

LIGHT MICROSCOPIC AND ULTRASTRUCTURAL FINDINGS IN LIVER BIOPSIES FROM 8 PSORIATICS BEFORE AND AFTER METHOTREXATE THERAPY

A Preliminary Report of a Prospective Study

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Abstract. Liver biopsies before and after methotrexate (MTX) therapy (single, weekly, oral doses) from each of 8 patients with disabling psoriasis were examined by light and electron microscopy for evidence of damage. Light microscopy showed that the number of Ito cells increased in a statistically significant way ($p < 0.01$) following the drug therapy. At the ultrastructural level MTX appeared to injure the hepatocytes, as evidenced by steatosis, membrane whorls and debris in the bile canaliculi. Megamitochondria with crystalline inclusions were found in many hepatocytes both before and after therapy. Changes were also seen in 6 of the 8 bile ducts after MTX therapy in the form of focal oedema, widespread mitochondrial damage and cell atrophy with enlarged intercellular spaces containing debris.

During MTX therapy the significant increase seen in the number of Ito cells, which may act as fibroblast precursors, suggests a mechanism which may explain the fibrosis seen after MTX therapy.

Key words: Methotrexate-treated psoriatics; Liver biopsies; Light microscopy; Electron microscopy; Fibrosis; Ito cells

It is becoming increasingly obvious that methotrexate (MTX) given orally is hepatotoxic even in the small doses given for psoriasis (2, 13), although some studies (24, 25) were negative in that respect.

Ultrastructural studies of liver biopsies from MTX-treated psoriatics are few, incomplete (3, 11), and not conclusive with respect to where the MTX damage takes place.

In this preliminary communication we report our findings in liver biopsies from psoriatics taken before and after MTX treatment.

MATERIAL

The material for this investigation is selected from a prospective study undertaken during the period 1969-1974 of liver biopsies from patients with disabling psoriasis before

and after MTX therapy. The criteria used in diagnosing psoriasis and evaluating the light microscopical findings in the patients are described elsewhere (12). MTX was given orally once weekly in a single dose of up to 25 mg. Serum aspartate aminotransferase, alkaline phosphatase and serum bilirubin levels were registered the day before the biopsy and at varying intervals during MTX therapy.

METHODS

The liver biopsies were all performed at the Finsen Institute with a Menghini needle. Part of each specimen was immediately cut into small pieces less than 1 mm in diameter and fixed in 4% glutaraldehyde, pH 7.2, dehydrated in graded alcohols, and embedded in Epon 812. The remainder was fixed in 4% formaldehyde and processed for light microscopy.

Thin sections were stained with lead citrate and uranyl acetate and were examined with an AE1 801B electron microscope.

Ito cells or lipocytes (4) were counted on thick (1 μ m) Epon sections with the fat droplets stained green by toluidine blue. Hepatocytes were counted similarly and the ratio of Ito cells per 1000 hepatocytes (Ito cell index) was determined. Eight to ten fields were examined from at least two blocks. Normal values range between 13 and 24 (3). The significance of the increase in Ito cells and the change in the biliary epithelium after MTX therapy was evaluated using the Wilcoxon matched-pairs signed-ranks test and the Sign test, respectively (18).

RESULTS

Patients. There were 7 females and 1 male with an average age of 45 years (range 25-65 years) at the first liver biopsy. All patients had severe psoriasis, with an average coverage of the skin surface of 32% (range 12-95%), 3 had psoriatic arthropathy and 4 itching, sufficient to disturb the sleep. The

Table I. Clinical data and light microscopy findings in liver biopsies from 8 psoriatics

f.c.: fatty change

n.s.r.h.: non-specific reactive hepatitis

Patient no.	Sex	Age (yr.)	Chief histological diagnosis		Total dose of MTX (mg)	Time between last MTX and last biopsy (days)	Ito cell index	
			1st Liver biopsy	2nd Liver biopsy			Pre-treatment	Post-treatment
Ny-279	♀	44	Mild f.c.	Mild f.c.	1 493	1	14	36
Ny-292	♀	40	Normal	Mild f.c.	3 218	1	6	105
Ny-306	♂	49	Normal	Normal	1 800	1	12	46
Ny-299	♀	33	Normal	Normal	1 074	2	13	47
Ny-295	♀	62	Mild f.c.	Possible cirrhosis	2 330	4	32	104
Ny-255	♀	65	Mild f.c.	Mild f.c.	2 233	6	25	118
Ny-301	♀	25	Normal	Normal	2 517	15	31	43
Ny-259	♀	46	Mild n.s.r.h.	Mild fibrosis	2 833	168	15	53

mean duration of psoriasis was 22 years (range 4–35 years).

