Ultrastructural and biochemical studies on the intestinal absorption of a medium-chain triglyceride (MCT), tricaprylin

Abstract The intestinal absorption of a medium-chain triglyceride (MCT) was studied by electron microscopy and biochemical analysis. In jejunal absorptive cells of rats fed tricaprylin, the smooth endoplasmic reticulum in the apical cytoplasm appeared to increase in number and contained one or two particles about 40–80 nm in diameter that were less electron dense and similar in size and profile to very low density lipoprotein. Similar particles were also observed packed in the dilated Golgi sacs and in the extended intercellular spaces. These particles were remarkably increased in number as compared with those in fasted rats. Biochemical analysis of lymph from the main intestinal lymph duct showed that caprylate was apparently demonstrated only in the lymph of rats given tricaprylin at the maximum rate 3 h after oral administration. The study strongly suggests that medium-chain triglyceride is at least in part transported via lacteal, possibly in the form of very low density lipoprotein.

Key words Electron microscopy · Medium-chain triglyceride · Intestinal absorption · Lacteal · Very low density lipoprotein (VLDL)

Introduction

Medium-chain fatty acid triglycerides (MCTs) have become widely available for clinical trial, particularly for malabsorption of fat. This is mainly based on physiological and biochemical studies showing that the absorption of MCTs is different in mode from that of long-chain fatty acid triglycerides (LCTs); that is, MCTs can be absorbed intact as triglyceride, and even medium-chain fatty acids (MCFAs) produced by either intraluminal or intracellular lipase are transported mainly by the portal blood vessels without being reesterified in the absorptive cells. However, recently small amounts of MCFAs have been found in chylomicrons in humans and animals fed MCTs, and the fact that oral administration of MCTs induces an increase in octanoyl Co A of nearly twofold in rat intestinal mucosa was demonstrated by Ohkubo et al. Their work has suggested strongly that some MCFAs produced by luminal lipase, if not all, are reesterified in the absorptive cells. If this is the case, these reesterified triglycerides must be absorbed in the form of lipoprotein particles seen under the electron microscope, as in the absorption of LCTs.

Ultrastructural morphology of intestinal absorption of LCTs has been well defined so far, whereas that of MCTs is poorly understood. The present study was carried out to determine whether reesterification and consequent formation of lipoprotein particles occur in the intestinal absorption of MCTs (tricaprylin), and, if those occur, to clarify the transport pathway of MCTs across the intestinal epithelium.

Materials and methods

Electron microscopy

Male Wistar rats weighing about 120g were used in this study. After fasting for 24 or 48 h, 10 rats were fed 1.5 or 2.0 ml of tricaprylin, a triglyceride of caprylic acids (Wako, Osaka, Japan), by means of a gastric tube inserted via the esophagus into the stomach. After 3, 5, 10, 30, or 60 min of administration, the rats were anesthetized by ethyl ether and the upper segment of the jejunum was removed. Immediately after removal, the materials were cut into small bits, and transferred into and fixed either in 2% paraformaldehyde and 2.5% glutaraldehyde buffered with 0.1 M phosphate at pH 7.4 for 2 h, followed by 2% osmium tetroxide in the same buffer for 2 h, or in 2% osmium tetroxide alone for
Control rats were fasted for 24 or 48h and then the upper jejunum was treated as described. All these specimens were dehydrated in graded ethanols and embedded in epoxy resin. Thin sections were made with a diamond knife in an ultratome, stained with uranyl acetate and lead tartrate, and then examined in a transmission electron microscope.

Fatty acid analysis of lymph

Animal treatment

Five male Wistar rats weighing 200–300g were given ad libitum a commercial nonpurified diet (CE-2; Nihon Clea, Tokyo, Japan) and drinking water. Rats under nembutal anesthesia were subjected to cannulation of the main intestinal lymph duct with tubing (clear vinyl tube SV-35), and an indwelling catheter (Polyethylene tube, 0.8 mm ID, 1.2 mm OD) was placed in the stomach. After surgery, animals were placed in restraining cages in a warm recovery room and allowed free drinking water containing physiological saline–5% glucose. In the next morning, after a collection of lymph for 2h (blank lymph), each animal was given 1ml of a test oil via stomach tube, and the lymph was collected at 3-h intervals for 24h. The test oil was 99% tricaprylin or soybean oil (Table 1). Lymph collected from the rats fasted overnight was prepared as described.

