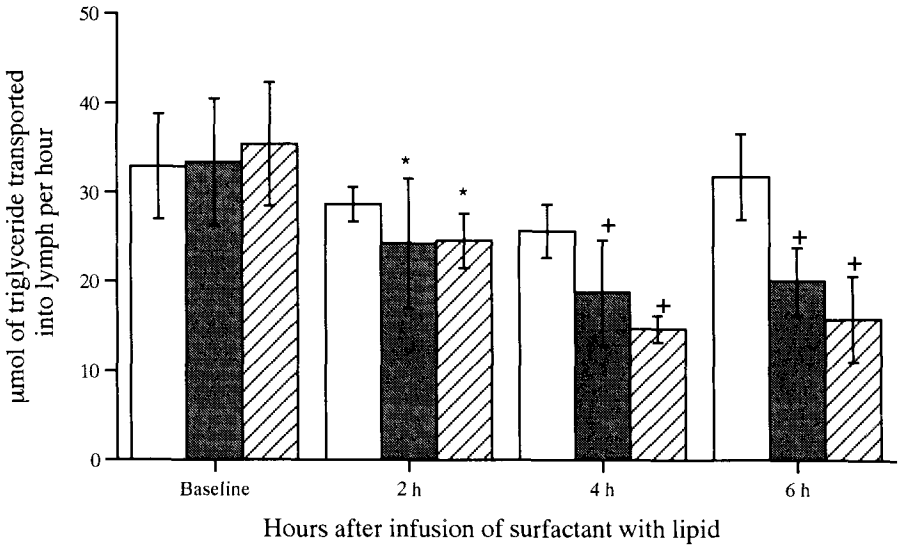

Figure 2. Secretion of triglyceride into intestinal lymph by Poloxalene treated rats given various lipid mixtures for absorption. Mesenteric lymph fistula rats were infused a standard lipid emulsion into the duodenum containing triolein (TO), 12 mM for 6 h. All rats were then infused with a lipid meal containing Poloxalene for the next 6 h. Gp 4 rats were given TO, 6 mM, and egg phosphatidylcholine (PC), 9 mM. Gp 5 was given TO, 9 mM, and PC, 4.5 mM, Gp 6 was given TO, 6 mM, and phosphatidylethanolamine, 9 mM. Lymph samples were collected in 2 h periods are assayed for triglyceride. The height of the bars represents the mean ±SD. Data are presented for baseline or the results of the last 2 h of the 6 h infusion of the standard lipid emulsion and for each 2 h period after starting Poloxalene. The mean of a given group for each period after starting Poloxalene is compared to the respective mean at the baseline for that group. *$p < 0.05$, $^{+}p < 0.01$. Open bar, Gp 4; solid bar, Gp 5; hatched bar, Gp 6.

turned to the same rate as observed at the end of 6 h of intraduodenal lipid infusion without Pol. Again, at no time period during Pol administration was the rate of triglyceride transport for this group significantly less than that observed at baseline.

Gp 5 received most of the fatty acid component presented for absorption along with Pol as TO (75%) with the remainder supplied as PC. In this group there was a more obvious decline of the triglyceride transport rate as a result of exposure to Pol. After 4 h of treat-

ment the transport rate fell about 40% to 18.8 ± 5.9 μmol/h ($p < 0.01$ as compared to baseline Gp 5). After 6 h of treatment the triglyceride transport rate was still quite low at 20.1 ± 3.8 μmol/h ($p < 0.01$ compared to the baseline).

The last group studied, Gp 6, received Pol along with a 50–50 mixture of TO and PE as the sources of lipid presented for absorption. Triglyceride transport rate rapidly declined so that after 4 h the rate of TG transport was only 45% of that observed just prior to starting Pol treatment. For all periods of lymph collection, after starting the Pol treatment, the values for triglyceride transport were significantly less than the baseline value for this group.

The phospholipid concentration in lymph at baseline and during the final 2 h period of Pol treatment was nearly similar in all groups. Values varied from 3.6 ± 0.8 μmol/h in Gp 1 to 5.7 ± 0.6 μmol/h in Gp 3. As shown in table 1 the ratio of TG to phospholipid (PL) dropped as the result of Pol treatment in each group so treated except for Gp 4 that received a 50:50 mixture of TO and PC.

