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In Vivo Methotrexate Transport in Murine Lewis Lung Tumor

K. H. YANG *, W. P. FUNG *, R. J. LUTZ ‡, R. L. DEDRICK ‡, and D. S. ZAHARKO *x

Received November 29, 1978, from the **Laboratory of Chemical Pharmacology, National Cancer Institute, and the* ‡*Biomedical Engineering and Instrumentation Branch, Division of Research Services, National Institutes of Health, Bethesda, MD 20014.* Accepted for publication February 5, 1979.

Abstract □ Methotrexate uptake by murine Lewis lung tumor was measured *in vivo* over a wide dose range. The data were analyzed according to a model previously developed for tissues in which methotrexate uptake is rate limited by transport across the cell membrane. Methotrexate transport in this tumor followed Michaelis–Menten kinetics with a rate constant for permeability (k/K) of 0.012 min^{-1}. The methotrexate binding capacity of dihydrofolate reductase in the tumor was not exceeded at any dose studied. A low membrane permeability in conjunction with a high dihydrofolate reductase level explains the resistance of this tumor to methotrexate.

Keyphrases □ Methotrexate—transport in murine Lewis lung tumor, *in vivo*, dihydrofolate reductase binding, tumor resistance □ Antineoplastic agents—methotrexate, transport in murine Lewis lung tumor, *in vivo*, dihydrofolate reductase binding, tumor resistance □ Lung neoplasms—Lewis, methotrexate transport, dihydrofolate reductase binding, *in vivo*

A previous study (1) found that when mice bearing Lewis lung tumor were infused with methotrexate to maintain a 0.01-μg/ml plasma level, the incorporation of ^{3}H-deoxyuridine into DNA was nearly completely blocked in the small intestine but virtually unaffected in the tumor. This difference was thought to occur because tumor cells contain a higher dihydrofolate reductase level and are less permeable to methotrexate than are intestinal cells. Subsequent measurements of this enzyme showed that its level in tumor cells is about six times higher than that in intestinal cells (2).

The question of membrane permeability remained. It is well known that many solid tumors are resistant to standard chemotherapeutic treatments (3). A relatively low permeability was recently demonstrated *in vivo* for methotrexate in spontaneous canine lymphosarcoma (4). The Lewis lung tumor, a solid metastasizing carcinoma insensitive to many chemotherapeutic agents (5), has been used as a valuable experimental tumor model (6, 7). Therefore, the membrane permeability of this tumor to methotrexate *in vivo* was examined to gain insights into the nature of tumor resistance through model simulations.

THEORETICAL

A general model was described (8) for methotrexate uptake in normal tissues, in which the uptake rate is limited by the membrane permeability. This model is now applied to the Lewis lung tumor tissue (Fig. 1). Implicit in this tumor model are the assumptions that: (*a*) the capillary membrane offers no barrier to passage, (*b*) no methotrexate binding occurs in the extracellular compartment, whereas saturable binding to dihydrofolate reductase occurs in the intracellular compartment, and (*c*) instantaneous, complete mixing takes place in the extracellular compartment. For such a model, the following equations apply:

$$\left(\frac{V_e}{V}\right)\frac{dC_e}{dt} = \left(\frac{Q}{V}\right)(C_p - C_e) - \frac{jA}{V} \qquad \text{(Eq. 1)}$$

$$\left(1 - \frac{V_e}{V}\right)\frac{dq_i}{dt} = \frac{jA}{V} \qquad \text{(Eq. 2)}$$

$$dq_i = C_i + \frac{aC_i}{E + C_i} \qquad \text{(Eq. 3)}$$

where C is the free methotrexate concentration, Q is the blood flow rate, jA is the net transport rate of methotrexate over total area A, q is the total intracellular methotrexate concentration, a is the maximum dihydrofolate reductase binding capacity for methotrexate, E is the dissociation constant of the dihydrofolate reductase–methotrexate complex, and V

