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Pharmacokinetic Studies during Phase I Trials of High-Dose Thymidine Infusions

Daniel S. Zaharko,[1] Bruce J. Bolten, Delia Chiuten, and Peter H. Wiernik

National Cancer Institute, NIH, Bethesda, Maryland 20205

ABSTRACT

Thymidine infusions (75 g/sq m/24 hr) were administered to 12 cancer patients as part of a Phase I study. Thymidine and thymine measurements, by high-pressure liquid chromatography, were made on plasma and urine from eight of these patients. Only the pharmacokinetic aspects of these studies are reported in this paper. Millimolar thymidine and thymine concentrations were achieved in all patients and maintained for 120 hr during each of three courses of infusion. The half-life of thymidine was approximately 100 min following cessation of infusion. The half-life of thymine was much longer but could not be accurately determined because it did not decline as a first-order rate function. The cerebrospinal fluid:plasma ratios at steady state for thymidine and thymine were 0.29 and 1.03, respectively. Total body clearance of thymidine ranged from 95 to 266 ml/min/sq m, and 41 to 67% was by kidney clearance of intact thymidine. Calculations and comparison to other studies at lower infusion rates (micromolar plasma thymidine) indicate that thymidine is metabolized significantly by organs in addition to the liver and that, at millimolar plasma thymidine, total body metabolic processes of thymidine are saturated as is the secretory portion of kidney clearance.

INTRODUCTION

Several studies (7, 9, 11, 15) have shown that a high concentration of thymidine has adverse effects on cell growth and cloning ability of a variety of cell types *in vitro*. Beltz *et al.* (1) discussed the concept of using such naturally occurring growth inhibitors as potential antineoplastic agents. They emphasized the importance of maintaining therapeutically effective concentrations *in vivo* by continuous infusion. More recent studies indicate that thymidine is selectively lethal to certain human and mouse tumor cells *in vitro* as compared to their normal counterparts (5). A large quantity of thymidine infused into thymus-deficient (nude) mice was found to cause regression of human xenographs growing in these mice (6). The plasma concentration of thymidine in these mice was approximately millimolar (19). Subsequent studies in humans suggested that such concentrations of thymidine could be achieved and sustained for days by infusing approximately 100 g/sq m/24 hr i.v. without any dramatic toxicity (3, 19).

As a result of some of the above findings, a Phase I clinical trial of high-dose thymidine was initiated at the Baltimore Cancer Research Program, National Cancer Institute, in October 1978. In this paper, we report the results of some pharmacokinetic measurements made during these trials.

MATERIALS AND METHODS

Thymidine. The Drug Development Branch of the National Cancer Institute provided thymidine as a sterile solution in 500-ml bottles. Each bottle contained 15 g of thymidine made isotonic with NaCl. At the dose administered, 75 g/sq m/day, an average adult patient receives about 5 to 6 liters of this solution each 24 hr.

Patients. The characteristics of patients from which sufficient data were obtained to do pharmacokinetic analysis are shown in Table 1. Most of the patients received thymidine infusions for 120 hr for 3 successive courses with 5 days between each course. It was not always possible to collect plasma and urine samples from these very sick patients as planned. However, analyses of the available data do provide valuable insights.

