53. Studies on Fat-Storing Cells. I

Ingestion of Triton WR-1339 by Fat-Storing Cells

By Sakae Yumoto

Department of Anatomy, Faculty of Medicine,
University of Tokyo, Tokyo 113

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Summary. Triton WR-1339 was intraperitoneally injected into rats and its accumulation in hepatic lobule cells was observed under an electron microscope. In the lipid granules of fat-storing cells, much Triton accumulated during the first 30 min to 3 hr post-injection. Kupffer cells scarcely, and endothelial cells slightly, ingested Triton.

Introduction. Three kinds of cells are localized in the sinusoidal region of the hepatic lobule, i.e. Kupffer cells, endothelial cells and fat-storing cells (FSC). The latter, according to Ito, are localized in the perisinusoidal space of Disse, outside the sinusoidal wall; they have well developed cytoplasmic lipid granules and, unlike Kupffer- and endothelial cells, they do not ingest foreign substances such as metals, dyes and fat-emulsions.

In the present study, Triton WR-1339 was intraperitoneally (i.p.) injected into rats and the ingestion of this substance by FSC was examined. Triton WR-1339 is specifically accumulated in the lysosomes of hepatic parenchymal cells. Therefore, the substance is used for the isolation of lysosomes from hepatocytes.

The present paper demonstrates that FSC ingest foreign substances contained in the blood.

Materials and methods. Triton WR-1339 was purchased from Ruger Chemical Co. (Irvington, N.J., U.S.A.) or Sigma Chemical Co. (Saint Louis, Mo., U.S.A.). Male Wistar rats, weighing 200 to 250 g were obtained from Shizuka Laboratory Animal Center (Hama-matsu, Shizuoka, Japan). They were given a single i.p. injection of 100 mg Triton/100 g body weight in a 0.9% saline carrier. The animals were fasted for 12 hr and then decapitated at various intervals after the injection. Liver specimens from each animal were fixed in a solution containing 2.5% (w/v) glutaraldehyde, 2.0% (w/v) formaldehyde, 2 mM CaCl₂, and 0.1 M cacodylate buffer (pH 7.4). The samples were postfixed with 1% (w/v) osmium tetroxide in 0.1 M cacodylate buffer (pH 7.4), stained with 0.5% (w/v) uranyl
acetate in 0.05 M maleate buffer (pH 5.2), dehydrated by a graded series of ethanol, and embedded in Epon. Sections were cut on an LKB Ultratome III microtome and inspected under a Hitachi HU-12 electron microscope.

Results. From 30–60 min after the injection of Triton, electron-lucid areas appeared in the lipid granules of FSC. Inside these areas, membranous structure was frequently recognized (Fig. 1). These features are morphologically characteristic for intracellularly accumulated Triton WR-1339.21,4,17 The ingestion of this substance was not evidenced in the cytoplasm of the Kupffer- and endothelial cells.

From 2–3 hr post-injection, Triton accumulation further increased in FSC lipid granules, which increased in size (Figs. 2 and 3). On the other hand, cytoplasmic ingestion of Triton by Kupffer cells was scarcely seen (Fig. 3). Small accumulations of Triton were occasionally seen in the lysosomes of endothelial cells. At 2–3 hr post-injection, the ingestion of Triton by hepatic parenchymal cells began.
By this time, almost all of the mitochondria in FSC had become swollen; electron density was low in the mitochondrial matrix. No swollen mitochondria were noted in the Kupffer-, endothelial-, or

Fig. 2. Two hr after injection, the accumulation of Triton increased in the lipid granules of FSC. Mitochondria in FSC became swollen and mitochondrial matrix was lowered in electron density. Lipid granule (L), mitochondrion (m), rough endoplasmic reticulum (R). Bar, 1 µm. ×25,000.

Fig. 3. At 3 hr post-injection, Triton WR-1339 was remarkably accumulated in the lipid granules of FSC. On the other hand, no accumulation could be detected in adjoining Kupffer cells. Furthermore, no Triton was incorporated into the lysosomes of these cells (arrow). FSC (F), lipid granule (L), Kupffer cell (K). Bar, 1 µm. ×13,000.
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parenchymal cells. Autophagic vacuoles were frequently seen in the cytoplasm of FSC.

Discussion. Ponfick,10 who first reported the presence of cells specializing in the ingestion of a foreign substance (cinnaber) in the sinusoids of the hepatic lobule, postulated that these cells (Zinnoberzellen) were localized in the perisinusoidal space, outside the wall of blood vessels. Later, Kupffer10) maintained that cells with the ability to ingest foreign substances and erythrocytes directly faced the lumen of blood vessels and constituted the vascular wall. This concept has subsequently found wide acceptance.1,8,20,22) Ito9) reported that unlike Kupffer- and endothelial cells which directly face the lumen of blood vessels and ingest foreign substances, FSC are located outside the vascular wall and do not ingest these substances. At present, FSC are thought to be specialized cells for the storing of vitamin A.5,11,15,18,23)

Triton incorporation into the lysosomes of hepatocytes reportedly begins 3 hr after i.p. injection, and accumulation peaks 3–5 days thereafter.4,17) In the present study, a large amount of Triton was ingested by the lipid granules of FSC within 30–180 min of i.p. injection, while there was hardly any ingestion by Kupffer cells, despite the concept that these cells are highly active in the ingestion of foreign substances.2,20,22)

The present results indicate that, although FSC are located outside the blood vessel, FSC play a role in the ingestion of foreign substances in the hepatic sinusoids. The endothelial cells of the hepatic sinusoids contain many pores (0.1 μm in diameter) which form “sieve plates”.21) In addition, large intracellular fenestrations (1–3 μm in diameter) and gaps of various sizes between adjacent endothelial cells are present in the vascular wall.13,14)

Based on present observations, the following hypothesis is proposed: FSC, Kupffer- and endothelial cells have characteristic cell surface receptors resulting in unique mechanisms providing for the ingestion of extracellular substances. These 3 types of cells may constitute a cooperative system in the sinusoids of the hepatic lobule, facilitating the removal of various foreign substances and harmful substances from the blood.

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References