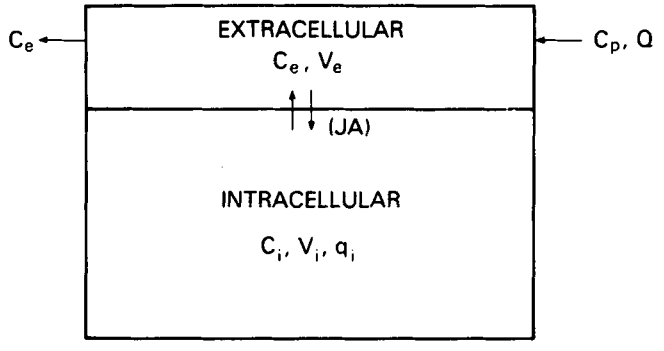

Figure 1—*Diagram of tumor model. See text for notations.*

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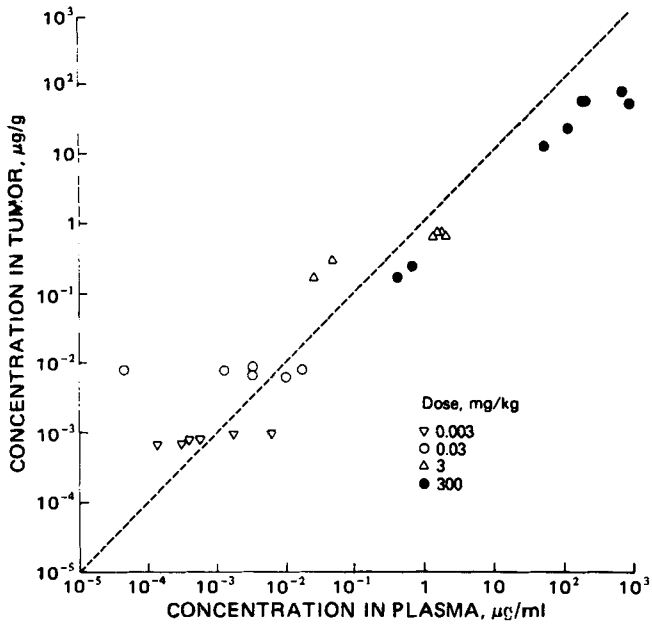

Figure 2—*Methotrexate distribution between plasma and Lewis lung tumor of mice at several doses and various times after dosing.*

is the total tissue volume. The subscripts e, i, and p denote extracellular, intracellular, and plasma, respectively.

Methotrexate transport across most normal and tumor cell membranes is generally assumed to be a carrier-mediated process (9, 10). At high concentrations, passive diffusion may play a role (11). With the assumption that the efflux and influx of the facilitated process are characterized by the same parameters, the net methotrexate transport rate into the cells is given by:

$$\frac{jA}{V} = \frac{kC_e}{K + C_e} - \frac{kC_i}{K + C_i} + b(C_e - C_i) \qquad \text{(Eq. 4)}$$

where k is the maximum transport rate per unit volume, K is the Michaelis constant for the transport, and b is the passive permeability coefficient per unit volume. At doses low enough so that virtually all methotrexate entering the cells becomes tightly bound to dihydrofolate reductase (*i.e.*, $C_i = 0$) and where the passive process is relatively insignificant compared with the facilitated process (*i.e.*, $b = 0$), Eq. 4 reduces to:

$$\frac{jA}{V} = \frac{kC_e}{K + C_e} \qquad \text{(Eq. 5)}$$

For low doses where $C_e \ll K$, Eq. 5 becomes:

$$\frac{jA}{V} = \left(\frac{k}{K}\right) C_e \qquad \text{(Eq. 6)}$$

Since $C_e \simeq C_p$, Eq. 6 predicts a linear increase of jA with C_p. For a time τ sufficiently large such that $C_e V_e \ll q_i V_i$, Eq. 6 leads to:

$$\left(\frac{k}{K}\right) \simeq \frac{C_T(\tau)}{\int_0^\tau C_p(t)\,dt} \qquad \text{(Eq. 7)}$$

where C_T is the average concentration of methotrexate in tumor cells and is given by:

$$C_T = (C_e V_e + q_i V_i)/V \qquad \text{(Eq. 8}a\text{)}$$

$$C_T = q_i + (V_e/V)(C_e - q_i) \qquad \text{(Eq. 8}b\text{)}$$

Equation 7 then gives a first estimate of k/K from $C_T(t)$ and $C_p(t)$, both of which can be determined experimentally. The resolution of the k/K ratio into its components was discussed in detail previously (4, 8). Based on the parameters k, K, Q/V, V_e/V, a, and E, the time course of methotrexate uptake in the tumor cell can be simulated through Eqs. 1–4.

EXPERIMENTAL

Lewis Lung Tumor-Bearing Mice—CDF_1 male mice, 18 ± 2 g, were transplanted subcutaneously with Lewis lung tumor of ~2 mm^3 in size.

