ULTRA-STRUCTURE OF THE LIVER IN PROPYLTHIOURACIL INDUCED HEPATITIS

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SUMMARY : A case of cholestatic hepatitis occurring in a 50 year old woman treated with propylthiouracil for 1.5 months is presented. Physical examination showed generalized icterus and nodular goiter. Electron microscopic examination of liver biopsy revealed dilated smooth and rough endoplasmic reticulum, mitochondrial inclusions, excessive lipofuchsin granules and vacuolar formation both in the cytoplasm and nucleus of the hepatocytes. The ultrastructural changes in the liver have never been described in detail in propylthiouracil induced hepatitis previously. In this article the possible pathophysiology of the disease is discussed.

Key Words : Propylthiouracil, liver, electron microscopy.

INTRODUCTION

Propylthiouracil (PTU) is a widely used drug for the treatment of hyperthyroidism. Hepatic damage is a rare side effect of PTU and there have been only a few reported cases of PTU induced hepatic injury (1-4, 9, 12, 14,17). The histologic hepatic damage has been recorded as acute periportal inflammation, bile stasis, sub-massive hepatic necrosis or chronic active hepatitis (5, 8, 9, 17). To our knowledge, the ultrastructural abnormalities in the liver have never been reported in detail previously. The aim of this report is to include the ultrastructural changes in the liver to the present data about the PTU induced hepatitis.

CASE REPORT

A 50 year old female was diagnosed as goiter and treated with 300 mg of propylthiouracil daily. Fatigue, pruritus, anorexia, nausea and vomiting began after 15 days of treatment. During the following days, her complaints became aggravated and she was referred to our hospital. Physical examination revealed generalized icterus and nodular goiter. Laboratory values at the time of admission were as fol-

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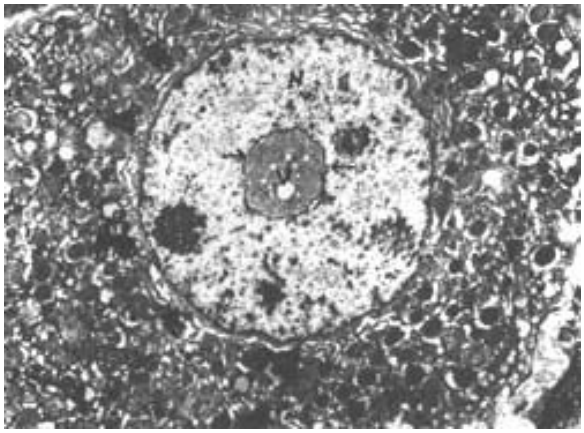
Figure 1: The cytoplasmic patches with variously sized vacuoles (V) surrounded by double membrane (arrows) are seen within the nucleus (N). Lipofuchsin granules (Lf). x 6000.



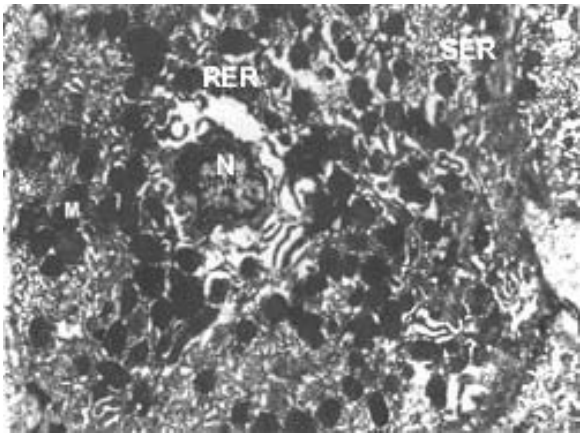
lows serum SGOT: 66 IU/L; SGPT: 116 IU/L; serum alkaline phosphatase: 414 IU/L; serum total bilirubin : 9.3 mg/dl; serum direct bilirubin 6.1 mg/dl; blood urea nitrogen: 19 mg/dl; serum creatinine: 0.9 mg/dl. Complete blood count showed Hb of 12.9 g/dl and hematocrit of 39.6%. The white blood cell count was 10.500 cells/mm^3. Serologic investigation included tests for hepatitis A, hepatitis B, hepatitis C, Epstein-Barr virus (EBV) and cytomegalovirus (CMV), all were negative. Determinations for antinuclear, anti-mitochondrial and anti-smooth muscle antibodies were not present. The thyroid function tests were as follows T_3 : 1.4 ng/ml; T_4 : 12 ng/ml; TSH : 0.08 ng/ml. The liver biopsy was performed for histologic examination and minute pieces from the liver biopsy were immediately placed in 5% glutaraldehyde buffered at pH 7.5 with phosphate buffer for 4 h. The pieces of tissue were subsequently fixed in 1% OsO_4 2 h. They were then dehydrated in graded ethanol and embedded in Araldite; 500 A° thick sections were cut by a Reichert OMU-3 ultra-microtome, stained evenly with acetate and lead citrate and examined with a Zeiss EM 900 electron microscope.

