J. clin. Path., 1976, **29**, 698-703

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

D. HOPWOOD AND A. NYFORS

From the Department of Pathology, Ninewells University Hospital, Dundee DD2 1UB, Scotland and the Department of Dermatology, the Finsen Institute, 2100 Copenhagen, Denmark

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 Brodthagen, 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.

Received for publication 4 November 1975

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

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 aminotransferase (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 μ 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):

$$P_T = \frac{0{\cdot}453 \,.\, (1 - V_v)}{V_v \,.\, (EV_v)^2}$$

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 30 000 points are counted this gives an error of 10%.

Similarly, after methotrexate therapy, if 20 000 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.

Liver biopsy number	*Chief histological diagnoses*	
	Pre-MTX	*Post-MTX*
349	Severe fc	Moderate fc
254-281-291		
293-299-301	Normal	Normal
302-306-311		
255-258	Mild fc	Mild fc
279-308		
296	Mild nsrh	Mild nsrh
256-292-321	Normal	Mild fc
249	Normal	Mild nsrh
303	Mild fc	Moderate fc
295	Mild fc	Possible cirrhosis
307	Moderate fc	Severe fc
294	Moderate fc	Cirrhosis
259	Mild nsrh	Mild fibrosis

Table I *Chief histological diagnoses before and after MTX therapy*

fc = fatty change; nsrh = non-specific reactive hepatitis
Sign test: 14 unchanged, 1 improved, and 9 with more pathological changes: 1-9: P = 0·011

Liver biopsy number	Ito cell index		MTX cumulative dose (mg)	Interval (days): last MTX dose to last liver biopsy
	Pre-MTX	Post-MTX		
299	13	47	1074	2
321	25	84	1385	1
279	14	36	1493	1
306	12	46	1800	1
349	33	53	1905	5
255	25	118	2233	6
293	29	117	2290	5
295	32	104	2330	4
294	21	47	2650	2
296	33	63	2895	4
292	6	105	3218	1
301	31	43	2517	15
258	23	46	3040	39
249	18	56	2100	122
302	63	61	1960	151
259	15	53	2833	168
311	31	38	763	183
307	25	47	875	240
291	20	73	1183	435
303	41	50	990	600
254	32	99	1450	670
308	47	83	735	731
281	44	97	1193	785
256	14	42	528	1120

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.

LIGHT MICROSCOPY

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

Pre-MTX				Post-MTX		
Liver biopsy number	Volume density	Standard deviation	Points/cell	Volume density	Standard deviation	Points/cell
299	0·0015	0·001	1·07	0·005	0·004	1·29
321	0·0019	0·001	1·17	0·0068	0·006	1·86
279	0·0025	0·002	1·37	0·0047	0·004	1·45
306	0·0017	0·001	1·03	0·0047	0·004	1·47
349	0·0015	0·001	1·07	0·005	0·004	1·42
255	0·0019	0·001	1·15	0·0074	0·006	1·54
293	0·0033	0·002	1·24	0·0109	0·007	1·90
295	0·0037	0·001	1·37	0·014	0·008	2·11
294	0·0019	0·001	1·17	0·0047	0·003	1·52
296	0·0034	0·002	1·13	0·0056	0·004	1·39
292	0·002	0·001	1·16	0·0034	0·002	1·24
301	0·0041	0·002	1·57	0·0053	0·004	1·69
258	0·0034	0·001	1·30	0·0112	0·008	2·00
349	0·0018	0·001	1·12	0·0035	0·003	1·11
302	0·0020	0·001	1·20	0·0056	0·003	1·38
259	0·0017	0·001	1·12	0·0048	0·003	1·31
311	0·0021	0·001	1·22	0·005	0·004	1·49
307	0·0014	0·0009	1·08	0·003	0·002	1·35
291	0·0026	0·001	1·44	0·0081	0·0059	1·65
303	0·0023	0·001	1·13	0·0048	0·004	1·44
254	0·0058	0·004	1·41	0·0105	0·006	1·63
308	0·004	0·002	1·21	0·0059	0·005	1·42
281	0·0022	0·001	1·57	0·0058	0·004	1·32
256	0·0025	0·001	1·15	0·0086	0·005	1·41