Table I—Percentage of Radioactivity Associated with Methotrexate in Plasma and Tumor Samples of CDF_1 Mice Given ^{3}H-Methotrexate Intravenously

Sample	Dose, mg/kg	Percentage[a] of Radioactivity Associated with Methotrexate after 30 min	180 min
Plasma[b]	0.03	82	10
	3	89	29
	300	—	24
Tumor[c]	0.03	—	93
	3	—	76
	300	—	32

[a] Based on the total radioactivity put on the column. [b] In control experiments, where ^{3}H-methotrexate was added to the pooled blood from the tumor-bearing mice and identical preparative procedures were followed, nearly 100% of the radioactivity was associated with methotrexate. [c] In control experiments, where ^{3}H-methotrexate was mixed with the homogenizing medium and added to the pooled tumors, the radioactivity associated with methotrexate was 96%.

Three weeks later, these tumor-bearing mice were used in the study. At this time, the primary tumors weighed 0.5–1.0 g.

Drug Solution Purification and Preparation—3′,5′,9′(*n*)-Methotrexate[1] was purified by diethylaminoethylcellulose chromatography (12) each time before use. The sodium salt of ^{3}H-methotrexate, 250 μCi, was taken up with 0.75 ml of 0.1 *M* ammonium bicarbonate buffer and applied onto a 0.7 × 9-cm column. The column was then eluted at 18 ml/hr with a linear gradient of the buffer at pH 8.3 from 0.1 to 0.4 *M*. The fractions containing methotrexate were pooled and lyophilized.

The lyophilized ^{3}H-methotrexate was reconstructed into a solution of the desired concentration with unlabeled methotrexate dissolved in an aqueous solution of 0.25% $NaHCO_3$ and 0.9% NaCl. The unlabeled methotrexate, of which the purity was checked by the column chromatography, was relatively free of impurities and was used without further purification. The specific activity of the solution, determined from measurements of its optical density at 302 nm and its disintegrations per milliliter, ranged from 1.6 to 17 μCi/μg.

Uptake Study—The tumor-bearing mice were injected with the drug solution *via* the tail vein at doses of 0.003, 0.03, 3, and 300 mg/kg. Then the animals were killed by decapitation at specified times. Blood samples were collected in heparinized tubes and centrifuged to separate the plasma. The tumors were removed surgically.

Aliquots of the plasma and of the nonnecrotic portion of the tumor were digested in a tissue solubilizer[2] and counted[3] for tritium in a counting cocktail consisting of 0.01% 1,4-bis[2-(5-phenyloxazolyl)]benzene[4] and 0.3% 2,5-diphenyloxazole[4] in methanol–toluene (6:14). The counting error was <5%. Appropriate corrections for quenching were made using the automatic external standard ratio method.

Two control mice, which received no drug, were processed for each dose.

Plasma and Tumor Sample Radiopurity—The radiopurity of the plasma and tumor samples was determined at several doses and time points by column chromatography. Six tumor-bearing mice were used for each time point. The drug solution was administered as in the uptake studies. Pooled plasma samples were heated in a boiling water bath for 10 min and centrifuged. An aliquot of the supernate thus obtained was applied onto the same column system described in the purification procedure and eluted similarly, starting, however, with a 0.05 *M* buffer. Each 0.75-ml fraction was collected, mixed with 15 ml of a scintillation cocktail[5], and counted for tritium content with appropriate corrections for quench. Pooled tumors weighing 1.5 g were homogenized with 2 ml of an ice-cold aqueous solution of 0.25% $NaHCO_3$ and 0.9% NaCl. The homogenate was treated similarly to the pooled plasma samples.

All procedures were carried out under minimal incandescent light to prevent the photodecomposition of these pteridine derivatives. The total radioactivity obtained from fractions that coincided with the absorbance of the coeluted methotrexate, which was added to the sample before fractionation as a marker, divided by the total radioactivity put on the column gave the fraction of remaining methotrexate in the samples. The radioactivity from these boiled plasma or tumor tissue extracts accounted

[1] NCS, Amersham/Searle.
[2] From Drug Research and Development Branch, National Cancer Institute.
[3] Packard Tri-Carb liquid scintillation spectrometer, model 3390.
[4] Packard.
[5] Aquasol, New England Nuclear.

for greater than 90% of the radioactivity as determined by digested and counted aliquots of similar tissues. Control experiments, in which the purified ^{3}H-methotrexate solution was added either to the blood pooled from the tumor-bearing mice receiving no drug or to the homogenizing medium for tumors from control mice, were also performed.

