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Effect of Route of Administration and Effusions on Methotrexate Pharmacokinetics[1]

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SUMMARY

The pharmacokinetics of i.v. and p.o. methotrexate (MTX) in humans is reported. The plasma concentration profile following the two routes of administration was parallel with a mean final half-life of 24.9 hr. Absorption of tritiated MTX from solution is complete at a dose of 30 mg MTX per sq m body surface area. At the higher dose of 80 mg/sq m only 31% of the dose was absorbed in the one patient studied. The amount of metabolites formed after i.v. administration is negligible at 6% of the dose. In contrast, following p.o. administration, 35% of the absorbed dose is excreted as metabolites. Metabolism of p.o. MTX in the gastrointestinal tract or during the first pass through the liver is suggested as possible events.

The presence of malignant effusions could contribute to inter-individual variation in pharmacokinetics.

INTRODUCTION

MTX[4] as an antitumor drug is effective either as a single agent or in combination with other chemotherapeutic agents. Advances in MTX therapy of malignant diseases have come through the development of different p.o. and parenteral schedules alone or in combined regimens. Thus, knowledge concerning the pharmacokinetics of MTX following different routes of administration is invaluable and should be helpful in developing optimal route and dosing schedules.

The absorption and metabolism of p.o. MTX in man is not well defined. Burchenal *et al.* (3) reported peak serum levels of approximately 10^{-7} M following ingestion of 5 mg, with 40 to 57% of the dose recovered from urine in 24 hr. An enzymatic method was used in this study. Freeman (4) reported rapid and complete absorption following the p.o. administration of 1 to 31 mg of MTX. A fluorimetric method was used in this study and plasma levels were 3 times higher than those reported by Burchenal *et al.* In a study using MTX-^{3}H, the absorption of MTX from solution was found to be complete at doses of 0.1 mg/kg. At larger doses, however, absorption was incomplete (5).

Gastrointestinal bacteria are probably responsible for metabolites of MTX found in urine and feces of mice (16, 17). Metabolites appear in the urine of man 24 hr after i.v. administration of MTX. We have previously suggested that the formation of metabolites of MTX in man is related to metabolism during enterohepatic cycling (6). If this is the case, differences in elimination between p.o. and i.v. MTX should exist.

The present study attempts to define the kinetics of absorption and elimination of p.o. MTX, compares elimination between p.o. and i.v. administered drug, and evaluates the influence of malignant effusions on pharmacokinetics.

MATERIALS AND METHODS

Subjects. Thirteen patients, 9 males and 4 females, with various types of cancers participated in the study. Patients admitted to the program had creatinine clearance greater than 50 ml/min. Kidney function was characterized by measurement of serum creatinine and the creatinine clearance was approximated from a normogram based on weight, age, and sex (13). Five patients had an elevated alkaline phosphatase due to skeletal metastases, and 1 patient had a high serum glutamate-oxaloacetate transaminase. All had normal bilirubin levels. Three patients had either a malignant pleural effusion (T. K.) or malignant ascites (M. W., J. P.). Five of the patients had no previous therapy, while 8 had been given various treatments. With the exception of codeine, volunteers did not receive other medication during the study. Informed consent was obtained from all volunteers after a complete discussion of the study and explanation of the hazards and inconveniences reasonably to be expected.

Drug and Administration. Tritiated MTX (Monsanto Chemical Co., St. Louis, Mo.) was purified by column chromatography on DEAE-cellulose (11). The fraction containing MTX was lyophilized, reconstituted in water for injection, sterilized by passage through a Millipore filter, and kept at −18° until used. The time between purification and use did not exceed 2 weeks. The MTX-^{3}H was mixed with a commercially available parenteral dosage form (Lederle Laboratories, Pearl River, N. Y.) immediately prior to administration. In each case, the dose of tritiated MTX was 200 μCi/sq m body surface area. The total dose of MTX was 30 mg/sq m. Seven of the patients received a single p.o. dose. Another 5 received the drug both i.v. and p.o. in a random order on 2 separate occasions at least 2 weeks apart. One patient received 30 mg/sq m i.v. and 80 mg/sq m p.o.