Assay. The assay system for thymidine and thymine is an adaptation of the method used for the measurement of nucleosides in hydrolysates of RNA and DNA (17). Plasma is precipitated under ice-cold conditions with an equal volume of 1 N perchloric acid. The supernatant is made to pH 9 to 11 (pH electrode) by addition of 4 N KOH. It takes 2 days at 5° for all the potassium salt to completely precipitate and the pH to stabilize. This supernatant is filtered (0.4 μm type HA with type A prefilter; Millipore Corp., Bedford, Mass.) and 20 μl or less are injected into a high-pressure liquid chromatograph. Urine and CSF[2] samples are treated differently than plasma. They are first diluted at least 10 times and then filtered at room temperature prior to high-pressure liquid chromatography analysis because of the potential insolubility at ice-cold conditions of thymine at the high concentrations seen in these fluids. The high-pressure liquid chromatograph column is 50 cm (length) x 4 mm (inner diameter) packed with Aminex A-7 cation-exchange resin with a particle size of 7 to 11 μm (Bio-Rad Laboratories, Richmond, Calif.). The eluant is 0.02 M $(NH_4)_2CO_3$ adjusted to pH 9.78 with NH_4OH. The pH must be adjusted daily by addition of NH_4OH. Ambient conditions of 25° at a pressure of 1000 psi result in a flow rate of 0.215 ml/min. Peak heights of thymidine and thymine at elution times of 23 and 37 min, respectively, were measured by absorbance at 254 nm in a 8-μl flow cell. Peak heights are proportional to the quantity of thymidine and thymine in the solution injected, if 20 μl or less are used. Standard curves were linear in the utilized range from micromolar to millimolar. The concentrations of thymidine and thymine in the unknown extracts were determined by extrapolation between 2 standards whose peak heights spanned the peak height of the unknown extract. Standards were run daily. With an injection volume of 20 μl, solutions with thymidine concentrations below micromolar are undetectable using the above extraction procedure with this high-pressure liquid chromatography system.

[1] To whom requests for reprints should be addressed, at Room 5A13, Building 37, NIH, Bethesda, Md. 20205.

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[2] The abbreviation used is: CSF, cerebrospinal fluid.

RESULTS

Plasma Concentration. The plasma concentrations of thymidine and thymine are illustrated in Chart 1 for 3 male patients and 4 female patients. Millimolar thymidine and thymine concentrations are attained within 12 hr and maintained during the infusion. Once the infusion stops, thymidine concentration rapidly decreases with a half-life of approximately 100 min (Table 2; Chart 1, *A* and *C*). A close look at Chart 1, *B* and *D*, indicates that thymine concentration in some cases actually rises or stays constant after the infusion stops. These observations suggest that the rate of decrease of thymine is not first order. The data in Table 2 illustrate this phenomenon more clearly and indicate that the calculated half-life for thymine is a function of the time interval used for the calculation. For example, for male patients, if a 4-hr interval after cessation of infusion is used, the mean half-life is 784 min for thymine, whereas if the 4- to 8-hr interval is used the mean half-life is 364 min for thymine. This lack of a first-order decline for thymine coupled with the thymidine half-life of 100 min (compared to 10 min at micromolar plasma concentrations) are indicative of saturable metabolic processes. The variation of values for both the plateau concentrations and half-lives of thymidine and thymine is greater for the female patients than for the male patients.

Table 1

Patient characteristics on thymidine infusions

Patient	Sex	Tumor	Wt (kg)	Body surface area (sq m)	Age (yr)
A	M	Melanoma	100	2.1	61
B	M	Colon carcinoma	67	1.8	40
C	M	Colon carcinoma	51	1.6	65
D	F	Colon carcinoma	68	1.8	50
E	F	Acute leukemia	53	1.6	58
F	F	Acute leukemia	52	1.6	39
G	F	Lung cancer	53	1.6	69
J	M	Melanoma	74	2.0	27

Volume of Distribution. Past studies indicate that, after thymidine administration, plasma thymidine declines exponentially with only one phase of disappearance. This suggests that an appropriate pharmacokinetic model is one compartment with first-order clearance by excretion and metabolism (2, 4). At millimolar plasma concentrations encountered in this study,