Three patients (279, 295, 301) had had corticosteroids prior to MTX therapy, and one (295) had received butazone (phenylbutazone) therapy for psoriatic arthropathy. Two patients (279, 301) had been treated with arsenic for their skin disease.

Five of the patients had been given possible hepatotoxic medicine prior to MTX therapy (13): one, phenytoin (299); 3, oestrogen (279, 292, 301); and one, chlorothiazide and methyldopa (295) (20).

The admitted alcohol intake in the years before and during MTX therapy among these 8 patients was: 7 occasionally and one 1–4 drinks per week. One drink is equivalent to 10 g ethanol.

No patients had had jaundice or diabetes, but one had had cholelithiasis.

No patients had hepatomegaly or ascites at the physical examination. Two patients (259, 295) were overweight (20%).

For each patient, sex, age, chief histological diagnosis of first and second liver biopsy, total dose of MTX, and time between last MTX intake and second liver biopsy (and liver function tests) are shown in Table I, while side-effects are reported elsewhere (10).

Laboratory tests. Liver function tests at the time of the second liver biopsy were normal.

While the patients were being treated with MTX the serum aspartate aminotransferase level was sporadically elevated, while 3 patients had a raised alkaline phosphatase level at some time. There was no increase in the serum bilirubin in any patient.

Light microscopy. The findings in the liver biopsies are shown in Table I. Similarly, the Ito cell index is given in Table I. There was a statistically significant increase ($p=0.0060$, Wilcoxon test) in the post-MTX Ito cell index (Fig. 1).

Electron microscopy. SEMI-quantitatively, no significant changes were found in post-MTX specimens. In 6 of 8 patients, bile ducts were affected.

The hepatocyte

All groups of cells showed evidence of damage. The steatosis noted by light microscopy was more widespread at the ultrastructural level. The mitochondrial crystals in the hepatocytes are one of the striking features.

Some of the mitochondria involved measured up to 1–2 μ m long and many had bizarre shapes. Not all the mitochondria in any cell contained crystalline arrays; the number of hepatocytes with such mitochondria varied from patient to patient in an apparently random way. There was a possible slight increase in the smooth endoplasmic reticulum which was dilated in places, as was the rough endoplasmic reticulum. Hepatocytes containing membrane whorls frequently showed autophagic vacuoles. Residual bodies—the ultrastructural counterpart of lipofuscin—were very easily found, sometimes in increased numbers. Two patients had nuclei with pseudo-inclusions. In all the patients many bile canaliculi contained particulate debris. A few contained membranous debris and some microvilli were oedematous. The cell membranes were unremarkable, as was the space of Disse.

