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Influence of Tetrahydrouridine on the Pharmacokinetics of Intrathecally Administered 1-β-D-arabinofuranosylcytosine

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ABSTRACT

Tetrahydrouridine (THU), a potent inhibitor of cytidine deaminase, has been shown to increase the antitumor activity of 1-β-D-arabinofuranosylcytosine (ara-C) both *in vitro* and *in vivo*.

In initial studies, which examined the cerebrospinal fluid (CSF) pharmacokinetics of intrathecally (i.t.) administered THU, the drug was found to be slowly cleared from the CSF with α and β half-lives of 1 and 8 hr, respectively. In subsequent experiments, both i.v. pretreatment with THU and concomitant i.t. injection of THU were found to retard the disappearance of i.t. ara-C from the CSF, although the effect of i.t. THU was more profound.

ara-C given alone was cleared from CSF with α and β half-lives of 27.5 ± 6.7 and 115.6 ± 0.4 (S.D.) min, respectively. Pretreatment with i.v. THU resulted in α and β half-lives of 10.4 ± 1.5 and 85.7 ± 11.1, respectively, whereas concomitant administration of i.t. THU resulted in a single half-life of 96 ± 0.7. The mean calculated clearance rates for ara-C alone, ara-C plus i.v. THU, and ara-C plus i.t. THU were 7.5, 6.2, and 4.2 ml/hr, respectively. This effect appeared to be primarily due to THU inhibition of ara-C deamination, since a decrease in formation of 1-β-D-arabinofuranosyluracil in the CSF was observed when ara-C was given in the presence of THU (either i.t. or i.v.). No acute neurotoxicity was noted after administration of either i.t. THU alone or i.t. THU with ara-C. The ability of THU to alter CSF ara-C pharmacokinetics may have potential therapeutic value.

INTRODUCTION

ara-C,[3] an effective systemic agent in the treatment of acute leukemia (1, 9), has also been used i.t. to treat central nervous system leukemia (18). Under normal circumstances, most systemically administered ara-C is rapidly deaminated by the enzyme cytidine deaminase to the inactive compound ara-U (3). THU, a reduced pyrimidine, is an inhibitor of cytidine deaminase (2). When administered systemically with ara-C, THU has been shown to significantly prolong the disappearance of ara-C from plasma in both animal species and humans (2, 7, 8, 13, 14). In addition, THU has been demonstrated to increase the net amount of ara-CTP, the active metabolite of ara-C, in human leukemic cells with high levels of cytidine deaminase (5, 11). Increases in intracellular ara-CTP have been correlated with increased cytotoxicity (6, 16, 17). In the present study, we have examined the influence of i.v. and i.t. THU on the pharmacokinetics of i.t. administered ara-C. These studies were performed in a subhuman primate model developed to facilitate the study of drug pharmacokinetics in the CSF (15, 20).

MATERIALS AND METHODS

Four adult male rhesus monkeys (*Macaca mulatta*), weighing between 6.9 and 8.4 kg, were obtained from the NIH Primate Center. Each animal was kept in a separate cage and fed Purina monkey chow and water *ad libitum*. In each animal, a Pudenz silicone catheter was surgically placed into the fourth ventricle and attached to a s.c. implanted Ommaya CSF reservoir. This system permits sterile ventricular CSF sampling over extended periods of time without requiring chronic immobilization and provides mixing of injected drugs with lateral ventricular CSF (20).

All injections and sampling were performed on conscious animals sitting in primate chairs. Venous blood samples were obtained from a heparinized indwelling femoral vein catheter. CSF samples were collected from the s.c. Ommaya reservoir by sterile technique. Monkeys 914 and 1965 were used to study the pharmacokinetics of i.t. and i.v. THU and the toxicity of repetitive doses of i.t. ara-C and i.t. THU. Monkeys 791 and 793 were used in the experiments in which ara-C was administered i.t. alone or in combination with i.v. and i.t. THU. At least a 2-week interval was allowed between each experiment. All tubes used to collect blood and CSF contained THU at a final concentration of 10 mg/ml to prevent further deamination after collection.