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[4] The abbreviation used is: MTX, methotrexate.

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All doses were administered after an overnight fast together with 250 ml water taken p.o. In the p.o. studies, the dose was administered via a nasogastric tube. Four hr following the administration of drug, patients were allowed tea and dry toast.

Sample Collection and Analysis. Blood and urine specimens were obtained prior to drug administration. All urine was collected at specified intervals for 96 hr, blood was collected into tubes containing heparin for 72 hr, and feces were collected for 4 days following administration of MTX-^{3}H. Blood, plasma, urine, and fecal samples were oxidized to ^3H_2O (Packard tissue sample oxidizer) and the radioactivity was determined by liquid scintillation spectrometry (Packard Tri-Carb liquid scintillation spectrometer) using a toluene-dioxane base scintillation fluid. Quench correction was by external standardization. The concentration of MTX in these samples was determined by relating the μCi of ^{3}H in that sample to the specific activity of the originally administered MTX.

Pleural or ascitic effusions were obtained by aspiration, and MTX concentration was determined as above. In 1 patient (M. W.), drug concentration in ascitic fluid was measured 24, 36, and 48 hr following drug administration.

Protein binding was determined on 4 to 5 plasma samples obtained during the 1st 8 hr from each patient using an ultrafiltration technique (2). Drug binding to ascitic or pleural fluid proteins was determined in the same manner.

To evaluate metabolism, aliquots of urine were chromatographed on DEAE-cellulose (10), and radioactivity in the various fractions was determined. In 1 patient (B. B.), blood (200 ml) was drawn only at the 24-hr sampling period during both the p.o. and i.v. studies. Plasma proteins were precipitated by isopropyl alcohol and the clear supernatant was concentrated by lyophilization. The concentrated material was then chromatographed to determine the presence of possible metabolites. All plasma and urine samples were stored at $-18°$ and chromatographed within 7 days.

RESULTS

Table 1 is a summary of some pertinent clinical data about the patients including diagnosis, previous therapy, and liver and kidney function.

P.O. Study (30 mg/sq m). The plasma concentration profile following p.o. administration of 30 mg/sq m to 7 subjects is shown in Chart 1. At least 70% of the dose was absorbed as determined by cumulative excretion in urine. Absorption was rapid with peak concentrations achieved by 1.5 hr. Excretion of drug in urine and feces at 72 hr accounted for 81% of this dose. The mean plasma clearance of drug (amount excreted

Table 1
Patient data

Patient	Sex	Diagnosis	Previous therapy	Clinical laboratory data: Creatinine clearance (ml/min)	Bilirubin (mg/dl)	Serum glutamate-oxaloacetate transaminase (units/ml)	Alkaline phosphatase (units/ml, Bessey-Lowry)
			p.o. Study				
A. P.	M	Small-cell cancer of lung	X-ray	79	ND[a]	ND	ND
P. G.	M	Cancer with squamoid tendencies primary unknown	None	78	0.4	94	11.8
J. P.	F	Papillary cystadenocarcinoma of ovary	Cyclophosphamide	65	0.1	22	4.7
B. B.	M	Metastatic anaplastic cancer primary probably lung	None	52	0.1	9	5.5
M. W.	F	Adenocarcinoma of ovary with peritoneal metastasis	Provera, X-ray	92	0.5	24	13.7
T. K.	M	Cancer of lung, undifferentiated	Nitrogen mustard	117	0.3	15	8.6
W. H.	M	Metastatic squamous cell cancer, primary unknown	Nitrogen mustard	82	0.4	15	6.3
			p.o. and i.v. Study				
V. B.	M	Adenocarcinoma of pancreas	None	76	0.5	10	4.4
E. G.	M	Squamous cell cancer of lung	X-ray	96	0.8	15	5.0
W. H.	M	Squamous cell cancer of lung	X-ray Cyclophosphamide	100	0.2	16	5.2
M. H.	F	Anaplastic cancer: cervix	X-ray Methyl-1-(2-chloro-ethyl)-3-cyclo-hexyl-1-nitrosourea	62	0.4	30	7.7
B. B.	F	Adenocarcinoma colon	None	107	0.7	10	3.6
O. S.	M	Metastatic adenocarcinoma, primary lung or pancreas	None	120	0.7	26	9.1

[a] ND, not done.

