Effect of methotrexate therapy in psoriasis on the Ito cells in liver biopsies, assessed by point-counting

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SYNOPSIS To evaluate the relationship, both quantitative and qualitative, between the Ito cells and methotrexate (MTX) therapy Ito cells were studied by light microscopy in 1 µm toluidine blue stained sections and by electron microscopy in 24 pairs of Menghini needle biopsies before and after MTX therapy of 24 consenting patients with severe psoriasis.

Light microscopy showed a statistically significant increase in pathological findings (p < 0.05) and in the number of Ito cells and their size (p < 0.0001) after MTX therapy. It was not possible to show a statistically significant correlation between the increase in the number of Ito cells and the cumulative dose of MTX.

Ultrastructural analysis of Ito cells showed no marked difference from pre- to post-MTX specimens.

The fibrosis and cirrhosis seen after MTX therapy in some liver biopsies from psoriatics and the post-MTX increase in the number of Ito cells direct attention to the possible role of Ito cells as fibroblast precursors.

The sinusoids of the liver are lined with endothelial cells, Kupffer cells, and the fat-containing lipocyte or Ito cell. These last cells are not well known, even though they were first described by von Kupffer (1875) and rediscovered by Maximow (1927). More recently, they have been characterized by Ito and Nemoto (1956). Fahimi (1970) distinguished Ito cells from the other littoral cells in experimental animals by cytological and histochemical techniques. The presence of Ito cells in man has been established and their variation in various diseases has been shown by Bronfenmajer, et al (1966). The physiological function of Ito cells remains obscure, although they may be related to vitamin A storage (Hruban et al, 1974). Various drugs have been found to increase the number of Ito cells present in the liver, including methotrexate (MTX) (Horvath et al, 1973).

MTX therapy has been reported to clear recalcitrant psoriasis vulgaris in 60-70% of cases (Roenigk et al, 1969; Nyfors and Brodhagen, 1970). Case reports of liver disease, particularly cirrhosis, observed after MTX therapy have aroused concern (Coe and Bull, 1968; Muller et al, 1969; Dahl et al, 1971). Prospective studies, including liver biopsy in psoriatics before and after MTX therapy and in the same patient at intervals during MTX therapy, have been undertaken (Weinstein et al, 1973; Nyfors and Poulsen, 1974). They revealed an increase in the number of pathological findings in post-MTX liver biopsies. Horvath et al (1973) found a post-MTX increase in the number of Ito cells per 1000 hepatocytes (Ito cell index), which was not apparently related to the dose of MTX received. We have confirmed this marked increase. The present study was aimed at evaluating the relationship, both quantitative and qualitative, between Ito cells and MTX therapy. This is part of an ultrastructural study of liver biopsies from psoriatics before and after receiving MTX therapy.

Patients and methods

Patients
The material comprises 24 pairs of Menghini needle biopsies. Biopsies were taken both before and after MTX therapy from each consenting patient with disabling psoriasis. The liver biopsies were performed at the Finsen Institute during the period August 1969 to March 1974. To be included in this study the patients had to fulfil the following criteria: a typical
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and prolonged psoriasis besides no lasting effect of other dermatological, mainly topical, treatments. Clinical and laboratory data concerning these patients are described elsewhere (Nyfors and Poulsen, 1976a, b). MTX was given orally once a week in a single dose of up to 25 mg.

LABORATORY TESTS
Liver function tests—serum aspartate amino-transferase (SGOT), alkaline phosphatase, and serum bilirubin were taken the day before liver biopsy and at varying intervals during MTX therapy.

LIGHT MICROSCOPY
Parts of each liver biopsy were taken for conventional light microscopy. The remainder of the biopsy was cut into 1 mm cubes, fixed in 4% glutaraldehyde at pH 7-2, and post osmicated and embedded in Araldite. 1 μm sections were stained with toluidine blue and examined under oil (× 1000). Ito cells containing numbers of small, blue-green lipid droplets can be recognized easily along the sinusoids.

POINT COUNTING
The volume density of the Ito cells in the liver was determined by point counting, using a 400 point array eyepiece graticule. Toluidine blue stained 1 μm plastic-embedded sections of the biopsies were used, at least five being examined from each biopsy. Ten consecutive non-overlapping fields were counted under oil with a × 10 eyepiece and a × 100 objective from each slide. The number of points falling on Ito cells and the number of cells within the graticule were recorded. The point counts are directly proportional to the Ito cells' volume in the liver.

