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The Protein Binding of Methotrexate in the Serum of Patients with Neoplastic Disease

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Summary. *1. Serum protein binding of methotrexate was studied in 14 patients with various forms of malignant disease and in eight age- and sex-matched subjects (control group) attending outpatient clinics for various clinical conditions.*

2. Protein binding was determined by continuous ultrafiltration and methotrexate concentrations by double-antibody radioimmunoassay.

3. Protein binding of the drug is critically dependent on albumin concentration, as shown by results in individual subjects and a significant regression of methotrexate binding on albumin concentration. Moreover, at high methotrexate concentrations drug binding becomes non-linear, resulting in disproportional elevation of free methotrexate levels. Both these findings have important implications for the treatment of hypoalbuminaemic patients.

4. Two classes of binding sites were observed in both groups of patients, viz a high-affinity, low-capacity group and a low-affinity group with higher capacity.

5. No significant difference was found between patient and control groups either in the percent bound drug or in the binding parameters.

6. In conclusion, while there appear to be no factors specific to malignant disease which perturb methotrexate's protein binding, it may be important to determine the extent of drug binding before methotrexate can be used judiciously, particularly when total drug level is related to likely toxicity and in the design of an appropriate folinic acid rescue regimen after high-dose therapy.

Introduction

Methotrexate has been reported to bind predominantly to serum albumin [14, 17]. Since albumin metabolism [15, 19] and electrophoretic behaviour [6, 9, 11] can be considerably modified by the presence of neoplastic disease, alterations in the nature and extent of methotrexate-albumin binding might theoretically occur due to aberrant protein structure or conformation. Anomalies in binding could influence distribution and excretion, as has been reported for other drugs [2, 12, 18], and subsequently alter drug effects such as efficacy and toxicity. This study describes the binding of methotrexate to albumin in the serum of patients with various forms of neoplastic disease.

Patients and Methods

Serum was obtained, before initiation of any chemotherapy, from 14 patients recently admitted to hospital for treatment of a variety of neoplastic diseases (Table 1). Patient data were compared with results obtained from an age- and sex-matched control group of patients attending an outpatient clinic for a number of non-malignant conditions (Table 1). Neither group had received drug therapy of any kind on the 3 days before withdrawal of blood. Serum was stored at −20° C until protein binding studies could be performed.

The serum albumin concentration was measured in a Technicon auto-analyser with the bromocresol green method, while methotrexate concentrations were measured by double-antibody radioimmunoassay [8]. Protein-methotrexate interaction was studied by continuous ultrafiltration [1] with the Amicon Multimicro Concentrator (MMC) apparatus and Amicon Diaflo XM_{50} ultrafiltration membranes, as described in a previous publication [13].

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Table 1. Clinical details of patients and controls

Patient	Sex	Age (years)	Disease	Serum bilirubin (μmol/l)	Serum albumin (g/l)
E. G.	F	66	Carcinoma, breast	5	45
E. L.	M	63	Carcinoma, lung	7	44
R. S.	M	48	Brain tumor	14	39
J. S.	M	40	Carcinoma, lung	6	38
E. R.	F	62	Carcinoma, breast	6	40
A. P.	F	49	Carcinoma, breast	6	46
D. P.	M	53	Osteosarcoma	6	41
H. B.	F	73	Carcinoma, caecum	7	32
M. McB.	M	63	Carcinoma, lip	4	36
M. D.	F	71	Melanoma	6	38
C. McS.	F	80	Lymphoma, colon	65	37
A. T.	F	64	Carcinoma, lung	6	35
C. T.	F	77	Carcinoma, breast	4	29
M. C.	F	56	Carcinoma, ovary	9	43
Mean ± S.D.		61.79 ± 11.56		10.79 ± 15.80	38.79 ± 4.89
Controls					
M. R.	F	67	Diabetic hypertensive	11	39
E. B.	F	79	C.V.A.	7	32
E. C.	M	70	C.V.A.	6	36
R. J.	M	45	C.V.A.	15	37
M. B.	F	64	C.V.A.	7	40
W. A.	F	56	Coeliac	11	28
J. A.	F	56	Coeliac	29	34
J. P.	M	76	Angina	11	35
Mean ± S.D.		64.13 ± 11.35		12.13 ± 7.4	35.13 ± 3.87

C.V.A. – Cerebrovascular accident

Curve fitting of the Scatchard plots was carried out by means of a least-squares fitting programme. Comparisons of parameter values between control and patient groups were made by the Mann-Whitney U test.

Results

The mean protein binding at a total concentration of 50 μmol/l was 92.04% ± 4.04 (SD) in the controls, as against 92.77% ± 4.50 (SD) in the patients (Table 2). Low serum albumin levels were found in one patient (C. T.: 29 g/l) and one control subject (W. A.: 28 g/l). The percent drug bound at 50 μmol/l in both these subjects was considerably reduced at 81.10% and 82.97% bound, respectively. Considering patients and controls together there was a highly significant regression ($P = 0.07$; $r^2 = 0.314$) of percent methotrexate binding at 50 μmol/l on serum albumin. A high serum bilirubin (65 μmol/l) was observed in patient C. McS. (Table 1), and while the percentage of methotrexate bound was slightly reduced at 88.85%, against a group mean of 92.77% (± 4.50), this did not appear to affect the binding parameters markedly.