Electron microscopic findings

The electron microscopic appearance of liver biopsy material varied considerably from moderate structural changes to cytoplasmic lysis in the hepatocytes.

The most striking feature in the nucleus of some of the hepatocytes was the occurrence of cytoplasmic patches. There were electron lucent vacuoles of various size surrounded by double membrane delimited by heterochromatin (Figure 1). Most of the hepatocytes exhibited dilated smooth and rough endoplasmic reticulum forming variously sized vacuoles throughout the cytoplasm (Figure 2). The mitochondria were diffusely distributed and their matrices were highly electron dense. The intercristal spaces appeared shrunken in many but not all mitochondria (Figure 3). Shapes and sizes of mitochondria varied considerably and some were even of giant size, elongated, ballooned as they appeared in the micrographs. The more striking structural abnormality of mitochondria was the presence of electron dense inclusions. Paracrystalline or parallel filamentous inclusions were found in the matrices of normal sized

Figure 2: There were dilated smooth (SER) and rough (RER) endoplasmic reticulum cisternae in the cytoplasm of hepatocyte Nucleus (N), mitochondria (M). x 8800.



mitochondria, but mostly and particularly in the giant mitochondria. They were usually arranged in parallel rows along the long axis of elongated mitochondria. They were situated centrally or peripherally and connected to the inner mitochondrial membrane, but were randomly scattered in the matrix (Figures 4 and 5).

The peroxisomes appeared as integrated with other cytoplasmic organelles, in particular with the endoplasmic reticulum. They are randomly distributed in the cytoplasm and contained homogenous, amorphous, finely granular matrix (Figure 3).

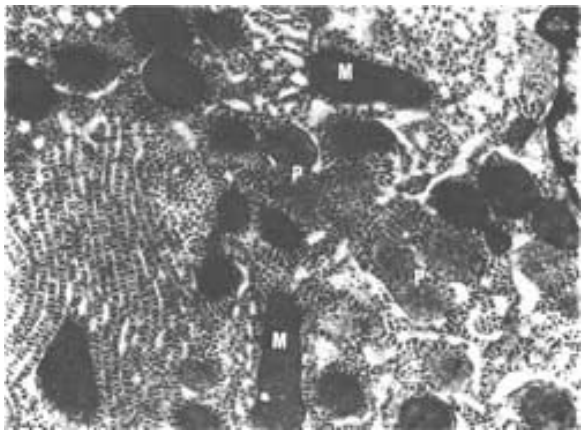
One other prominent feature was the occurrence of variously sized vacuoles in the cytoplasm of hepatocytes. Some of the vacuoles contained a membranous structure surrounded by a single membrane. They were connected with the cisterna of endoplasmic reticulum and mitochondria, some of which fusing each other formed larger vacuoles. The glycogen particles were present throughout the cytoplasm, but particularly around the vacuolar spaces and electron dense mitochondria (Figure 6).

In micrographs, the cytoplasm of some of the neighboring hepatocytes were more electron dense than the others and exhibited excessive lipofuchsin granules (Figure 4); some of which were enormously large, almost reaching to the size of nucleus. Lipofuchsin granules containing numerous, membrane bound tiny lipid droplets were frequently noted (Figure 7). In some of the hepatocytes, the cytoplasm became lytic and the cytoplasmic organelles highly degenerated. The inner and outer membrane of mitochondria were fragmented and their matrices were lytic. These cells also had large numbers of lipofuchsin granules, degenerated endoplasmic reticulum profiles, membranous structures and variously sized vacuoles. It is interestingly noted that these parenchymal cells did not exhibit any glycogen particles (Figure 8).

The cytoplasm of the parenchymal cells neighboring the Disse space were highly electron dense and a few small glycogen particles were generally located at the periphery of the cytoplasm of these cells. In this area, the bile canaliculi were dilated and contained finely granular and electron dense material aggregations (Figure 9).