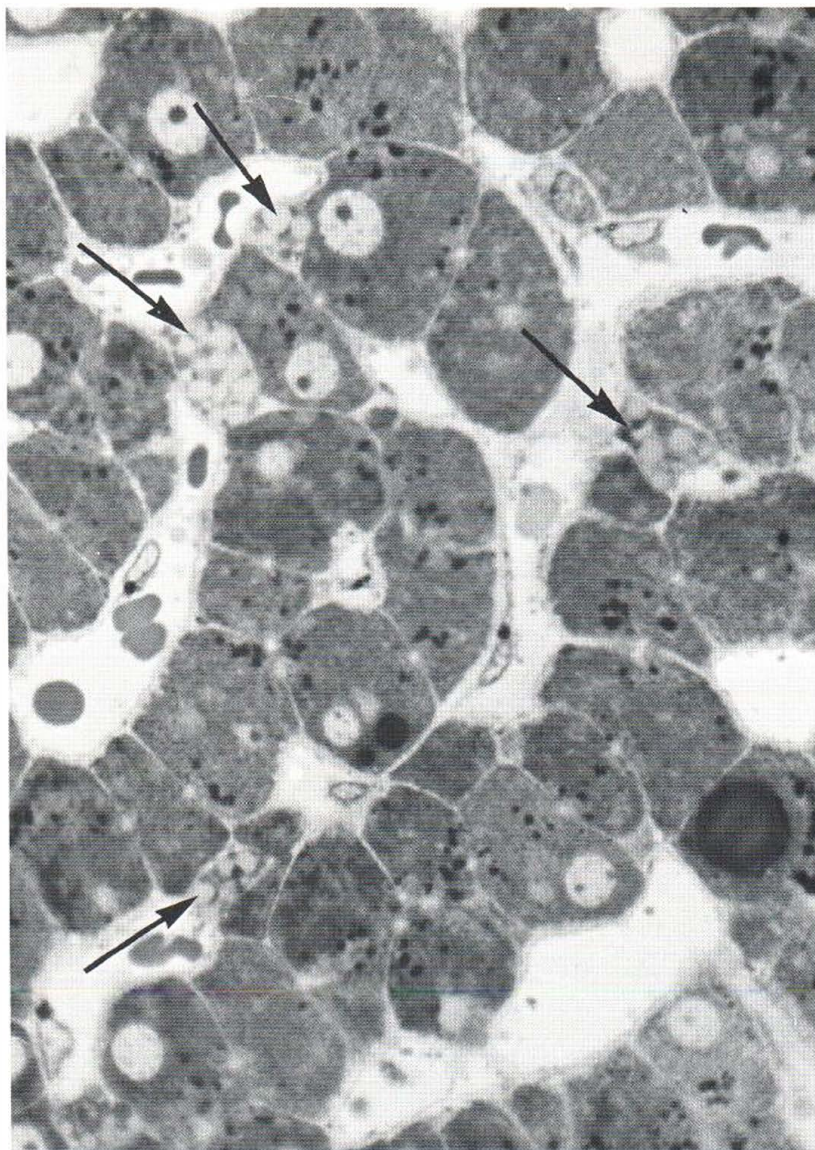


Fig. 1. Section of liver, 1 μ m, Epon embedded, stained with Toluidine Blue, from a patient undergoing Methotrexate therapy. Several Ito cells (arrows) are present in the field, $\times 16\,000$ (Ny-306).

Bile ducts

The bile ducts showed sign of injury (Fig. 2). Light and dark epithelial cells were atrophic. Mitochondrial damage was fairly widespread and some cells had dilated rough endoplasmic reticulum. Most biliary epithelium contained varying numbers of residual bodies. The specimens from 2 patients contained membrane whorls. Some biliary epithelium contained numbers of basal pinocytotic vesicles. The intercellular spaces (Fig. 3) were often widened and some contained particulate and membranous debris (Fig. 2), although some contained

no debris at all. The basement membrane of the bile ducts was duplicated in places and thickened in others. No gaps were seen.

Other cells

Ito cells were increased in number and contained large numbers of lipid droplets (Fig. 4). The sinusoids contained occasional polymorphonuclear leukocytes and in 1 patient, 2 mast cells. Some Kupffer cells contained a large number of residual bodies.