Inspection of the Scatchard plots generated by both groups suggested two classes of binding sites. Analysis of these curves for the control group indicated 0.19 ± 0.04 (SD) binding sites (N_1), with an intrinsic association constant (K_1) of $45.04 \times 10^4\ M^{-1}$ ± 7.34 (SD) in the high-affinity group (Class I). In the low-affinity group (Class II) $N_2 = 2.31 \pm 1.53$ (SD) and $K_2 = 0.16 \times 10^4\ M^{-1} \pm 0.10$ (SD) (Table 2). In the patients with malignant disease $N_1 = 0.19 \pm 0.04$ (SD); $K_1 = 51.29 \times 10^4\ M^{-1} \pm 30.10$ (SD); $N_2 = 3.06$ ± 1.79 (SD); and $K_2 = 0.11 \times 10^4\ M^{-1} \pm 0.10$ (SD) (Table 2).

There were no significant differences between the percent bound drug in the control group and in patients with malignant disease or in binding parameters between the groups (Table 3). While there was a scatter of values around the mean for K_1 in the patient group (Table 3), no biochemical or obvious clinical abnormalities explained this observation.

Finally, the results of this study confirmed the non-linearity of methotrexate protein binding, associated with total drug concentrations greater than 50 μmol/l, as we have described previously [13].

Table 2. Individual protein binding data of patients and controls

Patient	% Bound[a]	Class I		Class II	
		N_1	$K_1 \times 10^4\ M^{-1}$	N_2	$K_2 \times 10^4\ M^{-1}$
E. G.	92.99	0.26	12.20	4.13	0.03
E. L.	91.73	0.23	14.70	5.56	0.03
R. S.	98.01	0.17	133.00	1.86	0.12
J. S.	97.64	0.20	90.20	3.11	0.05
E. R.	96.13	0.17	50.00	5.73	0.05
A. P.	93.30	0.18	48.00	4.02	0.05
D. P.	90.75	0.17	51.18	5.52	0.05
H. B.	92.77	0.21	35.07	3.96	0.04
M. McB.	90.06	0.13	56.76	1.19	0.38
M. D.	91.50	0.13	51.35	1.39	0.23
C. McS.	88.85	0.16	36.00	1.04	0.20
A. T.	96.83	0.24	50.18	0.70	0.06
C. T.	81.10	0.22	37.87	2.86	0.05
M. C.	97.09	0.18	51.50	1.75	0.15
Mean ± SD	92.77 ± 4.50	0.19 ± 0.04	51.29 ± 30.10	3.06 ± 1.79	0.11 ± 0.10
Controls					
M. R.	93.90	0.16	44.72	0.55	0.31
E. B.	92.01	0.18	55.95	5.04	0.04
E. C.	93.60	0.23	38.91	4.01	0.09
R. J.	93.58	0.14	32.88	1.44	0.21
M. B.	96.02	0.21	49.63	1.93	0.13
W. A.	82.97	0.27	43.78	1.70	0.15
J. A.	90.16	0.18	42.73	2.72	0.06
J. P.	94.11	0.15	51.73	1.06	0.25
Mean ± SD	92.04 ± 4.04	0.19 ± 0.04	45.04 ± 7.34	2.31 ± 1.53	0.16 ± 0.10

[a] Values refer to binding at a total drug concentration of 50 μmol/l

Table 3. Group comparison of patients and controls

Group	Age (years)	Serum albumin (g/l)	% Bound[a]	Class I		Class II	
				N_1	$K_1 \times 10^4\ M^{-1}$	N_2	$K_2 \times 10^4\ M^{-1}$
Patients	61.79 ± 11.56	38.79 ± 4.89	92.77 ± 4.50	0.19 ± 0.04	51.29 ± 30.10	3.06 ± 1.79	0.11 ± 0.10
Controls	64.13 ± 11.35	35.13 ± 3.87	92.04 ± 4.04	0.19 ± 0.04	45.04 ± 7.34	2.31 ± 1.53	0.16 ± 0.10
Mann-Whitney-'U'-test	N.S.	N.S.	N.S.	N.S.		N.S.	N.S.

[a] Values refer to binding at a total drug concentration of 50 μmol/l

Discussion

The estimate of methotrexate's protein binding obtained here is considerably higher than some earlier published results [3, 4, 17] for the methodological reasons we have discussed previously [14].

Neoplastic disease per se did not overtly modify the interaction of methotrexate with albumin. Thus no statistically significant differences in percent methotrexate bound at 50 μmol/l or in the drug's binding parameters were observed between patients with malignant disease and the control group. While there was greater variability in respect of the K_1 estimates in the patient group the explanation for this is unclear, although it may be related to the wide range in overall severity of illness at the time of clinical presentation of malignancy.

The importance of serum albumin concentration in determining the plasma protein binding of methotrexate deserves emphasis. This is illustrated by the regression of methotrexate binding on albumin levels and the marked rise in *free* drug concentration at 50 μmol/l in two subjects with abnormally low albumin levels.

Midler [5] and Steinfeld [15] observed a progressive fall in plasma albumin with progression of malignant disease, and the influence of cachexia, a common accompaniment of malignancy, on circulating albumin levels is well known. Alteration in serum protein binding can profoundly influence the distribution and excretion of drugs [2, 18], but drug tissue binding plays an important part in this balance. It is clear, moreover, as has been demonstrated for phenytoin [12], that interpretation of a particular total serum level of a drug may be critically dependent on ambient serum protein binding. This concept may be particularly relevant to methotrexate when concentration/toxicity correlations are considered and when folinic acid rescue is used after high-dose therapy, where it is known that total concentrations over 1×10^{-6} *M* at 48 h after the start of infusion are associated with a high risk of toxicity [7, 10, 16]. As free drug levels determine pharmacological effect and toxicity, the influence of serum albumin concentration and the non-linearity of methotrexate protein binding at high drug levels should assume greater importance in assessing efficacy/toxicity likely to be associated with a particular total circulating methotrexate concentration.

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