RESULTS AND DISCUSSION

Plasma and Tumor Sample Radioactivity—Methotrexate is significantly metabolized in rodents by the host intestinal bacteria (13–15). Thus, the measured radioactivity is the sum of the radioactivity associated with both methotrexate and its metabolites. The radioactivity fraction associated with methotrexate in the samples, however, will vary with the dose given, the administration route, and the time after injection, as was observed in this study.

As can be seen in Table I, at times shorter than 30 min, methotrexate remained largely intact regardless of the dose. By 3 hr, only 10–20% of the radioactivity measured in the plasma (depending on the dose) was associated with methotrexate; the remainder was associated with metabolites and, in a few cases (plasma sample at 3 hr and 300 mg/kg; tumor sample at 3 hr and 300 mg/kg), with ^3H_2O. However, nearly all tritium measured in the tumor at 0.03 mg/kg at 3 hr was in methotrexate. The same result is to be expected for the 0.003-mg/kg dose. The lower percentage found in the tumor sample at 3 mg/kg can be attributed to the relatively significant amount of metabolite in the extracellular compartment, which is insignificant at lower doses. At 300 mg/kg, most of the radioactivity in both samples came from ^3H_2O, which largely accounted for the low percentages associated with methotrexate. These findings were used to make corrections in the uptake studies.

Membrane Transport as Rate-Limiting Step—After corrections were made in accordance with Table I, the methotrexate concentrations in tumors at different time points for all doses were plotted against the corresponding concentrations in plasma (Fig. 2). The tumor methotrexate concentration was not a unique function of its concentration in the plasma, which would be expected if the uptake were flow limited. The dependence of the tumor methotrexate concentration on the dose given as well as on the plasma concentration strongly suggests that membrane resistance can be a rate-limiting factor for methotrexate uptake in this tumor. Similar observations were made with such tissues as the small intestine, spleen, and bone marrow of the rat (8) and lymphosarcoma of the dog (4).

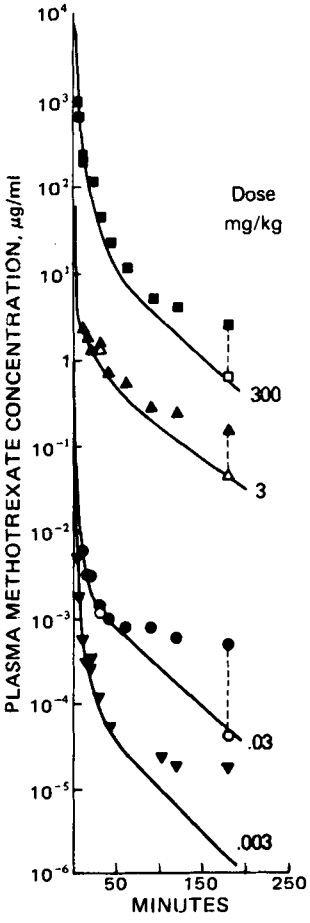

Figure 3—*Plasma methotrexate concentrations* versus *time following several intravenous doses. Dark symbols are experimental data. Open symbols are corrected data. Solid lines represent triexponential curve fitted as described in the text. Each point is from one mouse.*

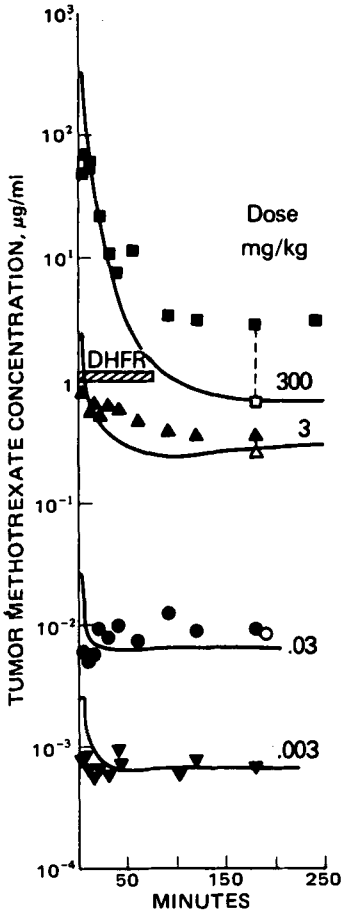

Figure 4—*Average tumor methotrexate concentrations* versus *time following several intravenous doses. Dark symbols are experimental data. Open symbols are corrected data. Solid lines represent model simulations. Shaded bar represents dihydrofolate reductase level. Each point is from one mouse.*

