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Lipid vehicles for intestinal lymphatic drug absorption

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The lipoprotein fractions in mesenteric lymph were monitored following intraduodenal administration of arachis oil and oleic, linoleic and linolenic fatty acids to rats. An increase in the chylomicron fraction, but not the VLDL or LDL fraction, was observed with each lipid. The greater the degree of unsaturation of the fatty acid, the more rapid the onset of chylomicron synthesis. The administration of linoleic acid and arachis oil produced the highest concentration of chylomicrons in the lymph. These results reflect differences in the rate of absorption and biochemical metabolism of the lipids and have implications for the selection of vehicles for the delivery of drugs by the lymphatic route.

The absorption of drugs into the lymphatic system following oral administration is of particular interest for compounds which are subject to first pass metabolism, as well as for anticancer agents directed to metastases within the lymphatics. Sieber et al (1974) and Noguchi et al (1985a) have suggested that the lymph concentration of chylomicrons (lipoprotein units produced in the enterocytes) is a major factor in controlling the uptake of lipophilic molecules into the lymphatic vessels. During the digestion of lipids containing long chain fatty acids, the synthesis of chylomicrons increases. These lipids can therefore be used to promote the intestinal absorption of drugs selectively absorbed via the lymphatic system (Palin & Wilson 1984). In the present study the chylomicron concentrations in rat mesenteric lymph were determined following intraduodenal administration of the triglyceride arachis oil and each of its constituent fatty acids; oleic, linoleic and linolenic acid. High performance size exclusion chromatography was employed to isolate the chylomicron fraction.

Materials and methods

The lipids used were oleic acid ($C_{18:1}$), linoleic acid ($C_{18:2}$) and linolenic acid ($C_{18:3}$) (Sigma Chemicals, Dorset) and arachis oil B.P. (Thornton and Ross, Huddersfield, Yorks).

Male Wistar rats were starved for 18 h, with free access to water, before anaesthesia with sodium pentobarbitone (Sagatal, 90 mg kg^{-1} i.p.). The superior mesenteric lymph duct was cannulated using a polyethylene cannula (0·8 mm i.d., 1.0 mm o.d.) as described by Noguchi et al (1985b). The inferior mesenteric lymph duct was cut and occluded using cyanoacrylate adhesive. An initial lymph sample was collected and then 0·3 mL phosphate buffered saline (pH 7·4), or lipid, was injected into the duodenum. The lymph output from the mesenteric duct was collected every 30 min for 240 min and stored at 4 °C before analysis.

Aliquots (20 μL) from each of the 30 min lymph samples were injected onto a TSK 6000PW aqueous exclusion column (Anachem, Luton, Beds) equilibrated in 0·9% w/v sodium chloride and eluted at 0·7 mL min^{-1}. The absorbance of the eluate at 290 nm was monitored; typical elution profiles are shown in Fig. 1. The area under the elution peaks was used to determine the proportion of the different lipoprotein fractions in the lymph samples. UV calibration curves were produced for serial dilutions of chylomicron peaks collected at different times during fat absorption. These followed Beer-Lambert behaviour and showed no significant differences following administration of the different vehicles. Alterations in size or chemical composition of the eluting lipoproteins were therefore assumed to have no effect on the extinction coefficient.

Results and discussion

The three major lipoprotein fractions in lymph are chylomicrons, very low density lipoproteins (VLDL) and low density lipoproteins (LDL). Analysis of the particle size in each eluate fraction by photon correlation spectroscopy (Malvern Instruments), gave 'z' average diameter values of 280 nm, 100 nm and <30 nm. These agree with previously quoted values for chylomicrons, VLDL and LDL, respectively (Counsell & Pohland 1982). The elution profiles obtained for the VLDL and LDL fractions were similar to those produced from plasma samples by previous workers, who found good correlation with other methods of lipoprotein separation, such as density gradient ultracentrifugation and soft agarose gel chromatography (Wehr et al 1982; Carroll & Rudel 1983). Repeated chromatography of the lipoprotein peaks, obtained after administration of arachis oil, indicated greater than 90% recovery of all three lipoprotein classes.

