Ultrastructural Changes Produced by Triparanol in Morris 5123 and Novikoff Hepatomas

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Summary

Force feeding of large amounts of triparanol to rats bearing transplanted hepatomas results in appearance of myeloid bodies in liver cells and in hepatoma cells. The myeloid bodies are most numerous in liver cells, less abundant in the cells of Morris 5123 hepatoma, and rare in the cells of Novikoff hepatoma. The quantity of myeloid bodies can be correlated with development of endoplasmic reticulum and with duration of treatment.

Introduction

Triparanol is a known inhibitor of the final stages of cholesterol synthesis (2, 25). Administration of triparanol is followed by accumulation of desmosterol in various tissues (9) and by cytologic alterations in the adrenal cortex (28). Large doses of triparanol produce characteristic changes in the ultrastructure of liver cells and of pancreatic acinar cells (15). Clinical studies have shown that triparanol may influence the growth of some carcinomas (6, 18).

Triparanol could be of value as a chemotherapeutic agent in spite of its toxic side effects (1, 5, 19, 29) if a significant difference was shown between its cytotoxic effects on normal and on neoplastic cells. An attempt to demonstrate differences in ultrastructural changes produced by large doses of triparanol in 2 transplantable hepatomas and livers was the purpose of the present study. Alterations in the cytoplasm were most pronounced in the liver cells, less marked in the cells of Morris 5123 hepatoma, and slight in the cells of Novikoff hepatoma.

Materials and Methods

Adult male rats of comparable age and weight were used in each experiment. The experimental and control diets were force-fed by a stomach tube (30) in 3 equal daily doses at 6-hour intervals for 3 or 5 days, respectively. The experimental diets contained 250 mg of triparanol/kg body weight/day in a liquid diet previously described (30). The proteins in the diet were supplied in the form of casein hydrolysate. Triparanol was supplied in powder form by the Wm. S. Merrell Company. Triparanol was in the form of casein hydrolysate. Triparanol was supplied in powder form by the Wm. S. Merrell Company. Triparanol was lacking in the control diet. The rats were fasted overnight before the onset of experimental feeding and before killing.

Adult male Buffalo rats were used for studies on the Morris 5123 hepatoma. The tumor was implanted s.c. into rats weighing 215-240 g and allowed to grow for approximately 1 month. At that time the tumor size ranged from 1.6 to 2.3 cm in diameter. Holtzman rats weighing 315-340 g were similarly inoculated with the Novikoff hepatoma cells. Experimental feeding was started on the 7th day when the tumor was about 1.8-2.3 cm in diameter. The individual groups are listed in Table 1.

Following treatment, the rats were fasted overnight, and samples of liver and hepatoma were taken from each rat under light ether anesthesia. The tissues were fixed in 1% osmium tetroxide in s-collidine buffer (4), pH 7.4, at 5°C for 1 hr. The tissues were dehydrated in graded alcohols and embedded in methacrylate with 2% benzoyl peroxide as catalyst. Thin sections were cut on a Porter-Blum microtome and stained with lead by Karnovsky Method A (16). The specimens were photographed in the RCA EMU 2 and RCA EMU 3 electron microscopes.

Additional samples of livers and hepatomas were used for light microscopic studies. The formalin-fixed tissues were used for hematoxylin and eosin stains. Livers fixed in formaldehyde-calcium solution were used for Baker's acid hematein test to demonstrate phospholipids (3). Other pieces of livers fixed in weak Bouin's solution (3), treated with pyridine, and subsequently stained with acid hematein were used as controls.

Results

The ultrastructural changes produced by large doses of triparanol in hepatoma cells and in liver cells were similar structurally, but differed quantitatively. The cells of animals treated with triparanol contained characteristic myeloid bodies, which were more abundant in liver cells than in the cells of hepatomas.

Light Microscopy Studies

Clear cytoplasmic vacuoles were found in hematoxylin and eosin stained sections of liver cells (Fig. 1). The number of these vacuoles was increased with duration of treatment. Cytoplasmic inclusions were seen in thick sections of methacrylate-embedded livers when viewed in the phase microscope. These inclusions were also demonstrated by the Baker's acid hematein method (Fig. 2), and they contained phospholipids extractable by pyridine.