Plasma Data—The plasma methotrexate concentrations obtained for each dose up to 3 hr are shown in Fig. 3. Corrections for tritium not associated with methotrexate were made in accordance with Table I. For the lowest dose, a correction was made at 3 hr in proportion to that made for 0.03 mg/kg. Based on these points and with the assumption of 100% radiopurity at time zero, a linear interpolation provided the points between 30 min and 3 hr. The resulting corrected plasma data sets for each dose were then curve fitted (16) with the following triexponential equation:

$$C_p(t) = a_1 \exp(-b_1 t) + a_2 \exp(-b_2 t) + a_3 \exp(-b_3 t) \quad \text{(Eq. 9)}$$

where $C_p(0)$ for each dose was obtained from the whole animal model developed earlier (17). These equations thus served to evaluate the denominator in Eq. 7 and as input functions for tumor model simulations.

Tumor Data—The measured tumor methotrexate concentrations as a function of time are plotted for each dose in Fig. 4. Also shown are the corrections made in accordance with Table I. The tumor methotrexate concentrations were indeed proportional to the doses at the two lowest doses, indicating the validity of Eq. 6. As the dose increased, the tumor concentration did not increase proportionally, and the transport mechanism apparently became saturated.

The data obtained at either of the two lowest doses were then used, together with the corresponding plasma data, in Eq. 7 to estimate k/K. Equations 1, 2, 6, and 8 can be solved simultaneously on a digital computer for C_T as a function of time for the two lowest doses, using the estimated value of k/K once V_e/V and Q/V are determined. The value of V_e/V was conveniently evaluated from Fig. 2, where $V_e/V \simeq C_T/C_p$ at short times following the highest dose when most of the drug is confined in the extracellular space due to transport saturation. To evaluate Q/V, it is noted that the change in C_T during the first few minutes following the highest dose can be approximated by:

$$\frac{dC_T}{dt} \simeq \left(\frac{Q}{V}\right)(C_p - C_T) \quad \text{(Eq. 10)}$$

Table II—Model Parameters for Murine Lewis Lung Tumor

Parameter	Value
Q/V, flow rate for unit volume, min^{-1}	0.1
V_e/V, fractional extracellular volume	0.2
a, maximum binding capacity, μg/ml	1.0[a]
E, dissociation constant, μg/ml	0.00001[b]
k, maximum transport rate for unit volume, μg/ml/min	0.0036
K, Michaelis constant for transport, μg/ml	0.3

[a] Reference 2. [b] Reference 8.

An approximate value of Q/V was thus found from the best fit of Eq. 10 to the experimental data under such conditions. Since the uptake is not flow limited, a precise knowledge of Q is not required. These parameters, along with other model parameters, are given in Table II. The predicted C_T values were made to best fit the experimental data at the two lowest doses by slightly adjusting the initial k/K estimate. The final k/K value was compared to that of the small intestine in Table III.

The nonresponsiveness of the Lewis lung tumor to methotrexate clearly results from a combination of at least two factors: higher dihydrofolate reductase and lower membrane permeability. With drug doses that are not lethal to the mouse, there is probably always free dihydrofolate reductase present in the tumor to produce sufficient coenzymes for the utilization of nucleotides in DNA and RNA synthesis. The molecular mechanism of decreased tumor permeability as compared to normal tissues certainly cannot be elucidated from this study alone. As an indication, however, it is seen in Table III that since the K's are the same for different tissues, the difference in the permeability thus reflects the difference in k's as shown. Acceptance of the hypothesis of a carrier-mediated transport (18) suggests that the carrier quantity and/or accessibility is less in the tumor cell membrane than in the normal cell membrane.