[*U*-^{14}C]Cytidine (specific activity, 467 mCi/mmol) was purchased from Amersham (Arlington Heights, Ill.), and [^{3}H]ara-C (specific activity, 14.3 Ci/mmol) labeled at positions 5 and 6 was purchased from New England Nuclear, Boston, Mass. The compounds were 98% pure by paper chromatography analysis. Pharmaceutical grade ara-C (Cytosar) was obtained from The Upjohn Company (Kalamazoo, Mich.). [^{14}C]THU (specific activity, 45 mCi/mmol) labeled at position 5 and unlabeled THU were kindly supplied by Dr. Robert Engle, Developmental Therapeutics Program, Division of Cancer Treatment, National Cancer Institute, Bethesda, Md. Thin-layer chromatography showed the nonradioactive material to be approximately 80% THU, 14% THU dimers, 5% deoxy THU, and 1% H_2O. Cytidine and ara-U were purchased from Sigma Chemical Co. (St. Louis, Mo.). Unlabeled THU was stored as a bulk powder at −10°. Samples for administration were dissolved in sterile water and passed through Millipore filters immediately prior to use; 250 μCi were administered to monkeys in each experiment. Aquasol scintillation solution was purchased from New England Nuclear. Silica gel thin-layer chromatography sheets, type SA, were purchased from Gelman Sciences, Inc. (Ann Arbor, Mich.).

^{14}C radioactivity was measured by adding 10 ml of Aquasol to 0.1 ml of sample fluid and counting the mixture in a Packard Model 541 scintillation counter (Packard Instrument Co., Downers Grove, Ill.). Total tritium radioactivity in each sample ([^{3}H]ara-C plus [^{3}H]ara-U) was determined in a similar manner. The relative amounts of ara-C and ara-U in each sample were measured by chromatographing the sample on type SA silica gel thin-layer sheets using an *n*-butyl alcohol:water solvent (86:14 v/v) (8). Samples of unlabeled ara-C (R_f = 0.38) and

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[3] The abbreviations used are: ara-C, 1-β-D-arabinofuranosylcytosine; i.t., intrathecal(ly); ara-U, 1-β-D-arabinofuranosyluracil; THU, tetrahydrouridine; ara-CTP, 1-β-D-arabinofuranosylcytosine 5′-triphosphate; CSF, cerebrospinal fluid.

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ara-U (R_f = 0.57) were included on each sheet as standards. After the chromatographic pattern was developed, the positions of the standard materials were identified using an UV lamp. The amounts of labeled ara-C and ara-U in the sample were then measured by cutting out 0.5-cm strips of the chromatography sheet in positions corresponding to the standards, placing them in scintillation vials, adding 1 ml of water and 10 ml of Aquasol, and counting the final mixture in a Packard scintillation counter as above. Counting efficiency was 53% for ^3H and 79% for ^{14}C.

Cytidine deaminase activity was assayed by the method described previously by Chabner *et al.* (4). Pharmacokinetic parameters for ara-C and THU were derived from curves which were least-squares linear fits of the observed data (10). Squared correlation coefficients (r^2) were greater than 0.95 for all phases of the fitted curves. Clearance of ara-C was obtained by dividing the initial dose by the area under the curve (10).

RESULTS

Intravenous Administration of THU. Chart 1 shows the plasma levels of [^{14}C]THU following an i.v. bolus (100 mg/kg). The drug disappeared from plasma in a biphasic pattern with α half-lives of approximately 7 and 8 min and β half-lives of 80 and 90 min, respectively, in 2 monkeys. Corresponding THU levels in the CSF were below the limit of assay sensitivity and thus less than 1% of the concomitant plasma levels. Plasma concentrations remained above 10^{-5} M for more than 11 hr. No studies on possible metabolites of THU were done since it has been shown that i.v. THU is excreted unchanged (13).