In the parenchyma of the whole series there was

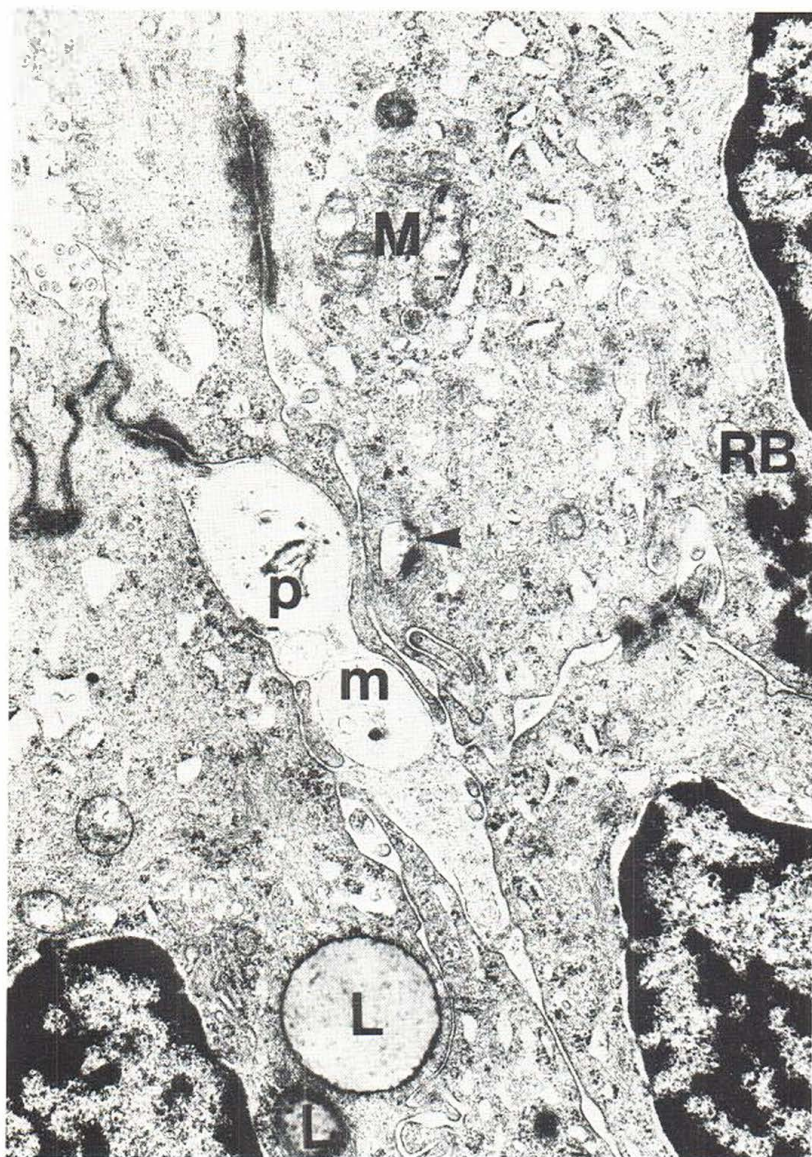


Fig. 2. Biliary epithelium showing membranous (*m*) and particulate (*p*) debris between the cells. The cytoplasm contains damaged mitochondria (*M*), autophagic vacuoles (*arrow head*), residual bodies (*RB*) and possible lipid droplets (*L*), $\times 20\,000$ (Ny-259).

an occasional cell death, but no focal necroses were seen. The sublethal injury of hepatocytes and bile ducts varied from cell to cell in the parenchyma and not all cells showed damage. The degree of damage appeared to correlate with the interval from the last dose of the drug, but not with the cumulative dose of MTX.

DISCUSSION

MTX appears to be hepatotoxic (2, 13) even in the small doses given for psoriasis, although these

doses are only a few per cent of those given for leukaemia. The disadvantage of this treatment is that it might be prolonged in the severe and recalcitrant cases which may be complicated with psoriatic arthropathy. Patients with pathological liver histology before MTX therapy tend to deteriorate more often during MTX treatment than those with a normal pre-MTX liver biopsy (13). The presence of normal liver tissue can only be confirmed by a liver biopsy, as liver function tests such as the three mentioned previously may be normal even in cirrhotics.

