Cancer Chemother Pharmacol (1985) 14:165–167

Cancer Chemotherapy and Pharmacology

The pharmacokinetics of 7-hydroxymethotrexate following medium-dose methotrexate therapy

Alan L. Stewart[1], Jennifer M. Margison[1], Peter M. Wilkinson[1], and Samuel B. Lucas[2]

[1] Department of Clinical Pharmacology, Christie Hospital & Holt Radium Institute, Withington, Manchester, M20 9BX, England
[2] Faculty of Medicine, Computational Group, Manchester University, Manchester, England

Summary. *An unambiguous and specific HPLC assay was used to determine the pharmacokinetics of 7-hydroxymethotrexate (7-OHMTX) following the administration of moderate-dose methotrexate (MTX) 100 mg · m^{-2} to 37 patients with advanced head and neck cancer. There was marked interpatient variation but patient exposure to 7-OHMTX was considerable. There was, however, no correlation between the amount of 7-OHMTX produced and either tumour response or patient toxicity.*

Introduction

Methotrexate (MTX) has been widely used in the treatment of malignant disease for over 20 years. The initial pharmacokinetic studies in man were performed with low-dose tritiated MTX [2], and one conclusion drawn was that there was minimal metabolism of the drug by bacterial flora in the lower gastrointestinal tract, with the production of 2,4-diamine N10 methylpteroic acid [3]. These findings were accepted for many years, but more recent studies with high-dose MTX and more specific assays have shown that there is in fact significant biotransformation. One of the metabolites is 7-hydroxymethotrexate (7-OHMTX), which is produced by hydroxylation of the parent drug by a hepatic aldehyde oxidase [5].

It has been claimed that 7-OHMTX is produced only after the administration of high-dose MTX because of the low affinity of the responsible enzyme for the parent drug and the slow rate of the reaction [4], but Lankelma et al. [7], have demonstrated the formation of 7-OHMTX after IV administration of as little as 25 mg.

The development of HPLC methodology has permitted more accurate kinetic studies, and we report here the pharmacokinetics of 7-OHMTX following the administration of MTX for the treatment of advanced head and neck cancer.

Patients and methods

All patients had advanced head and neck cancer, recurrent after radiotherapy or too advanced at presentation for useful radiotherapy. No patient had received prior chemotherapy. All patients had a Karnofsky performance status > 50 and all had normal hepatic and renal function as measured by standard biochemical parameters prior to commencing therapy. Patients were treated with MTX 100 mg · m^{-2} given as an IV bolus every 2 weeks and continued until definite tumour progression was noted. No attempts were made to increase hydration or to alkalinise the urine. Folinic acid rescue was only given to those patients whose 24-h serum MTX concentrations were $> 4 \times 10^{-7}$ *M* and were, from our previous experience, considered likely to be at risk from serious toxic manifestations. Venous blood for estimation of MTX and 7-OHMTX concentrations was obtained via an indwelling cannula at 5, 10, 15, 30, and 60 min and 2, 3, 6, 9, 12, 24, 33, and 48 h after treatment. Serum was removed and assayed within 24 h or stored at −20° C and assayed within 14 days. Haematological and mucosal toxicity were assessed using the criteria described by the Eastern Cooperative Oncology Group. Renal toxicity was assessed by serial measurement of serum creatinine and creatinine clearance.

Drug assay

MTX and 7-OHMTX were measured in serum using a sensitive and specific reverse-phase high-pressure liquid chromatography method developed in our laboratory.

7-OHMTX was prepared by incubating the supernatant from homogenised New Zealand white rabbit liver with MTX for 1 h. Following deproteination the supernatant was passed through a DE 52 ion exchange column. Four major peaks were obtained, the last of which gave a UV spectrum identical with that obtained with pure 7-OHMTX (kindly provided by Dr B. Chabner, National Cancer Institute), and a single peak on HPLC analysis.