Intrathecal THU Pharmacokinetics. Chart 2 shows the CSF levels of [^{14}C]THU following an i.t. dose of 6 mg/kg. The drug was slowly cleared from the CSF with an initial half-life of approximately 1 hr and a terminal half-life of approximately 8 hr. Corresponding plasma levels of THU were below 10^{-7} M, the limit of sensitivity of our assay. Doses up to 15 mg/kg were utilized i.t. in experiments aimed at assessing possible THU neurotoxicity. Neither acute nor delayed neurotoxicity was noted in one monkey treated with 15 mg of THU given i.t. every week for 6 weeks.

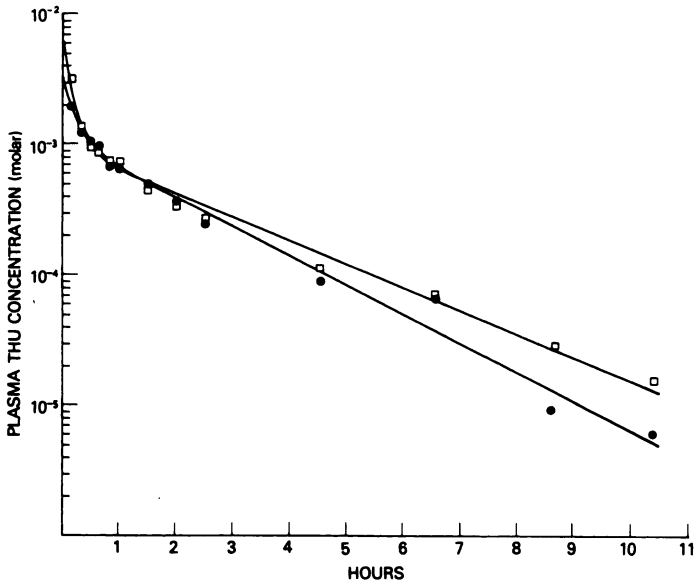

Chart 1. THU plasma concentration following an i.v. bolus of 100 mg/kg in 2 monkeys. See text for details.

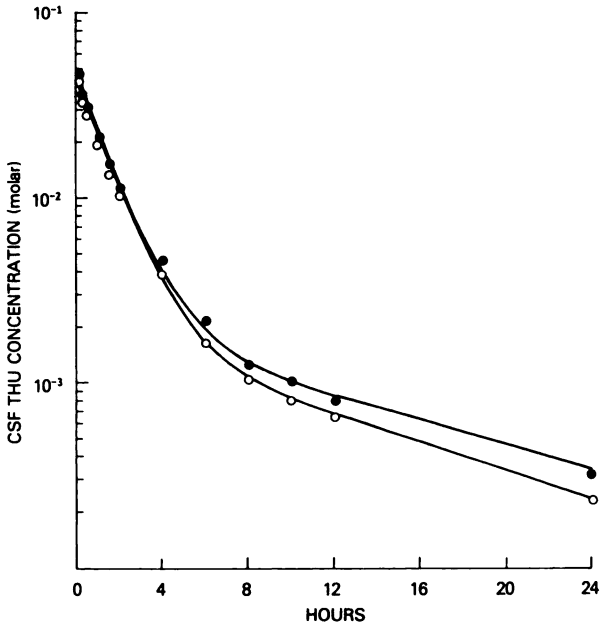

Chart 2. THU concentration in the CSF following i.t. administration of 6 mg/kg in 2 monkeys. See text for details.

Influence of Intravenous and Intrathecal THU on Intrathecal ara-C Pharmacokinetics. Chart 3*A* shows CSF levels of radiolabeled ara-C, ara-U, and ara-C plus ara-U measured in a monkey following i.t. administration of ara-C (1 mg/kg). In this experiment, CSF levels of intact ara-C declined over the first 6 hr with a half-life of about 22 min. The initial half-life was followed by a β half-life of about 116 min. α and β half-lives of 32 and 115 min, respectively, were noted when the experiment was repeated in a second animal. The amount of [^3H]ara-U present in the CSF increased rapidly; at 2 hr, ara-C accounted for only 10% of the total radioactivity present in the CSF.