Model Simulation—The simulations of the average methotrexate concentrations in tumors for all doses are shown in Fig. 4. The model simulates the experimental data over a wide concentration range, spanning six logarithms. The tight binding (19) of methotrexate to dihydrofolate reductase was recently shown to be ionic in nature (20), and the overwhelmingly high dihydrofolate reductase content of this tumor ensures a sink condition within the tumor cell and a one-way

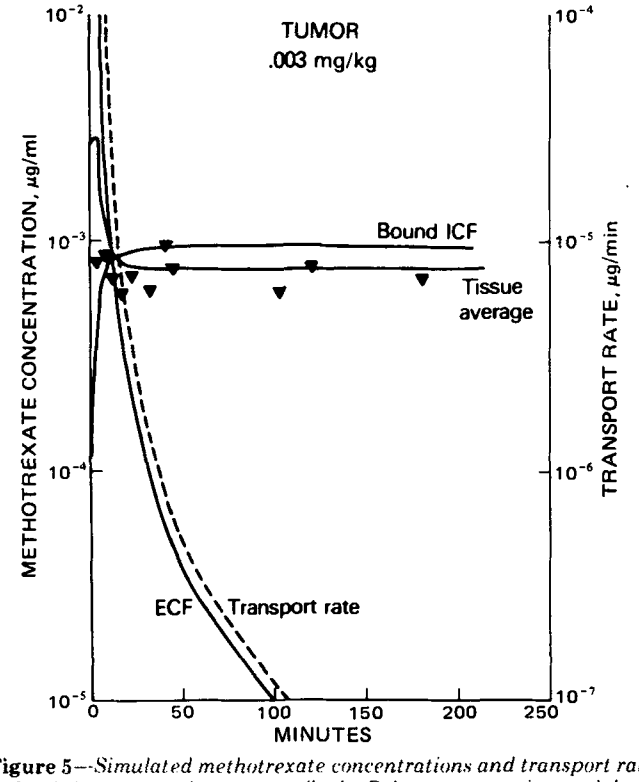

Figure 5—*Simulated methotrexate concentrations and transport rate in Lewis lung tumor for 0.003 mg/kg iv. Points are experimental data. ECF = extracellular fluid. ICF = intracellular fluid. The level of dihydrofolate reductase is at 1 μg of methotrexate equivalents/ml.*

Table III—Transport Parameters and Enzyme (Dihydrofolate Reductase) Activity for Murine Lewis Lung Tumor as Compared to the Small Intestine

Tissue	Permeability (k/K), min^{-1}	k, μg/ml/min	K, μg/ml	Enzyme Activity[a], nmoles of dihydrofolate/hr/mg of protein
Lewis lung tumor	0.012	0.0036	0.3	97
Small intestine	0.024[b]	0.0072[b]	0.3[b]	16

[a] Reference 2. [b] Bile-diverted mice (R. J. Lutz, D. S. Zaharko, K. H. Yang, and R. L. Dedrick, to be published).

transport involving influx only. This condition greatly simplifies the experimental system and may account for the apparent success of the simple model used here. Therefore, the conformity of the model predictions with the data should not be construed as indicating that the influx and efflux parameters of the transport system in the present case are indeed identical. The contrary, in fact, was suggested in some other tumor lines (21–23).

The events that take place are illustrated in Figs. 5 and 6 for 0.003 and 300 mg/kg, respectively. Figure 5 shows that methotrexate enters the cell quickly and binds to dihydrofolate reductase. With the extracellular concentration rapidly decreasing, very little methotrexate enters after 15 min; from then on, the measured tissue averages closely represent the bound intracellular concentrations. The transport rate is directly proportional to the extracellular concentration.

In Fig. 6, for the highest dose, the extracellular concentration dominates the measured concentration for the first 30 min. Only after ~3 hr does the intracellular concentration become significant. More impor-