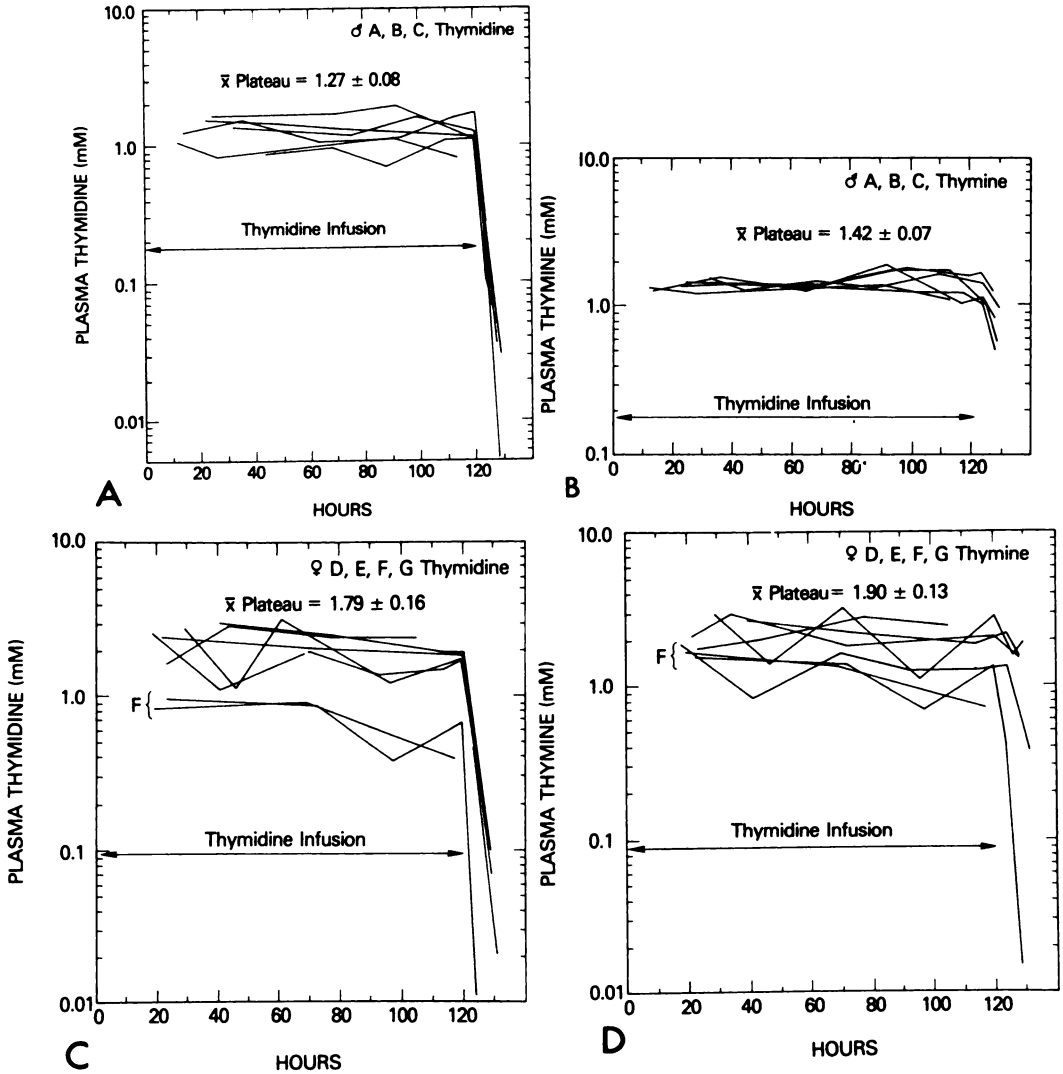

Chart 1. Plasma concentrations of thymidine and thymine during and following infusion of thymidine at 75 g/sq m/24 hr for 120 hr. *A* and *B*, male subjects, data from 2 courses of infusion, 5-day rest between infusions; *C* and *D*, female subjects, data from 2 courses of infusion (except Patient G, data from one course of infusion). Plateau value, mean ± S.E.