Chromatographic separation was carried out using a Waters' ALC 200 liquid chromatograph with a 250 × 5 mm column packed with ODS-Hypersil (Shandon – Southern Ltd), a C-18 bonded microparticulate silicate. Detection was by UV monitoring at a wavelength of 313 nm. The eluent was 0.1 *M* Tris phosphate buffer pH 6.7, containing 25% methanol, flow rate 1.2 ml · min^{-1}. Paraaminoacetophenone was used as an internal standard. A typical chromatogram is shown in Fig. 1. Serum samples were prepared by deproteination with methanol prior to injection. The retention time for MTX was 6.5–7 min and that of 7-OHMTX was 8–8.5 min. All assays were performed in duplicate.

The lower limits of detection for MTX and 7-OHMTX were 7×10^{-8} *M* and 5×10^{-7} *M*, respectively. The intra-assay coefficient of variation for 7-OHMTX was 5.2 at 8×10^{-7} *M* ($n = 20$) and the interassay coefficient of variation was 9.2 ± 1 at 8×10^{-7} *M* ($n = 20$).

Offprint requests to: A. L. Stewart

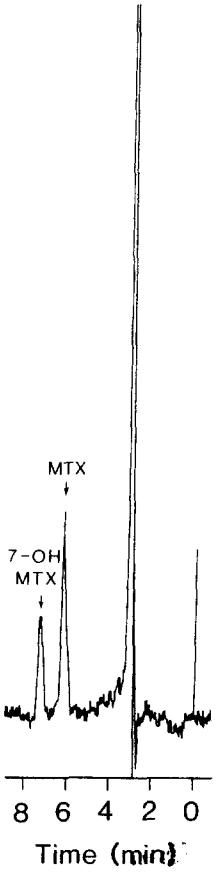

Fig. 1. Typical HPLC chromatogram: specimen taken 6 h after treatment shows MTX and 7-OHMTX peaks

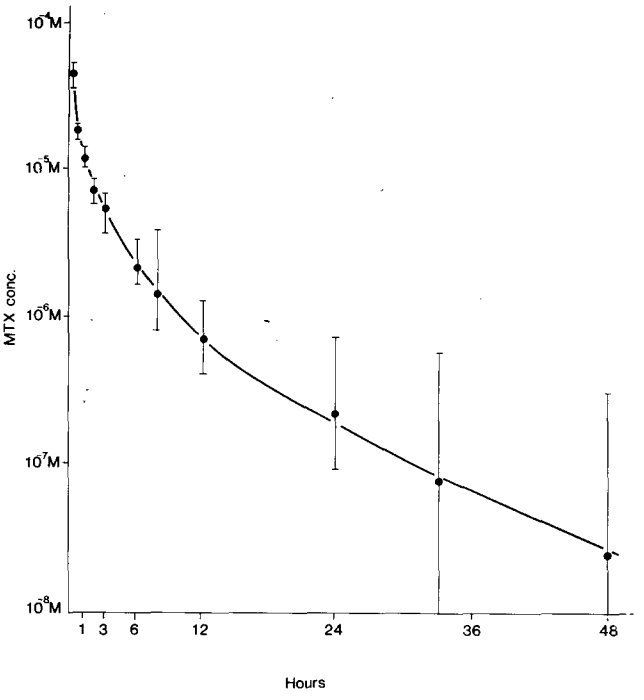

Fig. 2. Concentration/time decay curve for MTX median and interquartile range from 37 patients following IV bolus administration of MTX (100 mg/m^2)