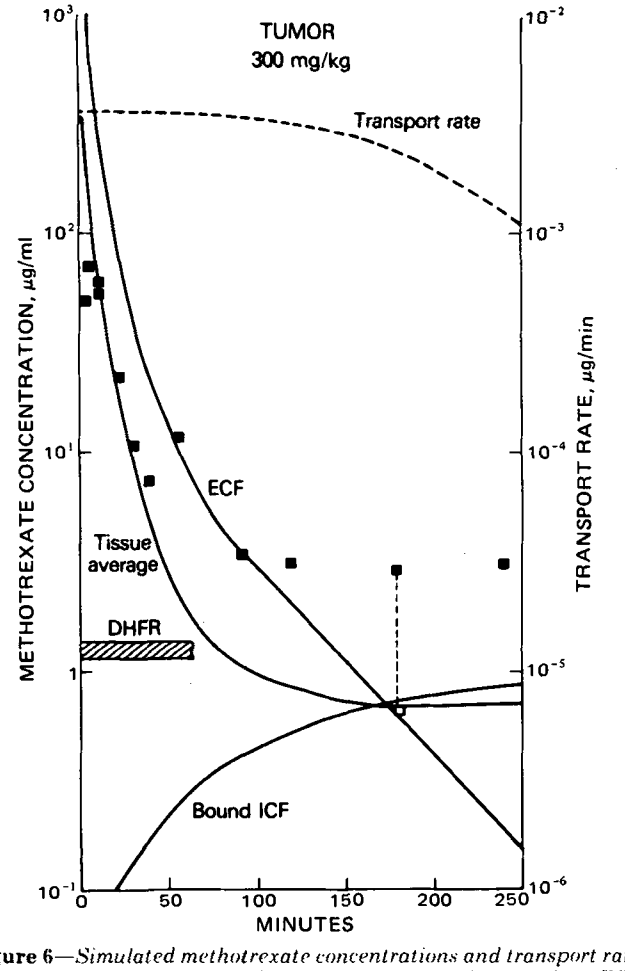

Figure 6—*Simulated methotrexate concentrations and transport rate in Lewis lung tumor for 300 mg/kg iv. Points are experimental data. ECF = extracellular fluid. ICF = intracellular fluid. The level of dihydrofolate reductase (DHFR) is at 1 μg of methotrexate equivalents/ml.*

tantly, there is insufficient methotrexate entering the cell to saturate all the dihydrofolate reductase present and, therefore, little free methotrexate exists inside the cell at any time. It has been demonstrated (24, 25), however, that the persistence of the free intracellular methotrexate accounts for its cytotoxic action. Thus, through model simulations, the measured concentrations in tumors are shown to consist almost entirely of extracellular and bound intracellular concentrations in various proportions, depending on the time and the dose. Since the transport rate has already reached its maximal value at this high dose (Fig. 6), a further increase in the dose would only shift the extracellular curve and, correspondingly, the tissue average curve upwards without a large concomitant increase in the intracellular concentration during the first 2–3 hr. The transport rate would be near the maximum level, only dropping at a later time.

These predictions will be altered, however, if a passive process plays an increasingly significant role as the dose is increased further. This role is still largely unknown at the present time. Nevertheless, what happens at even higher doses is probably of theoretical interest only. In practice, there is a limit to which the dose can be further increased; the LD_{50} of the drug in mice is 350 mg/kg (26).

REFERENCES

(1) D. S. Zaharko, R. L. Dedrick, A. L. Peale, J. C. Drake, and R. J. Lutz, *J. Pharmacol. Exp. Ther.*, **189**, 585 (1974).

(2) D. S. Zaharko, "Pharmacokinetics and Cancer Chemotherapy," Proceedings of the 7th International Congress of Pharmacology, Paris, France, July 1978.

(3) G. Zubrod, *Cancer*, **30**, 1474 (1972).

(4) R. J. Lutz, R. L. Dedrick, J. A. Straw, M. M. Hart, P. Klubes, and D. S. Zaharko, *J. Pharmacokinet. Biopharm.*, **3**, 77 (1975).

(5) J. G. Mayo, *Cancer Chemother. Rep., Part 2*, **3**, 325 (1972).

(6) J. M. Venditti, in "Pharmacological Basis of Cancer Chemotherapy, 27th Annual Symposium on Fundamental Cancer Research," Williams & Wilkins, Baltimore, Md., 1975, p. 245.

(7) T. Giraldi, P. J. Houghton, D. M. Taylor, and L. Nisi, *Cancer Treat. Rep.*, **62**, 721 (1978).

(8) R. L. Dedrick, D. S. Zaharko, and R. J. Lutz, *J. Pharm. Sci.*, **62**, 882 (1973).

(9) I. D. Goldman, *Ann. N.Y. Acad. Sci.*, **186**, 400 (1971).

(10) M. T. Hakala, in "Antineoplastic and Immunosuppressive Agents, Part 1," A. C. Sartorelli and D. G. Johns, Eds., Springer-Verlag, Berlin, Germany, 1974, p. 240.

(11) D. Kessel, T. C. Hall, and D. Roberts, *Cancer Res.*, **28**, 564 (1968).

(12) V. T. Oliverio, *Anal. Chem.*, **33**, 263 (1961).