Discussion

Results of these experiments confirm the inhibitory effects of hydrophobic surfactant on chylomicron secretion into lymph during periods when triglyceride is presented to the upper small intestine for absorption[1–3]. The 'mucosal block' for secretion of absorbed triglyceride into lymph is not complete, however, as very low density sized particles continue to be secreted as previously observed[2]. In the present investigation, therefore, it was expected that triglyceride output into lymph would fall with Pol treatment. While no morphologic studies on the TG-rich lipoproteins recovered in lymph were performed to confirm their small size the observation that the TG to PL ratio of lymph samples obtained under these conditions was relatively low indicates that the TG-rich lipoprotein particles were indeed small size. This is so as the total surface area of the lipoprotein particles is greater when a given mass of TG is transported in small rather than large lipoproteins. As PL is a component of the lipoprotein membrane that surrounds the TG core, the TG to PL ratio has to be relatively low when small TG-rich lipoproteins are employed to transport TG.

When PC was substituted for TO in Pol treated rats (Gp 3) there was only a transient decrease of TG transport rate into lymph that returned essentially to pretreatment values by the end of 6 h of Pol treatment. Results were basically the same for Gp 3 as those previously reported for rats treated with hydrophobic surfactant that were infused intraduodenally with PC as the only source of lipid presented for absorption except there was no transient decrease in the TG transport rate in the former study[8]. In the former study, however, rats were presented with only PC for absorption for 8 h prior to receiving the same intraduodenal infusate supplemented with surfactant. In the present study most of the lipid presented for absorption during the 6 h prior to starting surfactant treatment was in the form of TG. Thus this difference in the conduct of these two studies is at least one possible explanation for why no transient decrease in TG secretion occurred in the former study while it was quite obvious in this present one.

In the former study[8] it was noted that the particle size of triglyceride-rich lipoproteins recovered in mesenteric lymph was smaller when PC was the source of lipid presented for absorption compared to the particle size observed when triglyceride was absorbed under normal conditions. Apparently the largest of chylomicron particles are not secreted when the α-glycerolphosphate pathway is being used for the reesterification of absorbed lipids rather than the monoglyceride pathway which is the major pathway for reesterification when TG is being absorbed[13]. Others have made similar observations[14]. The reason for this is not clear, but it is known that this pathway is slower than the monoglyceride pathway[15]. The relatively slow rate of TG synthesis likely puts less demand on the enterocytes for rapid synthesis of lioprotein membrane for formation of TG-

Table 1. Ratio of triglycride to phospholipid in mesentric lymph from rats infused intraduodenally with various lipid mixtures plus Poloxalene.

Group	n	Pol	Lipid mixture*	TG to PL ratio		
				baseline	after Pol treatment	p
1	4	–	TO, 100%	6.8 ± 1.8	7.6 ± 0.9	NS**
2	5	+	TO, 100%	6.2 ± 1.7	2.8 ± 0.8	<0.005
3	4	+	PC, 100%	6.3 ± 0.7	4.5 ± 0.8	<0.005
4	4	+	TO, 50%:PC, 50%	8.8 ± 2.8	6.3 ± 1.4	NS
5	6	+	TO, 75%:PC, 25%	8.7 ± 2.7	4.5 ± 1.4	<0.01
6	4	+	TO, 50%:PE, 50%	7.8 ± 1.0	3.6 ± 0.6	<0.001

Values are mean ±SD. n is the number of animals studied.
*Percentage of fatty acid equivalents infused into the duodenum in the form of triolein (TO), phosphatidycholine (PC), or phosphatidylethanolamine (PE). **NS = not statistically significant.

rich lipoproteins. Thus there may be ample membrane components available such that there would be no compelling reason to form large TG-rich lipoproteins when only the α-glycerolphosphate pathway is being utilized to synthesize TG. Under these conditions then the enterocytes might be expected to secrete relatively small TG-rich lipoproteins. The TG to PL ratio was observed to be relatively low for Gp 3 which supports the prior observation[8] that TG-rich lipoproteins particles are relatively small when PC is the only lipid in the intestinal lumen available for absorption.