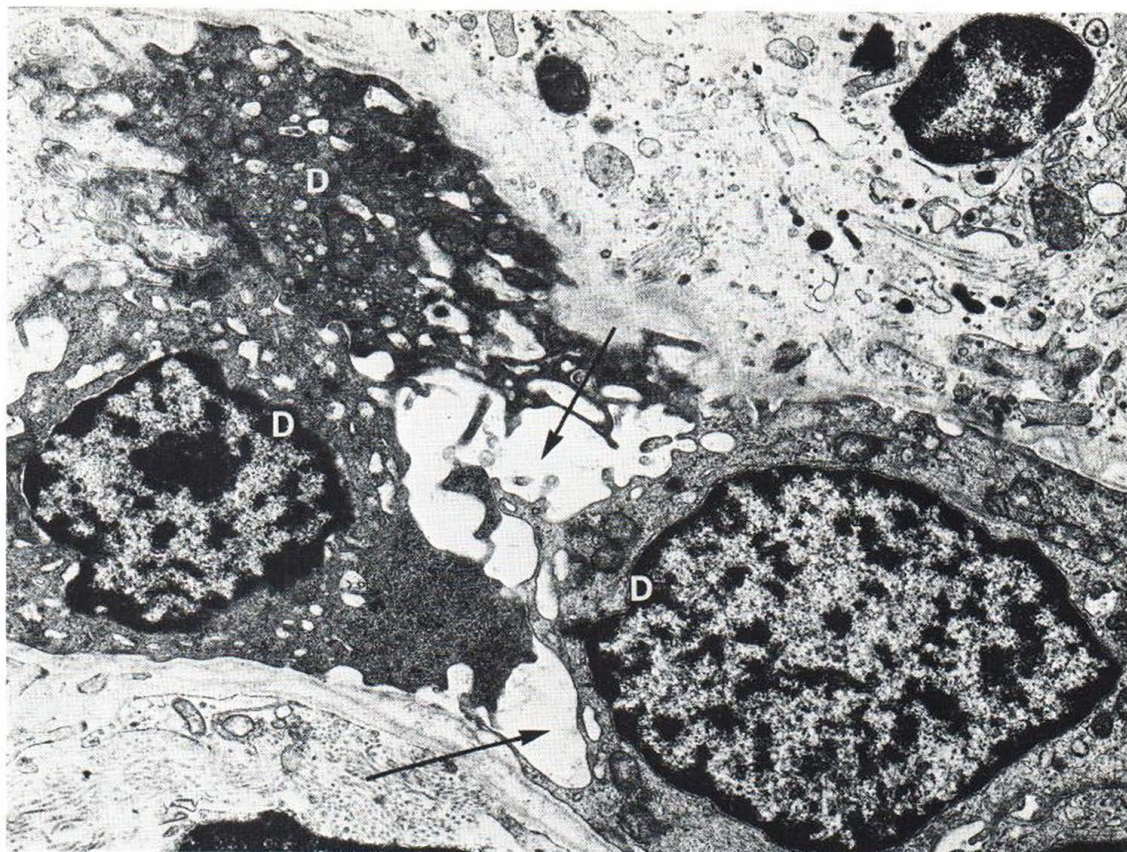


Fig. 3. Atrophic bile duct. There are large intercellular spaces (arrows) between the epithelial cells (D), $\times 46\,600$ (Ny-259).

In 1973, Horvath et al. (3) published a preliminary report on their study of liver biopsies from 9 MTX-treated psoriatics. Their most conspicuous finding was hyperplasia and hypertrophy of Ito cells. No statistically significant correlation was detected between the Ito cell accumulation and the extent of fat in the hepatocytes, the duration and regimen of medication, and severity of psoriasis. We confirmed these findings and found the increase in the number of Ito cells after MTX therapy to be highly significant statistically. Hruban et al. (4) have recently reviewed the response of the Ito cells to various drugs and diseases.

MTX is known to combine stoichiometrically with dihydrofolate reductase (9) which is present in large amounts in the cell sap of the liver (22). It can remain in this combined state for a long time.

The hepatocytes and bile ducts may show evidence of sublethal injury (21).

Hepatocytes

MTX has been shown to interfere with the replication of mitochondria (1, 23). The presence of megamitochondria with budding in more than two-thirds of this small series comes as no great surprise. Nyfors et al. (11) reported similar changes and pointed out that like alterations are produced by alcohol ingestion. Ethanol is known to induce megamitochondria (14). Only one of 8 patients in this study took alcohol regularly (1–4 drinks weekly). The mitochondrial crystals which have been seen previously in psoriatics receiving MTX (3, 11) may represent an inappropriate protein. Hepatic mitochondrial crystals have been noted in a number of diseases during and after the administration of various drugs (17, 19), but it is doubtful if they are significant. Other changes in the hepatocytes can be ascribed to injury by MTX, but the mechanism involved is not self-evident. These include the



Fig. 4. An Ito cell (*I*) between hepatocytes (*H*) and the sinusoid (*S*). The Ito cell contains numerous lipid droplets (*L*), $\times 12\,000$ (Ny-259).

membrane whorls and the dilatation of the rough and the smooth endoplasmic reticulum. As MTX is mostly excreted from man unchanged, the slight changes in the total amounts of the smooth endoplasmic reticulum compared with the normal state are to be expected (5, 8). The residual bodies are increased in number. Changes in the lysosomal apparatus have been noted following the use of certain drugs (6).

The accumulation of lipids in the hepatocyte is well known in patients who abuse alcohol (16), but is also known to occur following exposure to various drugs (7).

Bile ducts

There were also alterations in the bile duct epithelium. Some of these are cholestasis (15), light and dark cells, oedematous microvilli, widened

intercellular space, and thickened and duplicated basement membrane. Three patients showed a raised alkaline phosphatase level at some time during MTX therapy, and there was no light microscopic evidence of cholestasis. There was also evidence of cell injury in the biliary epithelium. Mitochondrial damage was found quite commonly and the endoplasmic reticulum was dilated. There was debris in the intercellular spaces. The atrophic biliary epithelium may result from direct injury by MTX. One may speculate on the atrophic biliary epithelium with widened intercellular spaces, the potential leakage of bile and the occurrence of portal fibrosis in the livers of patients treated with MTX.

The function of Ito cells is not clear, but the various theories as to their origin have been reviewed by Hruban et al. (4), who pointed out that

Ito cells may be fibroblast precursors. Whether they have this role in the fibrosis and cirrhosis reported following MTX therapy in psoriatics (2, 12) is open to question.

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