(13) D. S. Zaharko and V. T. Oliverio, *Biochem. Pharmacol.*, **19**, 2923 (1970).

(14) D. M. Valerino, D. G. Johns, D. S. Zaharko, and V. T. Oliverio, *ibid.*, **21**, 821 (1972).

(15) R. E. Kates and T. N. Tozer, *J. Pharm. Sci.*, **62**, 2056 (1973).

(16) J. E. Fletcher and R. I. Shrager, "Users Guide to Least Squares Model Fitting," Technical Report No. 1, Division of Computer Research and Technology, National Institutes of Health, Washington, D.C., 1968.

(17) K. B. Bischoff, R. L. Dedrick, D. S. Zaharko, and J. A. Longstreth, *J. Pharm. Sci.*, **60**, 1128 (1971).

(18) W. D. Stein, "The Movement of Molecules Across Cell Membranes," Academic, New York, N.Y., 1967, p. 148.

(19) W. C. Werkheiser, *J. Biol. Chem.*, **236**, 888 (1961).

(20) D. S. Saperstein, A. J. Rein, M. Poe, and M. F. Leahy, *J. Am. Chem. Soc.*, **100**, 4296 (1978).

(21) I. D. Goldman, N. S. Lichenstein, and V. T. Oliverio, *J. Biol. Chem.*, **243**, 5007 (1968).

(22) M. Dembo and F. M. Sirotnak, *Biochim. Biophys. Acta*, **448**, 505 (1976).

(23) F. M. Sirotnak and R. C. Donsbach, *Cancer Res.*, **36**, 1151 (1976).

(24) I. D. Goldman, *Mol. Pharmacol.*, **10**, 257 (1974).

(25) J. C. White and I. D. Goldman, *ibid.*, **12**, 711 (1976).

(26) D. S. Zaharko, in "Pharmacological Basis of Cancer Chemotherapy, 27th Annual Symposium on Fundamental Cancer Research," Williams & Wilkins, Baltimore, Md., 1975, p. 69.

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Effect of Enzyme-Inducing and Enzyme-Inhibiting Agents on Drug Absorption II: Influence of Proadifen on 3-*O*-Methylglucose Transport in Rats

WILLIAM R. RAVIS * and STUART FELDMAN ‡×

Received September 5, 1978, from the **School of Pharmacy, Auburn University, Auburn, AL 36830*, and the ‡*Department of Pharmaceutics, College of Pharmacy, University of Houston, Houston, TX 77004.* Accepted for publication January 22, 1979.

Abstract □ Studies utilizing the *in vitro* everted rat jejunum were performed to investigate the effects of proadifen pretreatment and fasting on active and passive intestinal transfer of 3-*O*-methylglucose. Animals were pretreated 24 hr prior to the experiments with 100 mg of proadifen/kg ip. With mucosal concentrations of 0.1 m*M* 3-*O*-methylglucose and ^{14}C-3-*O*-methylglucose, transfer to the sugar-free serosal buffer after pretreatment with proadifen was 50% greater than controls after a 24-hr fast and 120% greater than controls after a 48-hr fast. Everted intestinal segments obtained from unfasted control animals showed diminished ability to transfer the sugar derivative, with transfer rates approximately 50% less than those of segments obtained from 24-hr fasted control animals. Wet and dry tissue weights for 24- and 48 hr-fasted groups increased following proadifen pretreatment. The results suggest that proadifen enhances 3-*O*-methylglucose transport across the rat jejunum and also increases intestinal tissue weight.

Keyphrases □ Enzyme induction—effect of proadifen on intestinal methylglucose transport, rats, fasting □ Enzyme inhibition—effect of proadifen on intestinal methylglucose transport, rats, fasting □ Methylglucose—effect of proadifen on intestinal transport, rats, fasting □ Proadifen—effect on intestinal methylglucose transport, rats, fasting

Several monosaccharides, amino acids, vitamins, and clinically employed drugs are absorbed from the intestinal lumen by carrier-mediated processes. A 3-*O*-methyl derivative of *d*-glucose has been shown to be absorbed across the mucosal epithelia by an active transport mechanism (1, 2). Since this derivative is not utilized by animal tissue (1), as is *d*-glucose, it is a useful agent for specific examination of active sugar transport by intestinal tissue.

Chemical agents capable of altering cell metabolism influence active absorption processes in the intestine (3–8).

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