Results

Full kinetic data were obtained from 37 patients following the initial administration of MTX. Further data on a more limited basis were obtained on approximately half these patients on subsequent occasions. Figure 2 shows the median decay curve for MTX from 37 patients following the initial drug administration. Peak MTX concentrations were in the range $5-8 \times 10^{-5}$ *M* and the plot of serum MTX decay with time was fitted by a nonlinear regression analysis to a triexponential equation compatible with a three-compartment model as has been previously described [3]. The median total area under the concentration/time curve for MTX was 54.2 $\mu M \cdot l^{-1} \cdot h^{-1}$ (range 25.2–182 $\mu M \cdot l^{-1} \cdot h^{-1}$). The median half-times of the three decay phases were 0.3 h, 3.09 h, and 33 h, but there was considerable interpatient variation. The median total plasma clearance was 115 ml · min^{-1} and the median apparent volume of distribution at steady state was 37 l. There was marked interpatient variation in the amount of 7-OHMTX produced, and in one patient no measurable 7-OHMTX was detected.

Figure 3 shows the serum concentration/time curve for 7-OHMTX from 36 patients. 7-OHMTX was undetectable in serum in the 1st hour after administration, but thereafter the concentration increased rapidly, reaching maximum values of 1.2×10^{-6} *M* after 6–8 h. Following this, the concentration decreased slowly with a median $t_{1/2}$ of 13 h, compared with 9.5 h for the parent drug, between 12 and 24 h after administration. The median 7-OHMTX concentration

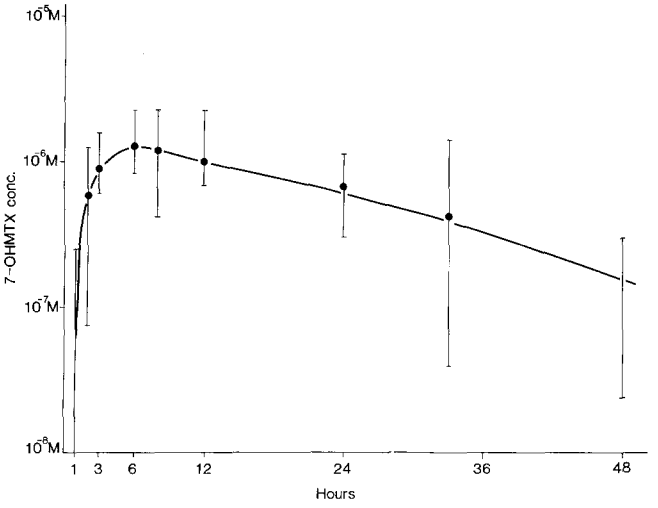

Fig. 3. Concentration/time decay curve for 7-OHMTX median and interquartile range from 36 patients following IV bolus administration of MTX (100 mg/m^2)

exceeded that of MTX at all time points after 10 h. Several patients with delayed MTX clearance had raised levels of 7-OHMTX, particularly at late time points. The median 7-OHMTX concentration at 24 h for those with normal MTX clearance (defined as serum MTX concentration $< 4 \times 10\,M$ at 24 h; was 6.5×10^{-7} *M*, which was significantly less than in

those with delayed MTX excretion (2.2×10^{-6} M; $P < 0.01$). Most patients had 7-OHMTX measured on subsequent occasions and a statistically significant ($P = 0.03$) increase in the amount of 7-OHMTX present was noted. The median 24-h 7-OHMTX concentration on first administration was 9.9×10^{-7} M and on second administration it was 1.6×10^{-6} M. This increase is presumably due to hepatic enzyme induction, as suggested by Lankelma et al. [7].

Patient exposure to 7-OHMTX was considerable. The median area under the concentration/time curve was 25.2 $\mu M \cdot l^{-1} \cdot h^{-1}$ (range 0–383 $\mu M \cdot l^{-1} \cdot h^{-1}$). The median value for the ratio of area under the concentration/time curve for MTX to that of 7-OHMTX was 2.46 : 1, and there was a significant positive correlation between total areas under the curves for MTX and 7-OHMTX ($r = 0.65$, $P = 0.001$). There was a significant inverse correlation between the rate of MTX elimination and the area under the curve for 7-OHMTX ($r = 0.43$, $P = 0.008$). There was no correlation between peak concentration or area under the curve for 7-OHMTX and serum urea, creatinine or creatinine clearance. There was no significant correlation between exposure to 7-OHMTX in terms of area under the curve and response to therapy ($r = 0.002$, $P = 0.33$) or to drug-induced toxicity ($r = 0.125$, $P = 0.248$).