Electron Microscopic Studies

The characteristic alteration in the cytoplasm of liver cells and hepatoma cells of rats fed triparanol was the appearance of myeloid bodies. These bodies were most abundant in liver cells,
The number of myeloid bodies increased with duration of treatment. Differences in the structure of myeloid bodies were also observed.

Liver Cells

The myeloid bodies in the liver cells of rats treated with triparanol for 3 days were about the size of mitochondria or larger (Fig. 3). They contained concentric layers of smooth membranes and were surrounded by a separate membrane. Connections between the myeloid membranes and endoplasmic reticulum were not found. The myeloid bodies were seen in many, but not in all, liver cells, and only a few bodies were found in a given cell. Most hepatocytes of rats fed triparanol for 5 days contained myeloid bodies, and the number of myeloid bodies in a single cell was markedly increased. In comparison with the simple myeloid bodies seen on the 3rd day, the myeloid bodies observed on the 5th day were often more complex. Two or more groups of concentric myeloid membranes appeared to form a large complex myeloid body (Fig. 4). Many myeloid bodies also contained homogeneous, moderately electron dense material which was occasionally connected with the myeloid membranes (Fig. 5).

Other changes observed in the hepatocytes of triparanol-treated rats were a diffuse distribution of the usually pericanalicular dense bodies, an increase of smooth endoplasmic reticulum, and a variable degree of disorganization of rough endoplasmic reticulum.

**Morris 5123 Hepatoma**

The structure of this hepatoma from untreated rats was the same as that previously described (7, 14). Myeloid bodies appeared in nonnecrotic Morris 5123 hepatoma cells after treatment with triparanol. These bodies were present in moderate numbers after 3 days of treatment and were more abundant after 5 days of treatment, but their quantity was in general smaller when compared with liver cells similarly treated.

Myeloid bodies from Morris 5123 hepatoma cells also showed minor structural differences. The membranes in the center of the body were often more densely packed than in the periphery of the body. Granular material was found in the center of some myeloid bodies (Fig. 7). Continuation of treatment with triparanol resulted in a moderate increase in number of hepatoma cells containing myeloid bodies, but even at 5 days some cells were free of these bodies. The myeloid bodies become more complex (Fig. 6), but in contrast to liver cells lipid material was not found in them. While most myeloid bodies were intracytoplasmic, in a few instances whorls of myeloid membranes or myeloid bodies were seen in bile canaliculi. These bodies may have formed within the canaliculi.

**Novikoff Hepatoma**

The fine structure of the previously described Novikoff hepatoma (12, 14) was least affected by triparanol treatment. Myeloid bodies were seen rarely in nonnecrotic cells even after 5 days of treatment. These bodies often contained granular material (Fig. 8), and some were very large. The myeloid bodies were distinctly different from the vacuoles containing granules and vesicles (Fig. 9, inset), which are present also in untreated Novikoff hepatoma cells (14). These vacuoles were seen more frequently, and were often larger after triparanol treatment (Fig. 9), than seen in untreated Novikoff hepatoma cells.

**Discussion**

Ultrastructural studies show a marked difference between the response of liver cells and hepatoma cells to administration of large doses of triparanol. Although the myeloid bodies appear characteristically as a response to triparanol in both normal and neoplastic cells, they are rare in the cells of Novikoff hepatoma and less numerous in Morris 5123 hepatoma cells than in the liver cells. Formation of myeloid bodies is not restricted to liver cells and their neoplastic derivatives, as they were found also in other tissues (13, 15).

The formation of myeloid bodies after treatment with triparanol may be related to (a) detoxification of triparanol, (b) direct injury of endoplasmic reticulum by triparanol, and (c) accumulation of desmosterol in place of the lacking cholesterol in the membranes of endoplasmic reticulum.

It is known that various drug-metabolizing enzymes are localized in the endoplasmic reticulum (8). The usual response of the liver cell to administration of drugs is hyperplasia of smooth endoplasmic reticulum (23) and formation of smooth fingerprints, as exemplified by phenobarbital (11, 23). While triparanol is localized in endoplasmic reticulum (10), the alterations produced by triparanol are quite different from those produced by phenobarbital (11). The well-formed myeloid bodies are usually sequestered by a limiting membrane and do not show transitions to the neighboring endoplasmic reticulum.

