Methotrexate Hepatotoxicity and Concentrations of Methotrexate and Folate in Erythrocytes—Relation to Liver Fibrosis and Cirrhosis

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Assessment of folate and methotrexate concentrations in erythrocytes has been suggested as criterion for when to institute liver biopsy surveillance during methotrexate treatment of psoriasis. We report an investigation of these parameters in thirty psoriatics on long-term methotrexate treatment. The patients were six psoriatics with histologically verified methotrexate-induced liver cirrhosis, eleven patients in which a blind histological evaluation had established a liver fibrosis in progression during treatment, and thirteen patients, who either had no liver fibrosis or no progression in the degree of fibrosis. There was no statistically significant difference in erythrocyte folate between the groups. When the two groups with progression were combined and compared with the group without progressive hepatic disease, erythrocyte methotrexate was found significantly higher in the patients with progression. Individual data, however, did not support the idea that assessment of folate and methotrexate in erythrocytes can limit the number of necessary liver biopsies during methotrexate treatment. (Received September 29, 1986.)

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It is now well established that methotrexate (MTX) may induce liver damage, which in some MTX-treated psoriatics will lead to fibrosis or cirrhosis (8, 11), but the biochemical basis for the hepatotoxicity is not known. It has recently been suggested, that folate-depletion may be an important factor. Changes found in the livers of folate-deficient rats have been reported to be similar to the MTX-induced changes of liver morphology in man. In psoriatics on long-term MTX folate may decrease and MTX accumulates intracellularly metabolized to polyglutamyl derivates in both liver cells and erythrocytes (3, 4).

Hendel et al. (5) studied changes in liver histology during MTX-therapy of psoriasis correlated to the concentrations of MTX and folate in erythrocytes. They found that MTX progressive changes in the liver were accompanied by high erythrocyte MTX and low erythrocyte folate. They also proposed that the application of these variables could prove valuable as decision criterion for performing liver biopsies. The present investigation was performed in order to study if erythrocyte MTX and erythrocyte folate in psoriatics with MTX-induced liver fibrosis or cirrhosis differed significantly from erythrocyte folate and erythrocyte MTX in psoriatics who did not develop fibrosis or cirrhosis.

MATERIAL AND METHODS

Thirty psoriatics, 16 females and 14 males, aged 28 to 83 years (median age 52 years) treated with MTX for 24 months or more were studied. All patients were started on the weekly divided oral dosage receiving 5 mg MTX three times with 12 h interval once weekly. Four patients when studied, however, were on MTX intramuscularly once weekly, because of subjective side-effects on the

former oral therapy. All patients had had their weekly dosage regulated according to clinical needs receiving from 0.06 to 0.029 mg/kg weekly (Tables I–III). Thirteen patients were still on their original dose of 15 mg MTX weekly. The weekly dosage had been unchanged for at least 5 weeks prior to the study. On the average the patients had been treated with MTX for a period of 7 years (range 1 to 16 years).

Erythrocyte MTX and erythrocyte folate was studied in all patients five to seven days following the previous MTX administration. Erythrocyte MTX was determined by enzymatic assay in a Cobas Bio centrifugal analyzer (9). When treatment is unaltered for at least 5 weeks only small variations have been found by this method (10). Erythrocyte folate concentrations were estimated by the method described by Mortensen (7).

All patients had liver biopsies taken during MTX-treatment with approximately one to two years' interval. In general the primary biopsy was a pre-MTX biopsy or a biopsy taken during the first 6 months of treatment. In all patients the histologic specimens were obtained by the Menghini technique. All biopsies were stained with hematoxylin-eosin stain, periodic acid–Schiff reagent, van Gieson's stain, Masson's trichromic stain, with Perl's method and reticular fiber stain a.m. Foot. To detect any progression in fibrosis towards cirrhosis all liver biopsies were assessed retrospectively by one of us (H. Sø.) without knowledge of individual patients, time of biopsy, or clinical and laboratory data. Cirrhosis was judged to be present or absent. Fibrosis was graded according to a four-step scala as either nor present, slight, moderate, or severe.