Table 2

Half-lives for thymidine and thymine following cessation of infusion

	Half-life from end of infusion to 4 hr (min)		Half-life from 4 to 8 hr (min)	
Patients	Thymidine	Thymine	Thymidine	Thymine
Male				
A_2[a]	113	1255	107	575
B_1	68	1126	230	243
B_2	101	∞[b]	81	593
C_1	135	∞	67	431
C_3	72	431	44	207
J_1	66	326[c]	ND	137[d]
Mean ± S.E.	92 ± 12	784[e] ± 237	105 ± 32	364[e] ± 80
Female				
D_1	120	863	119	1121
E_1	117	447	120	∞
E_2	115	∞	89	457
F_1	41	139	ND	50
G_2	126	∞	94	219
Mean ± S.E.	103 ± 16	483 ± 209	105 ± 8	461 ± 235

[a] Subscript, course of infusion.
[b] ∞, infinitely long when calculation is made from data in this time interval; ND, 8-hr plasma thymidine not detectable.
[c] From end of infusion to 8 hr.
[d] From 8 to 15 hr postinfusion.
[e] Significantly different ($p < 0.06$) with paired t test, excluding ∞ values.

the metabolism of thymidine appears to be saturated and thus zero order, and kidney clearance becomes the dominant form of first-order clearance. This model then can be represented mathematically by

$$\frac{dx}{dt} = (k_0 - k_m) - Kx \qquad (A)$$

where x is the quantity of thymidine at any time (t), k_0 is the input of thymidine, k_m is the zero-order metabolic clearance, and K is the first-order kidney clearance. Integration gives

$$x = \frac{k_0 - k_m(1 - e^{-Kt})}{K} \qquad (B)$$

As $t \rightarrow \infty$,

$$X_{ss} = \frac{k_0 - k_m}{K} \qquad (C)$$

where X_{ss} is the quantity of thymidine at steady state. Since

$$X_{ss} = C_{ss}V \qquad (D)$$

where C_{ss} is the plateau thymidine plasma concentration and V is volume of distribution, then by substitution of Equation D into Equation C

$$V = \frac{k_0 - k_m}{C_{ss}K} \qquad (E)$$

A sample calculation using the data in Chart 1*A* is revealing. k_0 is known (75 g/sq m/24 hr); k_m can be estimated from the data in Table 4 as the percentage of total body clearance of thymidine not accounted for by kidney clearance (mean value for male patients, 39%); C_{ss} is taken from Chart 1*A* for males (mean value, 1.27 mM); K is estimated from the thymidine half-life after the cessation of infusion (100 min) and

$$K = (0.693/t_{1/2})\frac{61}{100} = 0.00422 \text{ min}^{-1}$$

Therefore,

$$V(\text{ml}) = \frac{[0.4 \text{ mmol/min} - 0.39(0.4)]}{1.27 \times 10^{-3} \text{ mmol/ml} \times 0.00422 \text{ min}^{-1}} = 45{,}500 \text{ ml}$$

This volume represents about the total body water of an average male. Thymidine is not confined mainly to the plasma compartment, since the average male's plasma volume is about 4500 ml (12). Therefore, at steady-state millimolar thymidine concentration, there is 10 times as much thymidine in other places in the body as there is in the plasma. More data and further pharmacokinetic analysis are required to characterize the nature and disposition of this large extravascular pool of thymidine.

Plasma:CSF. Table 3 summarizes the data collected for CSF thymidine and thymine during infusion at steady-state plasma concentrations. The mean CSF:plasma ratio for thymidine is 0.29, whereas for thymine it is 1.03. It is apparent that CSF thymidine comes to equilibrium with plasma at a much lower concentration than does CSF thymine. This may be due to the lesser polarity and molecular weight of thymine relative to thymidine and, hence, its more ready access across the blood-brain barrier.