Table III *Ito cell changes with methotrexate therapy*

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

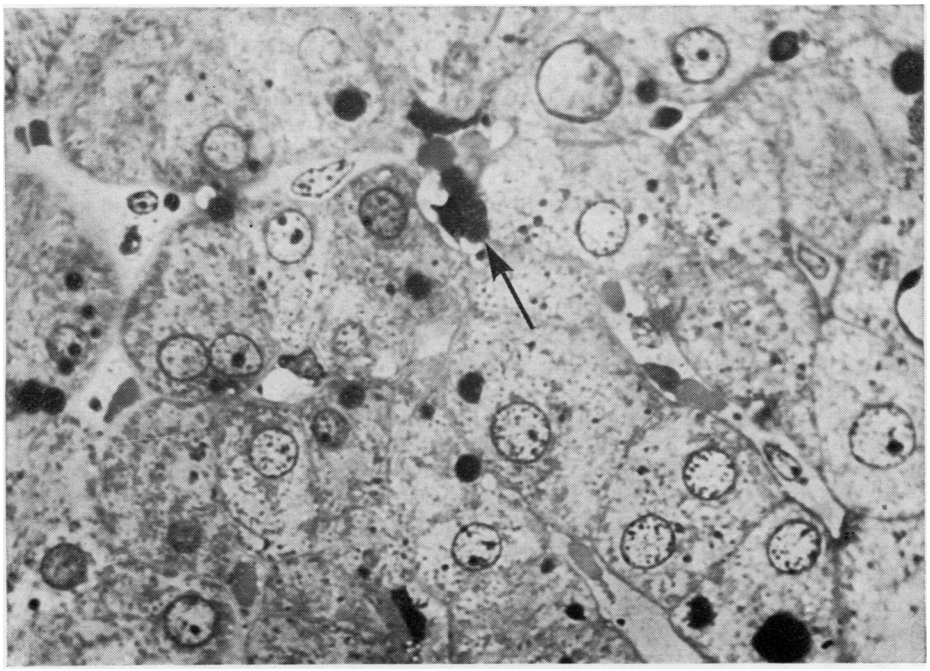

Fig 1 *Section of liver—1 μm Araldite embedded, toluidine blue stained—from a patient before methotrexate therapy. There is one Ito cell (arrow) towards the centre of the field. × 950.*

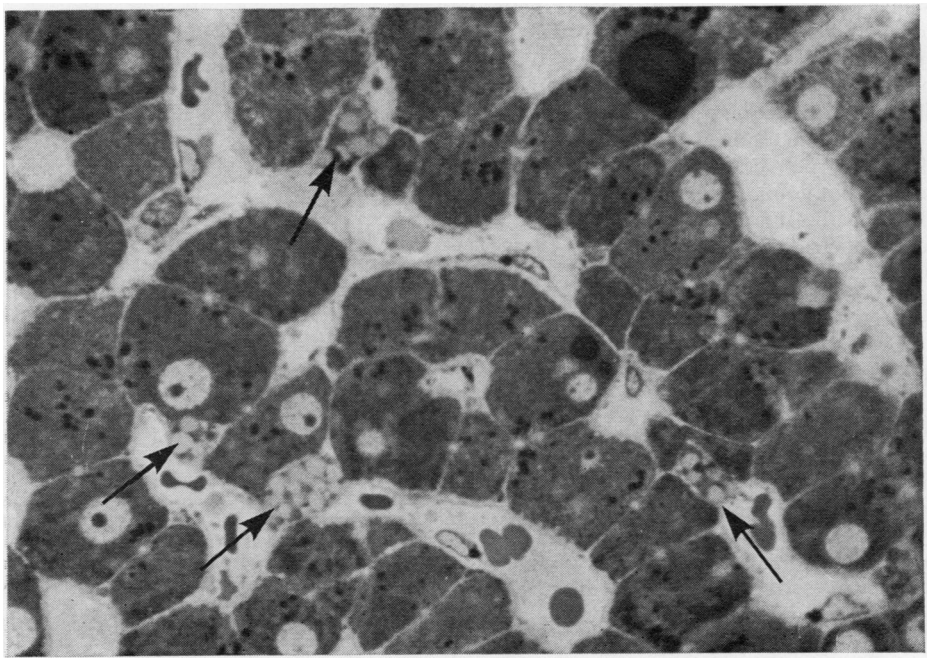

Fig 2 *Section of liver—1 μm Araldite embedded, stained with toluidine blue—from a patient during treatment with methotrexate. Several Ito cells are present in the field (arrows). × 840.*

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 open to 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.

We thank Mr G. Milne for his help with the preparation for electron microscopy, Mr R. Fawkes and Miss Sheila Nisbet for the photography, and Lederle for a research grant.