RESULTS

None of the primary liver biopsies showed cirrhosis. Six patients had liver biopsies which shoved progression to cirrhosis during their MTX treatment. Eleven patients had biopsies which showed progression in liver fibrosis, while 13 patients had no fibrosis or no progression in the degree of fibrosis.

The individual data on erythrocyte folate and erythrocyte MTX can be seen on Tables I–III and Fig. 1. The patients who had developed cirrhosis had been treated significantly longer than the other groups, however, they had developed their cirrhosis at a stage, which still would make them suitable for comparison (Table I). Patients without progression had lower mean erythrocyte MTX. The differences between the three groups were, however, not statistically significant. When the two groups developing progressive hepatic changes were combined and compared with the group of patients without progressive hepatic disease it was seen that the erythrocyte MTX was significantly higher in the patients with

Table I. *Erythrocyte folate and erythrocyte MTX in patients with MTX-induced liver cirrhosis*

Pat. no.	Age/sex	Cumulative MTX-dose (mg)	Cumulative MTX-dose at cirrhosis diagnosis (mg)	Latest dose (mg/kg)	Duration of treatment (months)	Duration of treatment at cirrhosis diagnosis (months)	Erythrocyte folate (nmol/l)	Erythrocyte MTX (nmol/l)
1	50/F	5 515	3 250	0.06	183	48	441	136
2	83/F	2 970	1 320	0.07	99	37	698	94
3	70/M	5 930	3 080	0.14	192	36	502	132
4	33/F	11 910	3 710	0.22	174	39	696	221
5	73/M	4 390	810	0.23	102	9	832	169
6	55/F	14 650	9 980	0.24	186	123	729	179
Mean[a] *n*=6	61±18	7 561±4 629	3 692±3 288	0.16±0.082	156±43.4	48±39	650±148	155±44.1

[a] Mean±SD.

progression than in the patients with no progression (Table IV) ($p<0.05$). The weekly doses of MTX in the two groups were comparable. The total dose of MTX and the duration of treatment, however, were stronger predictors of progressive hepatic changes. No correlation between erythrocyte folate and progressive hepatic disease could be demonstrated.

Table II. *Erythrocyte folate and erythrocyte MTX in patients with progression in MTX-induced liver fibrosis*

Pat. no.	Age/sex	Cumulative MTX-dose (mg)	Latest dose (mg/kg)	Duration of treatment (months)	Erythrocyte folate (nmol/l)	Erythrocyte MTX (nmol/l)
7	30/F	5 600	0.08	156	879	174
8	71/F	3 270	0.11	62	416	83
9[a]	66/M	5 850	0.12	117	300	153
10	71/M	1 720	0.16	43	540	120
11	35/F	2 980	0.16	71	337	122
12	63/M	1 830	0.16	33	301	169
13	44/F	13 520	0.21	139	688	182
14	39/F	5 210	0.22	89	422	94
15	40/F	4 290	0.23	91	491	136
16	70/F	3 270	0.24	58	373	217
17	35/M	6 720	0.28	112	686	202
Mean[b] $n=11$	51±17	4 939±3 281	0.18±0.062	88±40	494±187	150±43.2

[a] MTX given intramuscularly.
[b] Mean±SD.

Table III. *Erythrocyte folate and erythrocyte MTX in patients with no fibrosis or no progression in degree of liver fibrosis*

Pat. no.	Age/sex	Cumulative MTX-dose (mg)	Latest dose (mg/kg)	Duration of treatment (months)	Erythrocyte folate (nmol/l)	Erythrocyte MTX (nmol/l)
18[a]	64/F	2 160	0.08	48	241	107
19	62/M	2 410	0.09	64	425	83
20	45/F	4 320	0.12	102	269	94
21	71/M	1 690	0.12	24	235	157
22	47/M	3 280	0.13	81	919	94
23	65/F	4 760	0.17	91	390	62
24	54/M	2 580	0.17	70	738	127
25	38/M	4 290	0.18	66	233	136
26	34/M	1 540	0.19	24	841	118
27[a]	69/M	2 665	0.21	43	579	226
28[a]	28/M	4 320	0.25	79	325	136
29	40/F	2 220	0.25	37	466	108
30	32/F	3 180	0.29	54	590	84
Mean[b] $n=13$	50±15	3 032±1 086	0.17±0.065	60±24.6	480±236	118±42

[a] MTX given intramuscularly.
[b] Mean±SD.