In the cytoplasm of the Kupffer cells, numerous lipofuchsin granules, membranous structures, degen-

Figure 3: Dense appearance of mitochondria (M) and numerous peroxisomes (P) with finely granular matrix are seen. x 24000.



erated mitochondria and secondary lysosomes were found. In the same areas, the presence of electron dense, amorphous material aggregations were striking features (Figure 10).

DISCUSSION

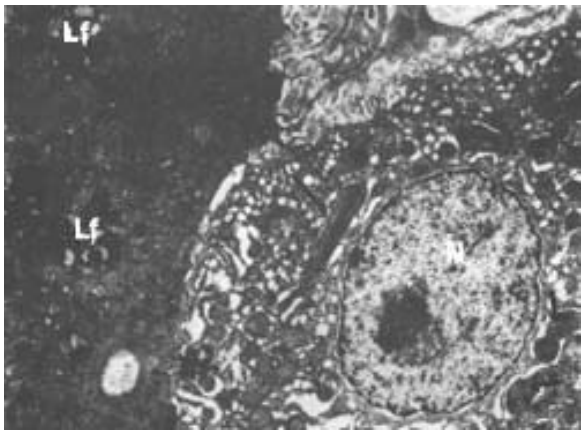
Thioamide type drugs were introduced in the 1940's for treatment of hyperthyroidism. Because of their therapeutic effectiveness and relatively few side effects on comparison with other Thiouracil derivatives, PTU has become the drug of choice for the treatment of hyperthyroidism (2, 4,16). Although PTU is widely used in the treatment of thyrotoxicosis. There are few reports of its toxic effects especially on the liver, in which as hepatocellular injury and rarely cholestasis was reported. PTU related hepatitis can be severe and has been fatal (5, 8,14).

Drug induced hepatic injury can be cytotoxic, cholestatic, or mixed; the mechanism of injury being either direct or secondary to host idiosyncrasy including hypersensitivity. The pathogenesis of PTU induced hepatic injury is unknown but it is postulated to be an allergic host response (4, 5,14).

Weiss *et. al.* (17) have reported cases of PTU induced hepatic damage in which auto-antibodies were demonstrated. Hayashida *et. al.* (6) have shown lymphocyte sensitization in patient with neonatal liver injury probably caused by placental transfer of PTU. All these reports agree in suggesting that hypersensitivity plays a role in the pathogenesis of liver damage associated with PTU therapy.

Hanson (5) proposed the following practical criteria for the diagnosis of drug induced hepatitis 1) clinical and laboratory evidence of hepatocellular dysfunction; 2) the onset of symptoms temporally related to drug therapy; 3) no serologic evidence for current infection with hepatitis A or B, CMV or EBV; 4) the absence of an acute hepatic insult such as shock or sepsis; 5) no evidence of chronic liver disease; and 6) the absence of other concomitantly administered drugs, especially known hepatotoxins. The presence of fever, rush, eosinophilia, or lymphadenopathy is supportive but not essential. The initial appearance of the disease is similar to that of viral hepatitis, characterized by nausea, vomiting and jaundice. The onset of symptoms ranges from two weeks to 6.5 months after institution of PTU therapy (4,5). These criteria were present in our patient except the systemic manifestation of hypersensitivity (i.e., eosinophilia, rash, or lymphadenopathy).

Figure 4: Note the electron dense matrix of mitochondria containing several para- crystalline inclusions (arrows). Nucleus (N), Lipofuchsin granules (Lf). x 8800.

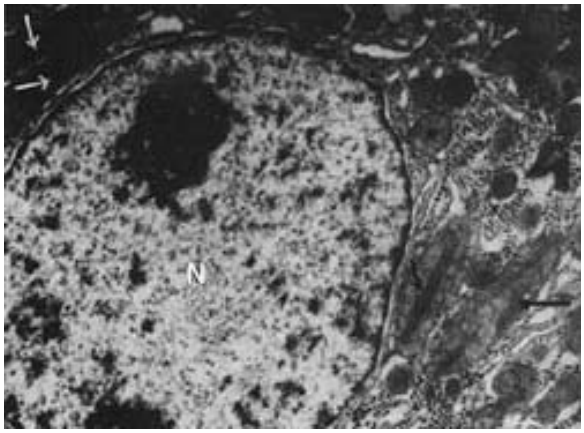


The PTU induced histologic damage in liver has been recorded as acute periportal inflammation with eosinophilic infiltration and bile stasis, sub-massive hepatic necrosis or chronic active hepatitis (5, 8, 9,17). In this study, ultrastructural changes such as dilatation of smooth and rough endoplasmic reticulum, formation of variously sized vacuoles, increased number of lipofuchsin granules and abnormality of mitochondria in the parenchymal cells were encountered frequently, whilst some of the hepatocytes were highly degenerative, including the presence of membranous structures and lytic areas in the cytoplasm. Some of these observations confirm the findings of Fedotin *et. al.* (3) who reported the clinical, laboratory and light and electron microscopic observations on a patient with chronic active hepatitis caused by the administration of propylthiouracil. There were dilatation of the smooth endoplasmic reticulum, abnormal mitochondria and increment of autophagic vacuoles and residual bodies in the hepatic parenchymal cells.