The theoretical accuracy of the point counting can be estimated using the equation given by Weibel (1963):

\[ P_T = \frac{0.453 \times (1 - V_v)}{V_v \times (EV_v)^2} \]

where \( P_T \) is the number of points counted, \( V_v \) the volume density of the Ito cells, and \( EV_v \) the error. Preliminary counts showed that the volume density of the Ito cells before therapy was about 0.25%. If 30000 points are counted this gives an error of 10%.

Similarly, after methotrexate therapy, if 20000 points are counted this gives an error of 5.5% for the volume density which rose to about 0.7%.

The Ito cell index was also determined using oil immersion (Table II). Up to 10 fields were assessed and the Ito cells and total hepatocytes were counted. The results were expressed as Ito cells per 1000 hepatocytes. The Ito cell index had become fairly constant.

ELECTRON MICROSCOPY
Thin sections were also cut with an LKB ultramicrotome, stained with lead citrate and uranyl acetate, and examined with an AEI 801B electron microscope at 60 kV.

STATISTICS
The significance of the difference between the histological findings before and after MTX therapy was assessed by the Sign test and the Wilcoxon matched-pairs signed-ranks test, respectively. The Sign test was used with data given in a nominal scale (eg present/absent), while the Wilcoxon matched-pairs signed-ranks test was applied to data in a rank (ordinal) scale (eg, fat: little, moderate, much). Correlation between the number of Ito cells and MTX total dose was tested by the Spearman rank correlation coefficient (Siegel, 1956). One-tailed probabilities were used.

Results

PATIENTS
The average age of the patients (8 males, 16 females) was 47 (range 25-77) years at the first liver biopsy, and the mean duration and extent (per cent of skin surface involved) of psoriasis was 21 (range 3-37) years and 27% (range 4-95%), respectively. The mean cumulative dose of MTX was 1810 mg (range 528-3218 mg), while the average duration of MTX therapy was 28 (range 5-41) months. The time between the last intake of MTX and the second liver biopsy was less than one week in 11, less than one month in 1, 1-4 months in 2, while it varied from 157 to 1120 days in the remaining patients.

<table>
<thead>
<tr>
<th>Liver biopsy number</th>
<th>Chief histological diagnoses</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre-MTX</td>
</tr>
<tr>
<td>349</td>
<td>Severe fc</td>
</tr>
<tr>
<td>254-281</td>
<td>Normal</td>
</tr>
<tr>
<td>293-299-301</td>
<td>Mild fc</td>
</tr>
<tr>
<td>302-306-311</td>
<td>Normal</td>
</tr>
<tr>
<td>255-258</td>
<td>Mild nshr</td>
</tr>
<tr>
<td>279-308</td>
<td>Normal</td>
</tr>
<tr>
<td>296</td>
<td>Mild fc</td>
</tr>
<tr>
<td>256-292-321</td>
<td>Normal</td>
</tr>
<tr>
<td>249</td>
<td>Normal</td>
</tr>
<tr>
<td>303</td>
<td>Mild fc</td>
</tr>
<tr>
<td>295</td>
<td>Moderate fc</td>
</tr>
<tr>
<td>307</td>
<td>Moderate fc</td>
</tr>
<tr>
<td>294</td>
<td>Moderate fc</td>
</tr>
<tr>
<td>259</td>
<td>Mild nshr</td>
</tr>
</tbody>
</table>

Table I  Chief histological diagnoses before and after MTX therapy
fc = fatty change; nshr = non-specific reactive hepatitis
Sign test: 14 unchanged, 1 improved, and 9 with more pathological changes: I-9: \( p = 0.011 \)
Table II  Relation of Ito cell index to MTX therapy

Wilcoxon matched-pairs signed-ranks test: increase in Ito cell index after MTX therapy: highly statistically significant: p < 0.0001, z = 4.26

LABORATORY TESTS
Liver function tests were normal at both liver biopsies in 20 patients. Two patients (295, 349) had a raised SGOT at the first liver biopsy, while one patient (307) had a raised alkaline phosphatase at the second liver biopsy.

Table III  Ito cell changes with methotrexate therapy

Wilcoxon matched-pairs signed-ranks test: increase in Ito cell volume density: z = 3.80, p < 0.001

The results are described in detail elsewhere (Nyfors and Poulsen, 1976a, b), while the chief histological diagnoses are shown in Table I. The number of Ito cells before and after MTX therapy, the cumulative dose of MTX, and the time between the last intake of MTX and the second liver biopsy are indicated in Table II. The volume density of Ito cells and their size before and after MTX therapy are shown in Table III and in figures 1 and 2. Some liver biopsies were taken at various time intervals after the last dose of MTX. Inspection of the tables shows that at 15 days both the volume density and the size of the Ito cells remain high. By 100 days the majority of the biopsies show that the size of the Ito cells returns to within normal range. The volume density of the Ito cells remains above the normal range but lower than the mean for the group on MTX therapy.