When Pol was given to rats receiving half the lipid meal as TG and half as PC (Gp 4) there was again a slight decrease in the rate of TG secretion into lymph as noted for Gp 3 with the TG secretion rate noted to return to normal by the end of 6 h of Pol treatment. As a considerable amount of TO was infused into these rats along with PC it is likely that the monoglyceride pathway of esterification was also being used as considerable amounts of 2-monoacylglyceride should have been produced from digestion of TO and absorbed. The ratio of TG to PL recoveries in lymph was relatively high approaching the value observed at baseline prior to starting Pol treatment at a time when TG absorption was taking place under normal conditions. This suggests that large chylomicrons were being secreted into intestinal lymph despite Pol therapy. Thus not all of the lipid presented for absorption has to be in the form of PC to reverse the inhibitory effects of hydrophobic surfactants on secretion of chylomicrons into lymph.

It is not clear from the results of Gp 5 (75% TO, 25% PC) whether smaller amounts of PC in relation to the TO given had any favorable effect on reversing the inhibitory action of Pol on chylomicron secretion. Output of TG into lymph fell by 44% of the baseline value after the Pol treatment but this decline was not as great as the nearly 60% drop of TG secretion observed in Gp 2 that received triolein only for absorption. During the last 2 h of Pol infusion for Gp 5 the TG secretion possibly improved slightly. The relatively low TG:PL ratio in lymph from the final 2 hours of Pol treatment indicates that the TG-rich lipoprotein particle size was relatively small.

It would appear, however, that the beneficial effects of phospholipid on chylomicron secretion in Pol treated animals is specific for PC as in Gp 6 (Pol with 50% TO 50%, 50% PE) the TG secretion rate into lymph fell to a level nearly as low as seen in Gp 2. For Gp 6 the TG transported into lymph declined 58% during Pol treatment. The TG transport rate during the last 2 h of Pol treatment in this group certainly did not approach the baseline transport rate as was the case for Gp 4 rats given the same mixture of triglyceride and phospholipid in the intestinal infusate with the exception that the phospholipid given to these rats was in the form of PC. It should also be noted that the TG:PL ratio of the final lymph sample of Gp 6 was quite low suggesting that only small TG-rich lipoprotein particles were being secreted under these conditions.

It is not known by what mechanism hydropholic surfactants inhibit chylomicron transport into lymph and it is also obviously unclear as to how PC reverses this action. Since PC can reverse this process even when significant amounts of TG are being presented for absorption as shown in Gp 4, it appears reasonable to conclude that PC maintains the normal pathway of TG metabolism and TG-rich lipoprotein formation and secretion of the enterocytes despite exposure to hydrophobic surfactants. These two surface active agents may in some way have a competitive action at an intracellular level where lipoprotein particles are being formed.

The present observations on the beneficial effects of PC on lipid absorption and chylomicron secretion by the enterocytes support prior observations that PC must be present to promote efficient transport of absorbed lipid into lymph[16]. Others have demonstrated that there are two pools of TG in the enterocytes during periods of lipid absorption, a pool that rapidly turns over and one that has a much slower turnover rate[17]. PC appears to have its major effect on the TG pool with the rapid turnover rate[18].

If hydrophobic surfactants and PC somehow compete at an intracellular site to affect lipid absorption it would be necessary for PC infused into the intestinal lumen to be rapidly digested and absorbed into the enterocytes in order to have this beneficial intracellular effect. PC is readily digested by pancreatic phospholipase A_2 to yield a free fatty acid molecule and lysophosphatidylcholine (lyso PC)[19]. The lyso PC has been shown to be quickly absorbed and reesterified back to PC[20]. Thus it is theoretically possible that infusion of PC into the duodenum will increase the intracellular pool size of PC in the enterocytes thus helping to overcome the inhibitory effects of hydrophobic surfactants on chylomicron formation and secretion.

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