References

Bronfenmajer, S., Schaffner, F., and Popper, H. (1966). Fat-storing cells (lipocytes) in human liver. *Arch. Path.*, **82**, 447-453.

Coe, R. O. and Bull, F. E. (1968). Cirrhosis associated with methotrexate treatment of psoriasis. *J. Amer. med. Ass.*, **206**, 1515-1520.

Dahl, M. G. C., Gregory, M. M., and Scheuer, P. J. (1971). Liver damage due to methotrexate in patients with psoriasis. *Brit. med. J.*, **1**, 625-630.

Fahimi, H. D. (1970). The fine structural localization of endogenous and exogenous peroxidase activity in Kupffer cells of rat liver. *J. Cell Biol.*, **47**, 247-262.

Hori, S. H. and Kitamura, T. (1972). The vitamin A content and retinol esterifying activity of a Kupffer cell fraction of rat liver. *J. Histochem. Cytochem.*, **20**, 811-816.

Horvath, E., Kovacs, K., and Ross, R. C. (1973). Liver ultrastructure in methotrexate treatment of psoriasis. (Letter.) *Arch. Derm.*, **108**, 427-428.

Hruban, Z., Russell, R. M., Boyer, J. L., Glagov, S., and Bagheri, S. A. (1974). Ultrastructural changes in livers of

two patients with hypervitaminosis A. *Amer. J. Path.*, **76,** 451-468.

Ito, T. and Nemoto, M. (1956). Morphologischen Studien über die "Fettspeicherungzellen" in Leber bei verschiedenen Wirbeltieren. *Okajima Folia anat. jap.*, **28,** 521-542.

von Kupffer, C. (1875). Ueber Sternzellen der Leber. *Arch. mikr. Anat.*, **12,** 353-358.

McGee, J. O. and Patrick, R. S. (1972). The role of perisinusoidal cells in hepatic fibrogenesis. *Lab. Invest.*, **26,** 429-440.

Maximow, A. (1927). Bindegewebe und Blutbindegewebe. In *Handbuch der mikroskopischen Anatomie des Menschen*, edited by W. von Möllendorff. Springer, Berlin.

Muller, S. A., Farrow, G. M., and Martalock, D. L. (1969). Cirrhosis caused by methotrexate in the treatment of psoriasis. *Arch. Derm.*, **100,** 523-530.

Nyfors, A. and Brodthagen, H. (1970). Methotexate for psoriasis in weekly oral doses without adjunctive therapy. *Dermatologica (Basel)*, **140,** 345-355.

Nyfors, A. and Poulsen, H. (1974). Histological findings in liver biopsies from 81 patients with severe psoriasis—before and after methotrexate therapy. A blind study. In *Proceedings of the 20th Scandinavian Dermatological Congress, Stockholm, 1974.* pp. 53-54.

Nyfors, A. and Poulsen, H. (1976a). Histological findings in liver biopsies from 123 patients with severe psoriasis admitted for methotrexate therapy. *Acta path microbiol. scand. (A)*, **84,** 253-261.

Nyfors, A. and Poulsen, H. (1976b). Histological findings in liver biopsies from 88 psoriatics before and after MTX treatment. *Acta path. microbiol. scand. (A)*, **84,** 262-270.

Roenigk, H. H., Jr., Fowler-Bergfeld, W., and Curtis, G. H. (1969). Methotrexate for psoriasis in weekly oral doses. *Arch. Derm.*, **99,** 86-93.

Siegel, S. (1956). *Nonparametric Statistics for the Behavioral Sciences.* McGraw-Hill, New York.

Wake, K. (1971). "Sternzellen" in the liver: perisinusoidal cells with special reference to storage of vitamin A. *Amer. J. Anat.*, **132,** 429-462.

Weibel, E. R. (1963). *Morphometry of the Human Lung.* Springer, Berlin.

Weibel, E. R. and Bolender, R. P. (1973). Stereological techniques for electron microscopic morphometry. In *Principles and Techniques of Electron Microscopy*, edited by M. A. Hayat, vol. 3. Van Nostrand-Reinhold, New York and London.

Weinstein, G., *et al.* (1973). Psoriasis-liver-methotrexate-interactions. *Arch. Derm.*, **108,** 36-42.

Requests for reprints: Dr A. Nyfors, Department of Dermatology Finsen Institute, 2100 Copenhagen, Denmark.