Clearance Determinations. By using the average plasma concentration over 24-hr intervals and the thymidine input and the urine output over this same interval, several clearance values were determined for thymidine and thymine using the formulas below.

$$\text{Total body clearance (ml/min)} = \frac{\text{Input (mmol/min)}}{\text{Plasma (mmol/ml)}}$$

$$\text{Kidney clearance (ml/min)} = \frac{\text{Urine (mmol/ml)} \times \text{Vol (ml/min)}}{\text{Plasma (mmol/ml)}}$$

These data are summarized in Table 4. Total body clearance ranged from 95 to 266 ml/min/sq m. There was no apparent relationship between sex and total body clearance of thymidine. However, the highest total body and kidney clearances of thymidine and kidney clearance of thymine were in Patient F, a female. This patient also had one of the highest percentages (37%) of total body clearance of thymidine not accounted for by kidney clearance of thymidine and thymine. This indi-

Table 3

Thymidine and thymine in the CSF during infusion of thymidine

Patient	CSF time (hr)	Thymidine (mM)			Thymine (mM)		
		Plasma	CSF[a]	CSF:Plasma	Plasma	CSF[a]	CSF:Plasma
A_1[b]	21	1.65[c]	0.30	0.18	1.44[c]	1.03	0.72
A_2	72	0.98	0.28	0.29	1.30	1.26	0.97
B_1	21	1.88	0.31	0.16	1.16	0.87	0.75
B_2	69	1.35	0.45	0.33	1.36	1.58	1.16
B_3	68	1.70	0.40	0.24	1.35	1.24	0.92
C_1	14	1.05	0.23	0.22	1.02	0.84	0.82
C_2	48	1.30	0.48	0.37	1.46	1.78	1.22
C_3	64	1.08	0.38	0.35	1.27	1.74	1.37
D_1	70	2.44	0.54	0.22	2.42	2.17	0.90
F_1	71	0.88	0.29	0.33	1.40	1.37	0.98
G_1	76	1.68	0.86	0.51	1.45	2.20	1.52
Mean ± S.E.				0.29[d] ± 0.03			1.03 ± 0.08

[a] Lumbar spinal tap.
[b] Subscript, course of infusion.
[c] Mean of 2 values bracketing the time of CSF sample.
[d] Significantly different ($p < 0.001$) for thymidine and thymine.

Table 4

Clearance of creatinine, thymidine, and thymine during high-dose infusions of thymidine

Clearance values are means ± S.E. calculated from three to six 24-hr urine collections during the plateau phase, usually the 24- to 48-hr and the 96- to 120-hr intervals. Each patient underwent three 120-hr courses of thymidine infusion and at least one, usually 2, clearance values were calculated for each course.

Total body clearance (ml/min/sq m)	Kidney clearance				
	ml/min/sq m			% of total body clearance	
	Creatinine[a]	Thymidine	Thymine	Thymidine	Thymine
208 ± 20	49 ± 7	110 ± 16	27 ± 6	52 ± 6	13 ± 3
156 ± 11	56 ± 8	98 ± 11	27 ± 3	63 ± 6	17 ± 2
128 ± 6	81 ± 23	86 ± 8	19 ± 1	67 ± 6	15 ± 1
95 ± 7	31 ± 8	52 ± 8	16 ± 2	56 ± 6	18 ± 2
97 ± 10	31 ± 4	40 ± 8	16 ± 2	41 ± 5	17 ± 2
266 ± 24	65 ± 10	124 ± 13	42 ± 4	47 ± 3	16 ± 2

[a] Plasma creatinine values were always ≤1.2 mg/100 ml.
[b] Patient died during the last course of treatment.

cates she had a greater capacity for metabolism of thymidine than did most other patients. These observations relate well to the fact that, as seen in Chart 1*C*, Patient F has the only distinguishable lower values from the clustered group values for thymidine plateau value and half-life.

In general, kidney clearance for thymidine was consistently greater than for creatinine, whereas thymine kidney clearance was consistently less than was creatinine clearance. This indicates that the kidney clears thymidine by both glomerular filtration and secretion, whereas thymine is filtered and reabsorbed. The secretory mechanism is most likely partially saturated at these millimolar thymidine concentrations, since our data indicate that thymidine clearance is only slightly greater than is creatinine clearance, whereas other data (2) indicate that at micromolar plasma thymidine, kidney clearance of thymidine is 4 times that of creatinine.