In many sections, where these changes were observed, the nuclei of the hepatocytes exhibited cytoplasmic patches which were contained in variously sized vacuoles surrounded by double membrane. In cross sections of hepatocytes, cytoplasmic invaginations frequently appeared as intra-nuclear vacuoles containing cytoplasmic patches. The cytoplasm of hepatocytes contained variously sized vacuoles. One of the most common causes of vacuolization is the presence of lipids within the cytoplasm. This is usually the result of a temporary metabolic defect or deficiency resulting in the accumulation of lipid droplets, which coalesce and form microscopically visible vacuoles. Administration of various substances and drugs may lead to vacuolization of cells and lead to cell death. Meiss and Robenek (10) reported a similar vacuolization in liver parenchymal cells of rats and mice following applications of phalloidine, amanitine, o-phenyl phenol, hexachlorophene and p-choloro-m-cresol and they suggested that the vacuoles in the liver cells originate from the invagination of the intercellular space.

Accumulation of electron dense and finely granular materials in the bile canaliculi may represent bile pigment deposition. The pathologic examination of

Figure 5: Giant mitochondria with parallel, filamentous, para-crystalline inclusions (arrows) are seen. Nucleus (N). x 14000.



the liver biopsy material of the patient was well adjusted cholestatic hepatitis. The cholestatic hepatitis was characterized by dilatation of bile canaliculi and deposition of bile pigment following obstruction of bile flow. Bile pigment is initially deposited in the endoplasmic reticulum of the parenchymal cells, later in the space of Disse and bile canaliculi (11). The presence of lipofuchsin granules, enlarged endoplasmic reticulum cisterna, dilatation of bile canaliculi and deposition of bile pigments are considered to be related with severe degeneration in the cells and disordered lipid and bilirubin metabolism in the liver.

In our study, the most striking feature of mitochondria was the presence of electron dense inclusions. Mitochondria of human hepatocytes from diseased livers frequently contained crystalline inclusions. Occasionally they have been observed in normal biopsy specimens. The origin and significance of the inclusions are unclear because their occurrence has not yet been related to any single factor. However, numerous toxic, pathologic, and possibly physiologic stimuli have been postulated as responsible for their formation. Ruffalo *et. al.* (13) have found the para-crystalline mitochondrial inclusions in the liver tissue obtained at necropsy within several hours following death and by needle biopsy of living patients. In their study, the mitochondrial inclusions were found only in patients with liver disease. Furthermore, similar mitochondrial matrix inclusions have been described by authors in a variety of human disease states including diabetes mellitus, primary amyloidosis, viral hepatitis, obstructive jaundice, alcoholism, Wilson disease and in normal subjects (15). Investigations of these structures have led to the conclusion that they are essentially normal structures in the human liver and may become numerically increased in diseased states. Since the liver plays a prominent role in the metabolic transformation of drugs and chemicals, in large amounts those substances can impose an increased functional strain on liver cells, resulting in increased enzyme production. Crystalline inclusions are thought to be over products of the mitochondria or crystallization of mitochondrial enzymes. In our opinion, the reason for the occurrence of para-crystalline mitochondrial inclusions could be related to the crystallization of enzymes caused by disordered liver metabolism due to the toxic effect of PTU.

Figure 6: Note the variously sized vacuoles (V), electron dense mitochondria (M) and dilated rough endoplasmic reticulum cisterna (RER). Glycogen particles (G1). x14000.