Discussion

This study has established that at this relatively low dose (100 $mg \cdot m^{-2}$) there is significant hydroxylation of MTX to the metabolite 7-OHMTX. However, the results show considerable interpatient variation in the amount of metabolite produced, and this is related to total exposure to the parent drug. The study does not support the theory that the hydroxylation of MTX is a dose-dependent phenomenon seen only at high dosage. The kinetic parameters reported are in general agreement with those recently described by Chan et al. [1].

The role of 7-OHMTX in cancer therapy is not yet fully elucidated. It is a less effective inhibitor of the enzyme dihydrofolate reductase, with a Ki of 6×10^{-9} M at pH 5.5, as against 3×10^{-11} for MTX [6]. It does not significantly add to the antitumour effect of MTX and may be thought of as an inactivation pathway. We have not investigated the interaction of MTX and 7-OHMTX at the cellular level, but Lankelma et al. have shown in vitro that the presence of 7-OHMTX in the tissue culture medium will reduce the intracellular MTX concentration by interference with membrane transport [7]. This will potentially reduce the antitumour effect, especially at late time points, when serum 7-OHMTX concentration is well in excess of that of the parent drug.

Our study has not shown any correlation between the degree of biotransformation and the response to treatment. The aqueous solubility of 7-OHMTX is three to four times less than that of MTX at physiological pH, and following the demonstration that 7-OHMTX precipitated in the renal tubules of monkeys receiving high-dose MTX this metabolite has been implicated in the development of MTX-related toxicity [5]. Our study has not demonstrated any association between the amount of 7-OHMTX produced either in terms of peak concentration or total exposure and increased mucosal haematological toxicity. There was no association between the amount of 7-OHMTX produced and change in renal functions as measured by serum urea ($P = 0.33$) or creatinine clearance ($P = 0.21$). In summary, there is evidence of considerable hydroxylation of MTX at relatively low dose administration, but we have been unable to demonstrate any significant biological effect from the metabolite 7-OHMTX in man.

Acknowledgements. This work was supported by a grant from the Medical Research Council. We thank Miss J.L. Parsons and Mrs M.A. Green for typing the manuscript.

References

1. Chan KK, Barrientos AR, Stolinsky D, Vales LM, Jamin D, Mitchell MS, Cohen JL (1982) Pharmacokinetics and metabolism of methotrexate revisited. Proc Am Assoc Cancer Res 23: 517
2. Henderson ES, Adamson RH, Oliverio VT (1965) The metabolic fate of tritiated methotrexate. II. Absorption and excretion in man. Cancer Res 25: 1018
3. Huffman DH, Wan SH, Azarnoff DC, Hoogstraten B (1973) Pharmacokinetics of methotrexate. Clin Pharmacol Ther 14: 572
4. Jacobs SA, Stoller RG, Chabner BA, Johns DG (1976) 7-Hydroxymethotrexate as a urinary metabolite in human subjects and rhesus monkeys receiving high-dose methotrexate. J Clin Invest 57: 534
5. Jacobs SA, Stoller RG, Chabner BA, Johns DG (1977) Dose-dependent metabolism of methotrexate in man and rhesus monkeys. Cancer Treat Rep 61: 651
6. Johns DG, Loo TL (1967) Metabolite of 4-amino-4-deoxy-N10-methylpteroylglutamic acid (methotrexate). J Pharm Sci 56: 346
7. Lankelma J, Van der Klein E, Ramaekers F (1980) The role of 7-hydroxy-methotrexate during methotrexate anticancer therapy. Cancer Lett 9: 133

Received September 21, 1983/Accepted August 14, 1984