Chart 3*B* shows the CSF levels of radiolabeled ara-C, ara-U, and ara-C plus ara-U in the same monkeys treated first with i.v. THU (100 mg/kg) and 30 min later with ara-C (1 mg/kg) i.t. In this experiment, intact ara-C was cleared from the CSF in a biphasic manner. An initial half-life of about 11 min was observed followed by a β-half-life of 77 min. Under identical experimental conditions in another monkey, initial and terminal half-lives were 9 and 93 min, respectively. Under these circumstances, ara-U formation was markedly decreased and never exceeded 20% of the total radioactivity.

Chart 3*C* shows the CSF concentration of radiolabeled ara-C, ara-U, and ara-C plus ara-U following i.t. injection of ara-C (1 mg/kg) and THU (6 mg/kg) in combination. Intact ara-C disappeared with a single half-life of approximately 96 min in both monkeys. Under these experimental conditions, catabolic degradation of ara-C to ara-U was almost completely inhibited. Throughout the experiment, ara-U never exceeded 5% of the total radioactivity.

When ara-C was administered i.t., a clearance of about 7.5 ± 0.4 (S.D.) ml/hr was observed (Table 1). In monkeys pretreated with i.v. THU, the clearance value fell to 6.2 ± 0.6 (S.D.) ml/hr. When ara-C was administered together with i.t. THU, the clearance rate was reduced to 4.2 ± 0.4 (S.D.) ml/hr.

The 2 monkeys in which the 2 drugs were used in combination at the dose of 1 mg/kg for i.t. ara-C, 100 mg/kg for i.v.

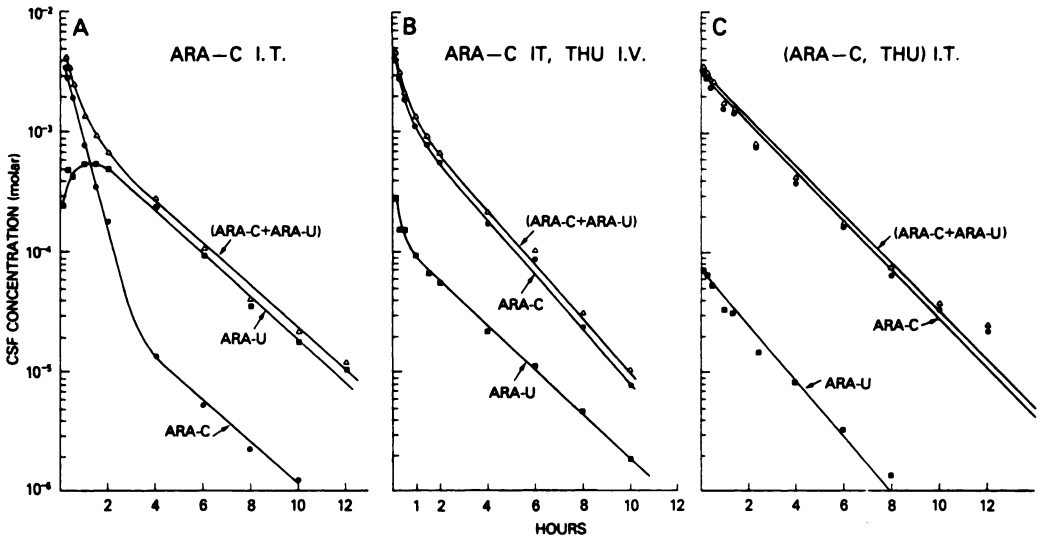

Chart 3. CSF profiles in the same monkey with radiolabeled ara-C, ara-U, and ara-C plus ara-U following i.t. administration of ara-C (1 mg/kg). *A*, ara-C injected i.t. alone; *B*, ara-C injected i.t. in monkeys pretreated (by 30 min) with i.v. THU (100 mg/kg); and *C*, ara-C and THU (6 mg/kg) injected i.t.