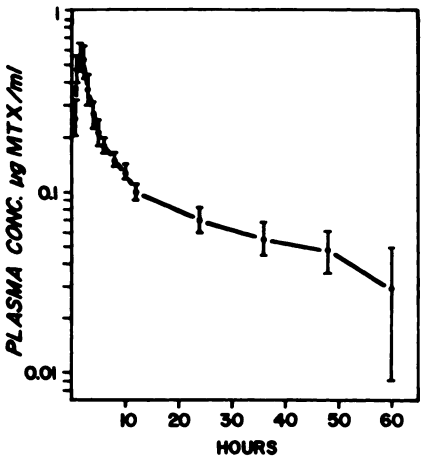

Chart 1. Radioactivity in plasma (mean ± S.E.) following p.o. administration of 30 mg/sq m. *N* = 7. *CONC.*, concentration.

per area under the plasma concentration time curve) was rapid at 80 ml/min, and the terminal plasma half-life was 44 hr. In 2 of the 3 patients with malignant effusions, the drug concentration in the effusions was higher than was the plasma concentration on all occasions that effusion fluid was obtained. The volume of effusion fluid removed from each patient varied considerably from 15 ml of pleural effusion to 2500 ml and 6500 ml of ascitic effusion in the 3 patients studied.

p.o. and i.v. Study (30 mg/sq m). The plasma concentration profiles of patients in the cross-over study who received 30 mg/sq m are given in Chart 2. Plasma concentration decline following the p.o. and i.v. doses was parallel. The data were fitted by a least-squares method to a 3-compartment system (6) and a mean final half-life value of 24.9 hr was obtained. Concentrations following the p.o. dose were lower than those following the i.v. dose, the concentration X time value (area) being 47.7% of the i.v. dose. At least 88% of the p.o. dose was absorbed as determined by cumulative excretion in urine.

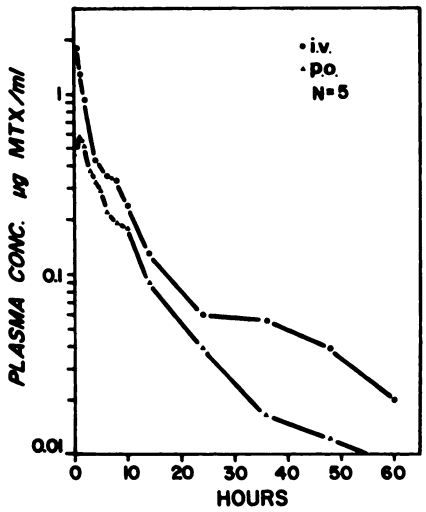

Chart 2. Radioactivity in plasma following i.v. and p.o. administration of 30 mg/sq m. *N* = 5. *CONC.*, concentration.

During the 1st 9 hr most of the radioactivity excreted in urine was in the form of unchanged MTX, regardless of the route of administration (Table 2). The amount of excreted radioactivity not associated with MTX increased with time and was much greater following p.o. dosing. Following i.v. administration, approximately 6% of the total radioactivity excreted in urine was associated with a metabolite. This is based on the observation that about 70% of MTX was excreted unchanged in 24 hr (Table 3), and the mean fraction of excreted radioactivity not associated with MTX was 0.20 beyond this time (1). In contrast, using similar calculations, the total amount of metabolite excreted in the urine following p.o. administration was 38.7% of the dose. This represents 34.2% of the amount absorbed. Following p.o. administration, 93% of the dose was recovered, 4.6% of which was in the feces. The DEAE-cellulose elution profile of radioactivity in urine of a patient given both i.v. and p.o. MTX is shown in Chart 3. Following i.v. administration, 86% was recovered from urine and 1.1% from feces. At 24 hr 33% of plasma radioactivity from 1 patient (B. B.) was associated with unchanged MTX isolated by column chromatography following both routes of administration. This patient excreted unusually high amounts of metabolite. During the 24 to 36 hr period, 46% and 85% of radioactivity in urine was associated with 1 or more metabolites.

i.v. (30 mg/sq m) and p.o. (80 mg/sq m). Absorption was erratic following the p.o. administration of 80 mg/sq m, with absorption still apparent at 72 hr (Chart 4). This patient excreted only 31% of the drug in the urine during the 96 hr of specimen collection. Consequently, fecal drug excretion was higher at 28.6% of the administered dose, indicating incomplete absorption of MTX at the higher dose of 80 mg/sq m.