The clearance of intact thymidine by the kidney when plasma concentrations are millimolar ranged from 41 to 67% of total body clearance, and kidney clearance of thymine accounted for an additional 13 to 18% of total body clearance. This is in contrast to 2% of the thymidine dose cleared by the kidney when plasma concentrations are micromolar in thymidine (2).

DISCUSSION

Knowledge of plasma concentrations of thymidine in patients, of the mechanisms by which these plasma concentrations are achieved, and of the variability among individuals in these mechanisms is necessary if rational chemotherapy with thymidine is to be attempted. Recent interest in the conjoint use of thymidine with methotrexate (16, 18) and 5-fluorouracil (8, 14), in addition to its use alone (6) in the treatment of experimental tumors, makes this kind of information in humans timely. It would appear logical that the concentration of thymidine is critical in determining its action, either as an agent to modify action of other drugs or to reverse toxicity of antimetabolites or as an antineoplastic agent itself.

Our investigations show that millimolar plasma thymidine can be achieved in most patients and maintained for 5 days without any major toxic manifestations. This is indeed remarkable when one considers that the normal plasma concentration of thymidine in humans is 10,000 times less, *i.e.*, 0.1 μM (10). At such low normal concentrations or even at concentrations 10 to 20 times higher, *i.e.*, 1.5 μM, clearance processes are different than at the high thymidine concentrations seen in this study. At micromolar plasma concentrations of thymidine, half-life was 10 min after the cessation of infusion (8 g/sq m/24 hr), and only 2% of the dose appeared in the urine (2). Calculations on such data indicate a total body clearance of thymidine of about 24,000 ml/min/subject. Contrast this to about 200 to 500 ml/min/subject from our data at millimolar plasma thymidine. These differences indicate the saturation of some clearance processes at millimolar concentrations. Further calculations using the fact that 98% of the thymidine at 8 g/sq m/24 hr is

cleared by metabolic processes indicate that 0.04 mmol/min are removed by metabolism. In contrast, our data at 75 g/sq m/24 hr indicate that 39% of the dose is removed by metabolism (0.2 mmol/min). Therefore, an increase in plasma thymidine concentration from micromolar to millimolar (1000-fold increase) only increased the amount of thymidine cleared by metabolic processes by a factor of 5 (0.04 to 0.2 mmol/min), an indication that metabolic clearance of thymidine at millimolar plasma concentration is completely saturated. This conclusion is also supported by data (Chart 1*B*; Table 2) which show that thymine concentration in several cases was maintained for some time after the thymidine infusion stopped. These data indicate that the first catabolite of thymidine, thymine, is being produced at a constant rate, even though its substrate, thymidine, is rapid declining. This is obvious, however, in only some patients, which suggests some variability in saturation from patient to patient. Ensminger and Frei (3) have recently shown that hepatic extraction processes for thymidine are saturable as dose rates increase from 16 to 32 g/sq m/day. As stated earlier, data from these same investigators showed that, at infusion rates of 8 g/sq m/day, total body clearance of thymidine was approximately 24,000 ml/min/subject. Since total liver blood flow is only about 1,400 ml/min/subject (13) (plasma flow, approximately 700 ml/min), then only 700/24,000 or 1/30 of the clearance of thymidine at micromolar plasma concentrations can be accounted for by liver metabolism. Thymidine must be metabolized by many other organs in the body. Our data indicate that other significant thymidine metabolic sites in the body are also saturated at millimolar thymidine concentrations. Once these sites become saturated, the primary route for elimination of thymidine becomes kidney excretion. It appears that the body can handle about 40% of the dose given (75 g/sq m/24 hr) by the metabolic route; however, some variation exists from patient to patient.

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