

**HUMAN FASCIOLIASIS : ULTRASTRUCTURAL STUDY
ON THE LIVER BEFORE AND AFTER BITHIONOL TREATMENT**

By

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ABSTRACT

The pathology of human fascioliasis was studied before and after bithionol treatment using light and transmission electron microscopy. Fine needle biopsies were taken from five patients, with established fascioliasis, before and after drug administration. By light microscope the pathology of human fascioliasis was similar to that reported in experimental fascioliasis. The ultrastructural picture revealed bile ductular hyperplasia, fibrosis of portal tracts, widening of the interhepatic spaces by many microvilli and dilated Disse space with collagen fibres. Bile ductular hyperplasia may be the initial factor to fibrinogenesis, which subsequently enhance the development of the microvilli on the surface of the hepatocytes. Both light and electron microscopic studies revealed regression of the picture of fascioliasis to normal after bithionol treatment with no sign of toxicity on the liver.

INTRODUCTION

Human fascioliasis is becoming a parasitological problem of increasing threat in the vicinity of Alexandria. The liver and biliary passages are mainly affected (Balci 1975 and Janes et al 1977). The literature on the experimental pathology of fascioliasis is extensive and has contributed to our understanding of the disease (Isseroff et al

1977, Masake et al 1978 and Abou Basha et al 1983). On the other hand few publications are available on the pathology of human fascioliasis. Diagnosis is based on autopsies (Acosta Ferreria et al 1979 and Duan et al 1986), surgical specimens or laparoscopic biopsies (Perry, et al., 1977, Moreto, and Barron, 1980 and Uribarrena, et al 1985).

The aim of the present work was to study the effect of human *Fasciola* and bithionol on the liver using light and transmission electron microscopy.

MATERIAL AND METHODS

Five patients with established fascioliasis were the subject of the present work. They received a course of bithionol treatment in a dose of 30 mg/kg body weight, every other day for 10 days. From each patient, needle liver biopsies were obtained before and one day after bithionol treatment. The specimens were rapidly fixed in 2% gluteraldehyde for 2 hours, post-fixed in 1% osmium tetroxide, dehydrated in ascending grades of acetone, cleared in propylene oxide then infiltrated and embedded in epon araldite mixture. Somithin sections were stained with toluidine blue and examined by the light microscope. Ultrathin sections were stained with lead citrate and urenyl acetate for electron microscopic examination.

RESULTS

LIGHT MICROSCOPIC PICTURE :

Before bithionol treatment, the liver parenchyma was traversed by fibrovascular tracks, surrounded by mononuclear cells, eosinophils and giant cells (Fig. 1a). Quite frequently foci of lymphocytic aggregate were encountered in the liver parenchyma, specially around vascular spaces (Fig. 1b). The portal tracts were enlarged by abundant fibroblastic proliferation, laying down collagen and including many capillaries, proliferating bile ducts and cellular infiltration mainly lymphocytes and mast cells (Fig. 2a, b). Frequent areas of complete liver necrosis with loss of nuclei and separated by fibroblastic proliferation (Fig. 2c). The liver cells close to the dilated portal tracts had a small pyknotic nuclei and deeply eosinophilic cytoplasm (Fig. 3a).

The necrotic areas were surrounded by granulomatous reaction with predominant histiocytes and epithelioid like cells (Fig. 3b). After bithionol treatment, the liver parenchyma regained its normal architecture. Liver cells appeared large polyhedral with rounded nucleus. Complete absence of liver necrosis and very infrequent degenerated liver cells were met with. The liver sinusoids were empty with few histiocytes. The portal tracts regressed, containing the triad and few mononuclear cellular infiltration (Fig. 3c).

ELECTRON MICROSCOPIC PICTURE :

I (a) Before treatment there was a considerable dilatation of intercellular spaces between hepatocytes, whose surfaces beared numerous microvilli over the entire surface. After treatment the normal parallel lines of the hepatocellular borders were regained including the bile canalicules between desmosomal attachments. (Fig. 4a, b, c). The sinusoidal surface of the hepatocytes before treatment showed slender and elongated microvilli. The Disse space looked wider than normal and contains minimal scattered collagen fibres. (Fig. 4a) After treatment, an almost complete recovery of the sinusoidal surface occurred, as the microvilli were more numerous and complex. (b) The mitochondria, in the infected liver, were big with election dense retained bodies. After treatment recovery of mitochondria with well formed cristae was noted (Fig. 5a). (c) Both smooth endoplasmic reticulum and glycogen content in the liver cells have decreased markedly in the infected liver, but returned to normal after treatment (Fig. 5a). (d) Both lysosomes and golgi apparatus have increased in the infected liver then returned to normal after treatment. (e) Nuclei of the infected liver contained abundant heterochromatin with inconspicuous nucleoli, and surrounded by serrated nuclear membrane, denoting signs pyknosis and necrosis. After treatment the nuclei contained abundant euchromatin with prominent nucleoli.

II — Disse space : In the infected liver, the Disse space, between the sinusoidal lining cells and the hepatocyt plate, appeared wider than normal and contained slender few microvilli, extending from the hepatic cell membrane. In the treated liver, the Disse space appeared narrower with numerous microvilli (Fig. 4a).