ELECTRON MICROSCOPY
Analysis of the morphology of the Ito cells showed no demonstrable qualitative difference. The lipid droplets were abundant, occupying most of the cytoplasm and indenting the nucleus. The normal types of organelles were present. The mitochondria did not show any giant forms or crystalline inclusions. There was no evidence of cellular damage.

STATISTICS
Analysis by the Sign test showed a significant increase (p < 0.05) of the light microscopy changes shown in
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**Fig 1** Section of liver—1 μm Araldite embedded, toluidine blue stained—from a patient before methotrexate therapy. There is one Ito cell (arrow) towards the centre of the field. × 950.

**Fig 2** Section of liver—1 μm Araldite embedded, stained with toluidine blue—from a patient during treatment with methotrexate. Several Ito cells are present in the field (arrows). × 840.
table I, and by the Wilcoxon test a highly statistically significant (p < 0.0001) increase in the number (table II) and volume density (table III) of Ito cells after MTX therapy. By means of the Spearman rank correlation coefficient it was not possible to show any statistically significant correlation between the increase in the number of Ito cells and the cumulative dose of MTX nor the number of hepatocytes with double nuclei.

Discussion

In the present study Ito cells were counted in 1 µm Araldite sections stained with toluidine blue. The present paper was prepared as part of an electron microscopic study. Ito cells can be seen in thin paraffin sections stained with haematoxylin, eosin, and aniline blue (Bronfenmajer et al, 1966). The general properties of the cells are those described previously by Ito and Nemoto (1956).

The point counting technique used showed that the volume density of the Ito cells in the normal liver biopsies had a mean of 0.25%. Immediately after treatment this rose to a mean of 0.66%, falling back to pre-therapy values by 100 days. The increase in volume density is due to hyperplasia and hypertrophy of the Ito cells. These cells were not estimated in the study of Weibel and Bolender (1973).

The problems of estimating small populations of infrequently occurring cells and organelles are well known (Weibel and Bolender, 1973).

Various methods have been used to assess Ito cells previously. Bronfenmajer et al (1966) counted the number of Ito cells per high-power field (∗ 560). The Ito cell index was introduced by Horvath et al (1973). They gave no details of their counting methods. We compared the Ito cell index determined from 8-10 fields (oil) with cell counts from the point-counting grid. The correlation was not good, probably due to inaccuracies in estimating the numbers of hepatocytes and the smaller numbers of fields counted. The uneven distribution of Ito cells within the liver acinus of experimental animals noted by Wake (1971) was confirmed in man.

**Implications of the Increase in the Number of Ito Cells**

The number of Ito cells present in human liver has been found to increase with a number of factors such as certain liver diseases and drugs. The number of Ito cells is increased in chronic hepatitis and extrahepatic biliary cirrhosis (Bronfenmajer et al, 1966). None of our patients had these diseases. Bronfenmajer et al (1966) described an increase in the number of Ito cells after prolonged steroid therapy. We saw this in two of our patients who were receiving corticosteroids (258, 302). Other drugs, such as chlorpromazine (Bronfenmajer et al, 1966) and chenodeoxycholic acid (Hopwood, et al, in preparation), have been reported to cause an increase in the number of Ito cells. We found that MTX produces an increase in Ito cells, confirming the report of Horvath et al (1973). An excess of vitamin A intake has been shown to create an increase in the number of Ito cells in the human liver (Hruban et al, 1974).

The function of Ito cells is not clear, but the various theories of their origin have been reviewed by Hruban et al (1974). They believe that the Ito cell plays a role in the storage of vitamin A although there is evidence contrary to this (Hori and Kitamura, 1972). Hruban et al (1974) also pointed out that Ito cells may be fibroblast precursors, possibly related to the fibrosis and cirrhosis which may develop in hypervitaminosis A. Whether this mechanism may play a role in the fibrosis and cirrhosis reported following MTX therapy (Dahl et al, 1971; Nyfors and Poulsen, 1974) is an open question. Certainly, McGee and Patrick (1972) believe that the Ito cell has greater potential than the storing of fat. How else do the present results fit in with the known function of Ito cells? At the moment one can only say that MTX is an example of another drug which causes an increase in the number of Ito cells in the liver. When more examples are known then a mechanism for Ito cell hyperplasia may become apparent and their normal role may be better understood.

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