Throughout the experimental period, the composition of the lymph in the control group remained constant, with the relative percentages of each lipoprotein fraction being: chylomicrons 39 ± 3·2%, VLDL 56·7 ± 3·1%, and LDL 4·3 ± 0·5% (mean ± s.d.).

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Following administration of each lipid, there was a significant increase in the percentage of the chylomicron fraction and a corresponding decrease in the VLDL and LDL percentages, compared with the control and the time zero samples. No significant differences were observed in the lymph flow rate. For each lipoprotein fraction in each sample, the ratio of the area under the elution curve to that of the sample taken before dosing was calculated. The ratios for both the VLDL and LDL fractions remained approximately one throughout the experiment for each lipid. The ratios for the chylomicron fraction showed significant, but differing increases with each lipid (Fig. 2). The ratio values were compared using a one way analysis of variance.

With each increase in the degree of fatty acid unsaturation, the lag-time to a significant increase in the chylomicron concentration was reduced compared with the control. This difference in the onset of chylomicron synthesis probably reflects a more rapid rate of absorption with increasing fatty acid unsaturation. The greater the degree of unsaturation of the fatty acid, the lower the melting point, the greater the fluidity at 37 °C and the less hydrophobic the molecule—all factors which will facilitate absorption. The longest lag time to increased chylomicron output was observed after administration of arachis oil. Unlike the fatty acids, arachis oil cannot be absorbed directly; it has to be hydrolysed by pancreatic lipase to the constituent fatty acids and 2-monoglycerides before uptake into the mucosal cells.

A steady state lymph chylomicron concentration was reached with each fatty acid, linoleic acid producing the highest concentration followed by linolenic acid and then oleic acid. These data probably reflect differences

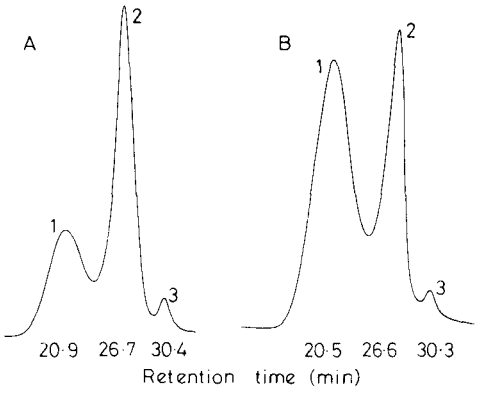

FIG. 1. The elution profile for (A) fasted rat and (B) rat dosed with arachis oil: 1, chylomicron peak; 2, VLDL peak; 3, LDL peak.

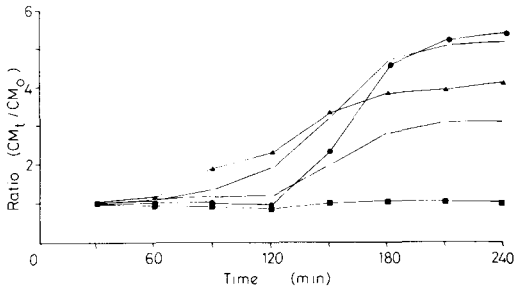

FIG. 2. The lymph chylomicron concentration (expressed as a ratio of the concentration at time t to that at time zero) following intraduodenal administration to rats of 0·3 ml volumes of ● arachis oil, □ oleic acid, ○ linoleic acid, ▲ linolenic acid, ■ phosphate buffered saline (pH 7·4), (mean, n = 6 per group).

in the biochemical pathways and rates of enzymatic reaction within the mucosal cell for the incorporation of the different fatty acids into chylomicrons (Brindley 1974). After a long lag time, by 240 min the lymph chylomicron concentration produced by administration of arachis oil was approaching a steady state equal to that of linoleic acid.

This study suggests that linoleic acid is the most suitable of the lipid vehicles investigated for the delivery of drugs to the lymphatic system since it rapidly stimulated high levels of chylomicrons in the lymph. Further investigations are required to assess the potential of fatty acids of different chain lengths and of mixed lipid systems.

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