Procedure for fatty acid analysis

Lymph (0.5ml) was mixed in chloroform:methanol (2:1 volume), and lipids were extracted by the method of Folch et al. Triglyceride was separated by thin-layer chromatography on a silica gel 60 F254 plate with fluorescent indicator (Merck, Darmstadt, Germany). After saponification, fatty acids of total lipid or the triglyceride fraction were extracted with hexane and methylated with a borofluorate-methanol mixture. Methyl pentadecanoate (GL Science, Tokyo, Japan) was added to each methylated fatty acid fraction as an authentic standard for the calibration of recovery. Fatty acid composition was analyzed by gas chromatography (Shimadzu GC-14; Kyoto) equipped with a 5% Shinchrom E71 column (3.2 mm × 3.1 m). For all fatty acid analysis, oven temperature was initiated at 140°C, elevated to 215°C by a temperature gradient of 3°C/min, and was then held for 60 min. Triglyceride concentration was assayed by triglyceride test (Wako), principally based on Fletcher's method.

Results

Electron microscopy

Jejunal absorptive cells in fasted rats

In the apical cytoplasm, the smooth endoplasmic reticulum was located just beneath the terminal web in the form of a loosely waven lattice of tubules, but was not so abundant (Fig. 1). Some vesicular elements of the smooth endoplasmic reticulum were slightly dilated but appeared empty. The ribosomes were not on its surface, and were distributed in close association with the smooth endoplasmic reticulum. The Golgi apparatus in the supranuclear cytoplasm consisted of flattened sacs, vacuoles, and vesicles, and their dilated sacs were packed with small particles about 40nm in diameter, which were of less electron opacity and quite similar in appearance to very low density lipoprotein (VLDL) (Fig. 2). Similar particles, although only a few, were frequently observed in the expanded intercellular spaces of the intestinal absorptive cells (Fig. 2) and in the central lacteal, but not in the blood capillary.

Absorption of tricaprylin

After the animals were fed tricaprylin, the smooth endoplasmic reticulum appeared to be increased in the apical cytoplasm of absorptive cells, which was tubular or vesicular in profile and contained one or two less electron dense particles 40–80nm in diameter, probably VLDL (Fig. 3). Cisterns of the Golgi apparatus were dilated and packed with the particles mentioned (Fig. 4). The intercellular spaces between neighboring absorptive cells were often extended, and here similar particles were accumulated (Figs. 4, 5). These particles then leaked out through gaps in the epithelial basal lamina into the lamina propria (Fig. 5). These particles in the lamina propria were also observed either in the intercellular spaces or in between fibrous components of the connective tissue. Further large numbers of similar particles were seen in the lumen of the lymphatics (Fig. 6).

Fatty acids of lymph

For lipid analysis, lymph was collected for 3-h intervals until 9h had passed from individual rats, and that pooled from 9 to 24h was used. Table 2 shows lymph volume in ml/h and triglyceride content in mg/h in each fraction. In comparison with pretest, lymph volume was slightly increased, although the difference was not statistically significant, after administration of MCT and soybean oil. Triglyceride content after administration of soybean oil was greatly increased, showing the maximum value after 3–6h, and the increase continued for the following 3h. A slight increase in triglyceride content was observed after administration of MCT,

<table>
<thead>
<tr>
<th>Fatty acid composition of oils</th>
<th>MCT</th>
<th>Soybean oil</th>
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<tbody>
<tr>
<td>8:0</td>
<td>99.9</td>
<td>0</td>
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<tr>
<td>16:0</td>
<td>10.3</td>
<td>3.8</td>
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MCT, medium-chain triglyceride

1Number of C; number of double bonds

Values are weight % of fatty acids