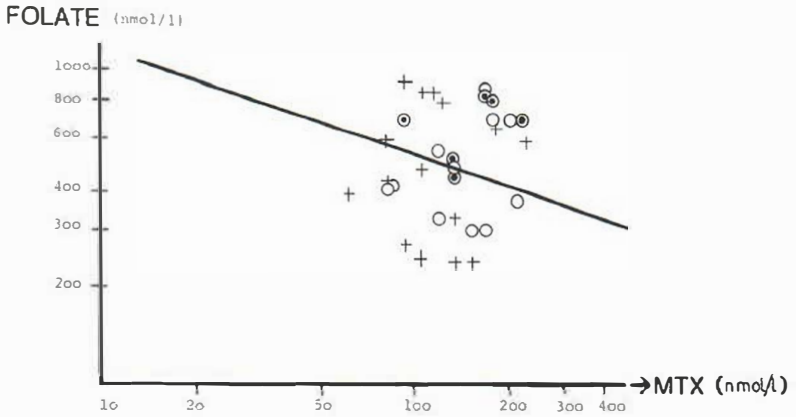

Fig. 1. Erythrocyte methotrexate concentrations and erythrocyte folate concentrations in methotrexate-treated psoriatics. +, no progression; ○, progression; ⊙, progression to cirrhosis.

DISCUSSION

In order to investigate for long-term hepatotoxicity in psoriatics on MTX repeat liver biopsies are recommended. Several attempts have been made to test non-invasive methods such as the galactose tolerance test (6), Tc 99m sulphur colloid liver scan (2), and attenuation of ultrasound (1). None of these methods, however, have been able to substitute histology. The approach by Hendel et al. (5) proposing that high erythrocyte MTX and low erythrocyte folate could be used as decision criterion for performing liver biopsies, has therefore been highly attractive.

The data from the present investigation, however, do not support the idea, that erythrocyte MTX and erythrocyte folate studies can limit the number of necessary liver biopsies. We found no significant differences within these parameters between patients with MTX-induced liver cirrhosis, patients with progression in liver fibrosis, and patients with no progression. Even though we found a significantly higher erythrocyte MTX in the combined group of patients with progressive hepatic changes compared with patients with no progression, we were not able to define a critical erythrocyte MTX concentration, above which the risk of developing hepatic damage was significantly increased. This is probably due to the fact that the erythrocyte MTX is determined by other factors as well, e.g. by the weekly dose of MTX (10), and by individual variations in the ability to metabolize MTX to polyglutamate forms in the red cell precursors of the bone marrow. Our data showed, however, that the cumulative dose of MTX and the length of treatment were stronger predictors of progressive hepatic disease than the erythrocyte MTX.

Table IV. *Comparisons between patients with progression in liver fibrosis or with cirrhosis and patients with no progression*

	Patients with progression (n=17)	Patients without progression (n=13)	p-values
MTX-dose (mg/kg/week)	0.172±0.068	0.173±0.065	NS
Ery-MTX (nmol/l)	152±42	118±42	$p<0.05$
Total MTX-dose (mg)	5 860±3 889	3 032±1 086	$p<0.01$
Treatment (months)	112±52	60±25	$p<0.01$
Ery-folate (nmol/l)	549±186	480±236	NS

Our patient material differs from the psoriatics studied by Hendel and co-workers (5) as we chose to study a group of psoriatics in which we knew there would be a number of patients with MTX-induced liver fibrosis or liver cirrhosis. This because the progressive changes described by Hendel and co-workers mainly were found within the fatty changes. Although fatty changes may be a manifestation of a process eventually proceeding to cirrhosis, this is not necessarily so. There have also been differences in dosage schedule. Most of our patients were on the weekly divided oral MTX-dosage, while all the patients from the study of Hendel et al. received one single weekly oral dose of MTX. Results from long-term studies with serial biopsies have, however, showed approximately the same frequency of liver cirrhosis in psoriatics on the two dosage schedules (11).

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