Protein Binding Studies. A mean of 0.45 (S.E., 0.041) of radioactivity in plasma was bound to proteins in the 12 patients studied. Because of assay limitations, only plasma from the initial 8 hr of the study was used. Plasma MTX concentration during this period ranged from 0.3 to 3 µg/ml. The binding observed in plasma following i.v. and p.o. administration was similar. On the other hand, a wide variation in binding to effusion fluid proteins was found, ranging from 0 to 17%.

DISCUSSION

The pharmacokinetics following i.v. administration of MTX (30 mg/sq m) has been studied previously (6). A large interindividual variation in pharmacokinetics was observed even following i.v. dosing. Variation of a similar magnitude is seen in the present studies. A previous study has shown that, following an i.v. dose, the disappearance of MTX from plasma was triphasic (6). In the current studies with p.o. MTX, the data did not justify the use of a complex 3-compartment model, especially in the presence of wide interindividual variation. Hence, only terminal half-life values are reported. A difference between effusion fluid and plasma terminal half-life was also observed in the 1 individual in whom it was studied. The half-life of MTX in effusion fluid in patient M. W. was 27.3 hr, whereas the plasma half-life was 9.2 hr. Because of

Table 2

Mean excretion of MTX and metabolites following i.v. and p.o. administration of MTX (30 mg/sq m)

Time (hr)	i.v. MTX (%)	i.v. Metabolites (%)	p.o. MTX (%)	p.o. Metabolites (%)	N
4–5	98.8 (97.5–100)[a]	1.2 (0–2.5)	92.4 (69.0–100)	7.6 (0–31)	5
7–9	100	0	92.0	8.0	1
11–13	100	0	43.0	57.0	1
13–24	96.4 (93.1–98.4)	3.6 (1.6–6.9)	62.6 (9.4–91.5)	37.4 (8.5–90.6)	3
24–36	85.6 (64.0–97.5)	14.4 (2.5–36.0)	13.1 (3.3–23.0)	86.9 (77.0–96.7)	3
36–48	59.5 (25.3–93.7)	40.5 (6.3–74.7)	4.3 (0–12.8)	95.7 (87.2–100)	3

[a] Numbers in parentheses, range.

Table 3

Cumulative excretion of radioactivity following administration of MTX (30 mg/sq m) i.v. and p.o. in cross-over studies

	Mean % of dose excreted			
	Urine		Feces	
Time (hr)	i.v.	p.o.	i.v.	p.o.
4	46.1	28.2		
5	51.3	34.3		
7	59.7	45.3		
9	64.3	48.8		
11	67.0	53.9		
13	68.9	58.3		
24	77.7	76.7		
36	80.5	81.6		
48	82.4	84.9		
96	85.9	88.1	1.1	4.6

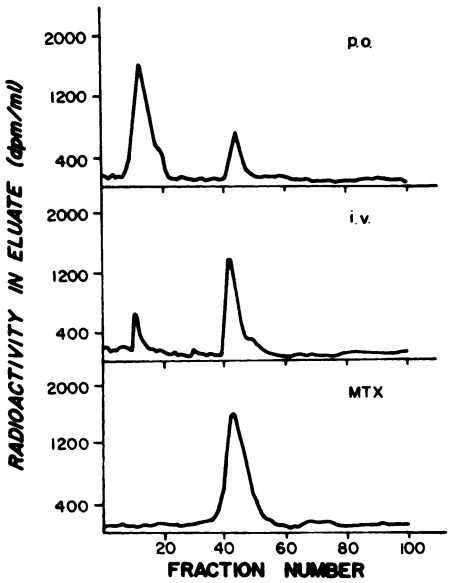

Chart 3. DEAE-cellulose elution profiles of MTX and urine from a patient (W. H.) given MTX (30 mg/sq m) p.o. and i.v. Urine collection period, 24 to 36 hr.