Table 1

Disappearance half-lives and clearance rates from CSF of i.t. ara-C injected alone and in combination with i.t. and i.v. THU

	Monkey	$t_{1/2}\alpha$	$t_{1/2}\beta$	Clearance (ml/min)	
ara-C (i.t.)[a]	791	22.8	116	7.8	
	793	32.3	115.3	7.2	NS[b]
ara-C (i.t.), THU (i.v.)[c]	791	9.3	93.6	5.8	$p < 0.01$[d]
	793	11.5	77.8	6.6	
ara-C (i.t., THU (i.t.)[e]	791		96.6	4.5	
	793		95.5	3.9	

[a] Total dose of i.t. ara-C was 7 mg in all experiments.
[b] NS, not significant.
[c] THU (i.v.) given at a dose of 100 mg/kg.
[d] Statistically significant by paired *t* test.
[e] THU (i.t.) given at a dose of 6 mg/kg.

THU, and 6 mg/kg for i.t. THU were observed for at least 6 months, and neither acute nor delayed neurotoxicity occurred.

Cytidine Deaminase Activity. Eight human and 5 monkey CSF samples were tested for cytidine deaminase activity. In all the samples studied, the enzyme activity was below 2 nmol/ml/hr, the limit of sensitivity of our assay.

DISCUSSION

In the present study, we investigated the interaction of ara-C and THU in the CSF, utilizing an experimental, subhuman primate model for study of CSF pharmacokinetics (15, 20). Our data indicate that, in monkeys, when administered alone, i.t. ara-C is almost completely metabolized within 4 hr and that both i.v. pretreatment and concomitant i.t. injection of THU substantially alter disappearance of i.t. administered ara-C from CSF. The disappearance of ara-C from the CSF is retarded by THU administration, resulting in higher CSF levels of intact ara-C. Both i.t. and i.v. administrations have similar effects; however, i.t. THU had a more pronounced influence on ara-C disappearance. The effect of THU on ara-C disappearance appears to be primarily due to the inhibition of cytidine deaminase, since the disappearance curve of the total radioactivity (ara-C + ara-U) is not modified in the presence of either i.v. or i.t. THU.

Clinical pharmacological studies conducted in humans with i.t. administered [^{3}H]ara-C have shown that, after 7 hr, only 10% of the total radioactivity is metabolized to the inactive compound ara-U (12). Therefore, the profound effect of i.t. THU on CSF ara-C pharmacokinetics observed in the monkey may not be as dramatic as that in humans since, in humans, only a fraction of i.t. administered ara-C is metabolized to ara-U. In the present study, the level of cytidine deaminase activity found in CSF was extremely low in both monkeys and humans. Therefore, the observed differences in the rates of deamination of ara-C between monkeys and humans (8) cannot be explained by differences in CSF cytidine deaminase activity. However, it is possible that this interspecies difference may be accounted for by differences in the concentration of cytidine deaminase in other sites such as the ependymal surface and/or choroid plexus.

Nevertheless, there is substantial rationale for the use of this combination in humans, since THU has a profound effect upon ara-C metabolism in human leukemic cells *in vitro* (6, 11). Ho *et al.* (11) have shown that THU induces a 7-fold increase in the intracellular level of ara-CTP in acute myelogenous leukemia cells exposed *in vitro* to THU and ara-C. This effect has been correlated with the high level of cytidine deaminase in myeloblasts. Therefore, it seems appropriate to consider combination i.t. ara-C and THU treatment for central nervous system involvement, particularly by neoplastic cells with high cytidine deaminase activity (*e.g.*, acute myelogenous leukemia or malignant melanoma).

Of the 2 approaches we have studied, the concomitant i.t. administration of ara-C with THU appears most appropriate. In our experiments, administration of a dose of 6 mg of THU per kg i.t. did not produce detectable neurotoxicity. Moreover, i.v. THU administration would probably result in significant systemic toxicity, since systemic ara-C concentrations have been found to be greatly increased in the presence of i.v. THU (19).

Our results suggest that the combination of i.t. ara-C with THU may have therapeutic applicability in humans.

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