III — Endothelial lining of the sinusoids : Before and after treatment, the sinusoidal wall consisted of a single sheet of endothelial cells which were elongated and thin, showing no stratification and no basement membrane like deposits, and interrupted in small gaps or poor fenestrations between the endothelial lining (Fig. 4a).

IV — The bile canaliculi in the infected liver, showed proliferation and dilatation and appeared as multiple widened intercellular spaces bound by cell membrane of the adjacent hepatocytes. Their lumina contained numerous complex microvilli. After treatment, regression of the number of bile canaliculi to normal was noted with few normal microvilli into their lumina (Fig. 4b, c).

V — The portal tract in the infected liver was filled with abundant collagen bundles, arranged in small or large bundles. Many active fibroblasts with large nuclei with abundant euchromatin and prominent nucleolus. Frequent lymphocytes had a prominent deep cleavage of the nucleus denoting activity (Fig. 5b, c).

DISCUSSION

The most prominent histopathological findings of human fascioliasis in the present work were the multifocal aggregates of lymphocytic infiltration in the liver parenchyma and in the perivascular areas; ductular hyperplasia; foci of liver necrosis and degeneration around the portal tracts and the periportal fibrosis with dense collagen fibre inside the portal tracts and predominance of lymphocytes, fibrocytes and mast cells. Similar picture was reported by many authors in experimental fascioliasis in mice (Masabe 1981). The observed wide spread lymphocytic infiltration specially perivascular, supports the views of a cell mediated response (Lang, 1967). The darkly stained cytoplasm and pyknotic nuclei in hepatic cells associated with lymphocytic responses probably resulted from a delayed hypersensitivity causing local tissue damage (Lang, 1967). The presence of large number of mast cells in liver biopsies of *Fasciola* patients may denote recruitment of cells and antibodies at site of inflammation that may injure the parasite (Eiseen 1980 and Vannier 1986). After bithionol, rapid regression of the lesion was dramatic as revealed by the binucleated hepatic cells, indicating hepatic regeneration and the disappearance of the perivascular lymphocytic infiltration and foci of liver necrosis.

Electron microscopic features of hepatic fascioliasis disclosed new information on the surface characteristics and altered interrelationships of the hepatocytes; as well as closer insight into the process of fibrinogenesis leading to fibrosis. A considerable dilatation of intercellular spaces between hepatocytes, whose surfaces bear microvilli over the entire surface; thus the normal parallel lines of the hepatocellular borders are not seen. Anomalous microvillous border was reported to be attributed to adaptation to the low oxygen tension prevailing in the blood and tissue fluids (Theron and Liebenberg 1959) or to increased anabolic demands due to enhanced collagen production. Enlargement of the Disse space, separating the sinusoidal wall from the hepatocytes, in the present work interferes with nutrient transport from the sinusoids to the hepatocytes (Andrade, 1986). The proliferating portal bile ductules is a main feature in the present work. The main centres of fibrinogenesis are the portal tracts and the proliferating portal bile ductules (Johannesson 1979). Popper et al (1960) and Schaffner et al (1963), have attributed great importance to the inducer role of ductular proliferation in fibrosis. The reduction in both SER and glycogen have contributed to a reduction of basophilia seen in light microscopy, as believed by Steiner et al (1980). The mitochondrial alterations seen in this study are not specific, as they are seen in many other pathological conditions as well as in normal hepatocytes (Soga et al 1970, Burns et al 1972).

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EXPLANATION OF FIGURES

- Figure 1.** (a) A fibrovascular track (T) traversing the liver parenchyma and surrounded by eosinophils (E), lymphocytes (L) and giant cells (G), H & E X 400. (b) A perivascular lymphocytic infiltration in between the liver cells. H & E X 300.
- Figure 2.** (a) Semithin section of the portal tract with proliferating bile ducts (D) and dense collagen fibres (F). Toluidine blue X 500 (b) Semithin section of the portal tract with dense collagen fibres (F) and many mast cells (M). Toluidine blue X 500 (c) Semithin section of necrotic liver cells (N), surrounded by collagen fibres (C) and fibroblastic proliferation. Toluidine blue X 500
- Figure 3.** (a) Fibrotic portal tract with nearby degenerated liver cells. H & E X300 (b) Granuloma cells predominantly made up of epithelioid (↑) cells and histiocytes (↑↑) H & E X300 (c) Liver cells after bithionol treatment, looking near to normal. H & E X300.
- Figure 4.** (a) Electron micrograph of three hepatocytes with dilated intercellular spaces (Is) bearing microvilli (m) on their surfaces. The hepatocyte close to the sinusoid shows dilated Disse space (D) with collagen fibres (C). X 5000. (b) Lateral surfaces of neighbouring hepatocytes, dilated and covered by numerous complex microvilli (m). X 8000. (c) Part of 2 neighbouring hepatocytes after bithionol treatment showing normal canalicule (C), bound by desmosomes (D). X 8000.
- Figure 5.** (a) Liver cytoplasm before treatment showing big mitochondria (M) with inclusion bodies (Ib). X 10000 (b) Electron micrograph of the portal tract with 2 active lymphocytes (L), having dense collagen in their nuclei (Cl). A fibrocytes (F) and dense collagen fibres (C). X 5000 (c) An active fibroblast in the portal tract with serrated nuclear membrane and prominent nucleolus (N1), laying down dense collagen fibres (C), X8000.