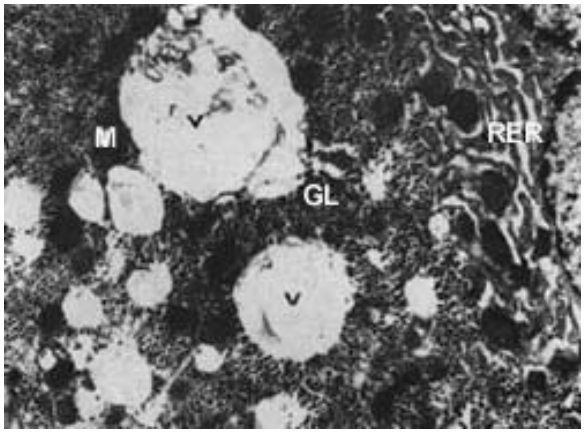
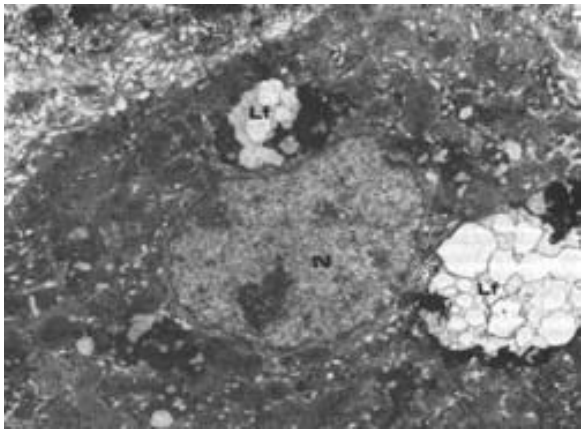


Figure 7: Enormously large lipofuchsin granules (Lf) are seen near the irregular outlined nucleus (N) of hepatocyte. x 8800.



Finally, PTU is an important drug in the management of hyperthyroidism, but may cause hepatocellular injury as in our case. Therefore, the potential danger of permanent hepatic damage and even fatal outcome should be kept in mind.

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Figure 8: There were degenerated cytop- lasmic organelles, membranous structures (Ms), lipofuchsin granules (Lf) and glyco- gen depletion in the cytoplasm of hepatocyte. x 14000.

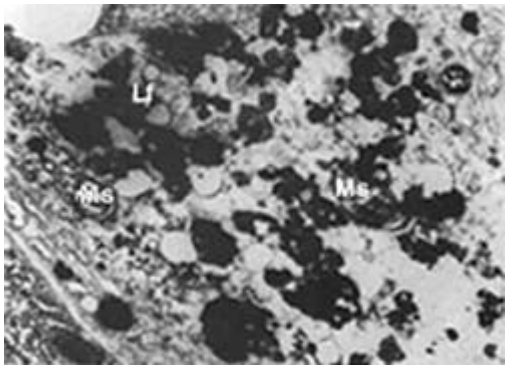

Figure 9: Dilated bile canaliculi (Bc) containing electron dense and finely granular material are seen. Mitochondria (M), glycogen particles (G1). x 8800.

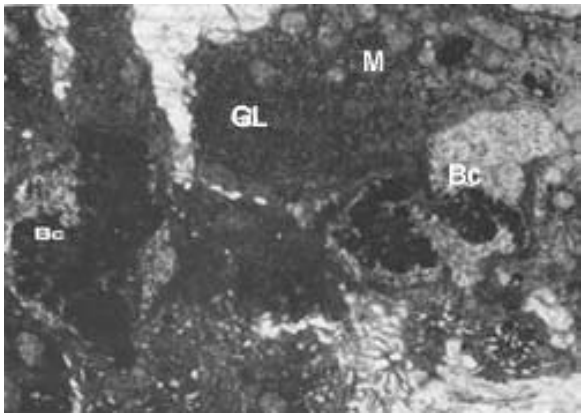

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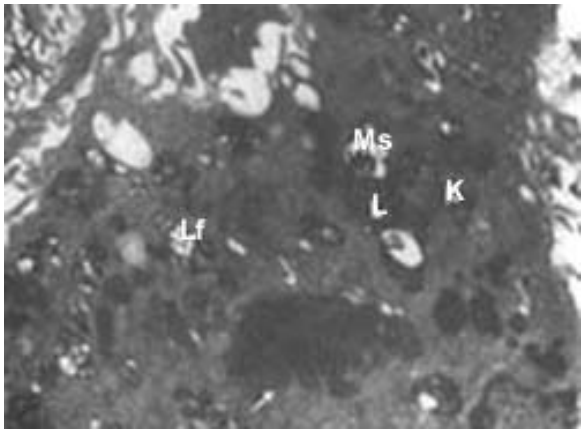
Figure 10: Note the lipofuchsin granules (Lf), membranous structures (Ms), lysosomes (L) and electron dense material aggregations (arrows) in the cytoplasm of Kupffer cell (K). x 14000.



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