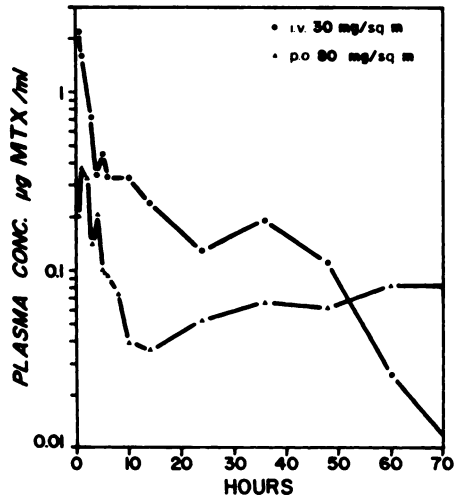

Chart 4. Plasma radioactivity and urinary excretion rates following the administration of MTX p.o. (80 mg/sq m) and i.v. (30 mg/sq m). *CONC.*, concentration.

low blood perfusion of effusion fluids and inadequate mixing, equilibration of drug between these 2 body fluids would necessarily be slow. The slow transfer of drug from effusion fluids to plasma could contribute to interindividual difference in pharmacokinetics, especially when the volume of effusion fluid present is large.

Absorption of a 30 mg/sq m dose from the gastrointestinal tract is essentially complete. Although the peak plasma concentration was rapidly achieved, secretion into bile and reabsorption continued throughout much of the study, resulting in secondary peaks in plasma concentration and excretion in urine. Although the plasma concentration decline was parallel following i.v. and p.o. administration of MTX, the area under the plasma concentration-time curve was smaller following p.o. administration. Since there was no statistical difference in excretion of drug in urine between the 2 routes of administration, it can be concluded only that the distribution and/or elimination of p.o. and i.v. MTX is different, with metabolites having a higher volume of distribution or higher renal clearance. Metabolism in the gastrointestinal tract or during the 1st pass through the liver are both possibilities for the difference in area. This supposition was further strengthened by the results of column chromatography of the urine. The amount of metabolite formed after i.v. administration is 6% of the dose, in contrast to p.o. administration where 35% of the dose is excreted as metabolites. The incomplete absorption of MTX at 80 mg/sq

m in the patient studied is in agreement with a previous report (5). Thus, attempting to compensate for increased metabolism by increasing the p.o. dose may be unsuccessful. However, further documentation of this phenomenon is necessary and is presently under investigation. Furthermore, these studies relate only to absorption of MTX from solution. The bioavailability of MTX from commercially available tablets remains to be studied.

In this presentation, plasma MTX concentration is used synonymously with plasma radioactivity. Radioactivity measurement may overestimate the quantity of active drug present in the p.o. studies and at the later time periods in the i.v. studies. While this overestimation may be unimportant in the i.v. studies (6), the difference may be significant following p.o. administration. A few species can metabolize MTX to 7-hydroxy-MTX, which migrates more slowly than does MTX on DEAE-cellulose (8). Since most of the metabolites we found in human urine migrated prior to MTX, it is unlikely that any of these is 7-hydroxy-MTX. Recently, several cleavage products, including 4-amino-4-deoxy-N^{10}-methylpteroic acid, have been identified in urine and fecal contents of mice (14, 15). The elution characteristics of these compounds are similar to some of the metabolites found in this study and also when MTX is incubated with human feces (6). In mice, the cleavage products are formed by intestinal bacteria during enterohepatic cycling. In the present study, the long lag time for appearance of metabolites plus the greater percentage of metabolite formed following p.o. administration is further evidence that enterohepatic cycling with metabolism by intestinal bacteria also exists in man (5–7). Various bacterial species are known to be able to cleave MTX at the amide bond (1, 9, 10, 12). Bacterial carboxypeptidase has been shown to have similar enzymatic activity (1, 9). It appears that such hydrolytic activity plays an important role in MTX metabolism in man. 4-Amino-4-deoxy-N^{10}-methylpteroic acid is 200 times less potent than is MTX in inhibiting dihydrofolate reductase. Therefore, differences in metabolism of MTX following p.o. and i.v. administration are quite important. i.v. administration appears to be the route of choice.