Inhibition of drug-metabolizing enzyme systems by triparanol (10, 17) may be explained either by a direct injury of endoplasmic reticulum by triparanol, which has been shown to be present in the liver (20), or by the effect of triparanol on cholesterol synthesis. Diethylaminomethylphenyl-n-propylacetate (SKF 525-A) and triparanol are both derivatives of β-diethylaminoethanol. Both compounds inhibit drug-metabolizing enzymes (17) and interfere with formation of hepatic cholesterol (26). Formation of myeloid bodies was, however, not observed after administration of SKF 525-A (24).
The present observations and the previously described stages in the formation of myeloid bodies (27) suggest that the cytoplasmic changes produced by triparanol represent an overproduction of lipoproteins by the endoplasmic reticulum and their subsequent sequestration. Triparanol interferes with formation of cholesterol from desmosterol (2, 25) in endoplasmic reticulum (10) and results in accumulation of desmosterol (9). The altered composition of lipoprotein membranes appears to be followed by an overproduction of membranes. The newly formed membranes apparently have abnormal composition, because they are sequestrated. The persistence of myeloid membranes in myeloid bodies suggests inadequate degradation of sequestered material.

The hepatoma cells contain in general fewer myeloid bodies than the liver cells. The neoplastic cells are either more resistant to injury by triparanol than is the normal liver cell, or the neoplastic cells do not react to the injury as markedly as does the liver cell. The myeloid body is a cytoplasmic lesion which can be interpreted as an attempt of the cell to compensate for the injury to membranes which is unsuccessful and is followed by the process of focal degradation (13).

The differences in the quantity of myeloid bodies in liver cells and hepatoma cells can be well correlated with the amount of rough endoplasmic reticulum in respective cells. Different metabolism of cholesterol in tumors (22) and absence of drug-metabolizing enzymes in tumors (21) may be contributory factors. Whether the diminished response of the hepatoma cell to triparanol also represents a greater resistance to injury must be decided in chronic experiments.

References
Fig. 1. Portion of liver from a rat treated with triparanol for 3 days. Numerous vacuoles are seen in the cytoplasm of hepatic cells. H & E, × 380.

Fig. 2. Portion of liver from a rat treated with triparanol for 3 days. The cytoplasm of liver cells shows numerous dark bodies of various sizes. Nuclei (x) are pale. Acid hematein stain, × 380.

Fig. 3. Myeloid bodies from a hepatocyte of a rat treated with triparanol for 3 days. The bodies consist of concentric layers of smooth membranes. × 37,000.

Fig. 4. Portion of 2 hepatocytes from a rat treated with triparanol for 5 days. Several myeloid bodies form complex bodies. Limiting membrane is seen at arrows. Other structures seen are microbodies (m), Golgi complex (G), mitochondria, and smooth and rough endoplasmic reticulum. × 26,000.

Fig. 5. Myeloid bodies containing amorphous material in a hepatocyte of a rat treated with triparanol for 5 days. Limiting membrane is seen at arrows. Other structures seen are multivesiculate bodies (v), microbodies (m), and mitochondria. × 26,000.

Fig. 6. Complex myeloid body from a cell of Morris 5123 hepatoma from a rat treated with triparanol for 5 days. A simple myeloid body is seen at the top. Other structures seen are nucleus (N), microbodies (m), mitochondria, and endoplasmic reticulum. × 37,000.

Fig. 7. Portion of a cell from Morris 5123 hepatoma from a rat treated with triparanol for 3 days. Myeloid bodies (B) are small and often fused with other bodies into larger complexes. Granular material (x) in the center of myeloid bodies. Single membrane (arrows) surrounds 1 group of myeloid bodies. Microvilli cover canalicular surfaces (L). × 26,000.

Fig. 8. Portion of a cell from the Novikoff hepatoma from a rat treated with triparanol for 3 days. Myeloid bodies (B) are small and contain granular material. Other structures seen are nucleus (N), annulate lamellae (A), Golgi complex (G), endoplasmic reticulum, and free ribosomes. × 25,000.

Fig. 9. Portion of a cell from the Novikoff hepatoma from a rat treated with triparanol for 5 days. Large vacuoles contain vesicles, granules, and membranous material. Other structures seen are mitochondria, annulate lamellae (A), fibrillar material (F), endoplasmic reticulum, and free ribosomes. × 37,000. Inset: Small vacuoles containing vesicles, granules, and membranous material from another cell. Rat treated as in Fig. 9. × 26,000.
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