REFERENCES

1. Bertino, J. R., O'Brien, P., and McCullough, J. L. Inhibition of Growth of Leukemia Cells by Enzymatic Folate Depletion. Science, *172:* 161–162, 1971.
2. Borga, O., Azarnoff, D. L., Forshell, G. P., and Sjoqvist, F. Plasma Protein Binding of Tricyclic Antidepressants in Man. Biochem. Pharmacol., *18:* 2135–2143, 1969.
3. Burchenal, H. J., Waring, G. B., Ellison, R. R., and Reilly, H. C. A Simple Method for Determination of Levels of Amethopterin in Blood and Urine. Proc. Soc. Exptl. Biol. Med., *78:* 603–605, 1951.
4. Freeman, M. V. The Fluorimetric Measurement of the Absorption Distribution and Excretion of Single Doses of 4-Amino-10-methylpteroylglutamic Acid (Amethopterin) in Man. J. Pharmacol. Exptl. Therap., *122:* 154–162, 1958.
5. Henderson, E. S., Adamson, R. H., and Oliverio, V. T. The Metabolic Fate of Tritiated Mtx. II. Absorption and Excretion in Man. Cancer Res., *25:* 1018–1024, 1965.
6. Huffman, D. H., Wan, S. H., Azarnoff, D. L., and Hoogstraten, B. Pharmacokinetics of Methotrexate. Clin. Pharmacol. Therap., *14:* 572–579, 1973.
7. Johns, D. G., Hollingsworth, J. W., Cashmore, A. R., Plenderleith, I. H., and Bertino, J. R. Methotrexate Displacement in Man. J. Clin. Invest., *43:* 621–629, 1964.
8. Johns, D. G., Iannotti, A. T., Sartorelli, A. C., and Bertino, J. R. The Relative Toxicities of Methotrexate and Aminopterin. Biochem. Pharmacol., *15:* 555–561, 1966.
9. Levy, C. C., and Goldman, P. The Enzymatic Hydrolysis of Methotrexate and Folic Acid. J. Biol. Chem., *242:* 2933–2938, 1967.
10. McNutt, W. S. The Enzymic Deamination and Amide Cleavage of Folic Acid. Arch. Biochem. Biophys., *101:* 1–6, 1963.
11. Oliverio, V. T. Chromatographic Separation and Purification of Folic Acid Analogues. Anal. Chem., *33:* 263–265, 1961.
12. Pratt, A. G., Crawford, E. J., and Friedkin, J. J. The Hydrolysis of Mono-, Di-, and Triglutamate Derivatives of Folic Acid with Bacterial Enzymes. J. Biol. Chem., *243:* 6367–6372, 1963.
13. Siersback-Nielsen, K., Molholm Hansen, J., Kamponann, J., and Kristensen, M. Rapid Evaluation of Creatinine Clearance. Lancet, *7709:* 1133–1134, 1971.
14. Valerino, D. M. Studies of the Metabolism of Methotrexate. II. Res. Commun. Chem. Pathol. Pharmacol., *4:* 529–542, 1972.
15. Valerino, D. M., Johns, D. G., Zaharko, D. S., and Oliverio, V. T. Studies of the Metabolism of Methotrexate by Intestinal Flora. I. Biochem. Pharmacol., *21:* 821–831, 1972.
16. Zaharko, D. S., Bruckner, H., and Oliverio, V. T. Antibiotics Alter Methotrexate Metabolism and Excretion. Science, *166:* 887–888, 1969.
17. Zaharko, D. S., and Oliverio, V. T. Reinvestigation of Methotrexate Metabolism in Rodents. Biochem. Pharmacol., *19:* 2923–2925, 1970.