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Letter to the Editor

Correspondence re: L. E. Damon *et al.*, Plasma and Cerebrospinal Fluid Pharmacokinetics of 1-β-D-Arabinofuranosylcytosine and 1-β-D-Arabinofuranosyluracil following the Repeated Intravenous Administration of High- and Intermediate-Dose 1-β-D-Arabinofuranosylcytosine. Cancer Res., *51:* 4141–4145, 1991.[1]

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The major metabolic product of Ara-C,[2] by deamination of the 5-amino group of 1-β-D-arabinofuranosylcytosine, is Ara-U (1, 2). The study by Damon *et al.* (3) suggested Ara-U as an important factor in the biological activity of Ara-C. Ara-U is the major metabolite of Ara-C appearing in plasma, cerebrospinal fluid, and urine with temporary ratios of Ara-U:Ara-C in plasma and cerebrospinal fluid of well over 100:1 (4, 5) and a large excess of urinary excretion as compared to Ara-C (2, 5). In the introduction to their report (3), Ara-U was quoted as nontoxic. Yet, Muller and Zahn reported in 1979 (6) significant cytotoxic activity of Ara-U for the growth of L5178Y cells *in vitro* at levels of 0.5 to 10 μg/ml and *in vivo* growth inhibition of L5178Y in mice at doses of 90 mg/kg. Of major importance was their finding of the formation of Ara-U 5′-mono-, di-, and triphosphates in the test cells which might well be responsible for the reported toxicity, including putative neurotoxicity.

One of the side effects of Ara-U, not mentioned in the report by Damon *et al.*, is the observation by Kessel and Shurin (7) of the inhibition of the uptake of Ara-C into cells. The rapid deamination of Ara-C was observed by Rustum *et al.* to reduce intracellular Ara-CTP (8), and a possible adverse effect of Ara-U on the treatment of lymphoblastic, myeloblastic, and myelomonoblastic leukemias was reported by Steuart and Burke (9). Also of clinical importance in the evaluation of the deamination reaction of Ara-C is the inducibility of the deaminase by Ara-C (9, 10).

While Ara-U is an inhibitor of cytidine deaminase (EC 3.5.4.5), its K_i for cytidine deaminase inhibition of 5.6×10^{-3} M (4) is substantially higher than the Ara-U plasma and cerebrospinal fluid levels reported by Damon *et al.* and others (3, 11). Thus, deamination inhibition by Ara-U cannot be efficient enough to have a clinical effect. Damon *et al.* did not comment on THU, which is a much more powerful deaminase inhibitor than Ara-U, with a K_i of 2.9×10^{-8} M (12) and a dissociation constant (K_D) of 4.5×10^{-8} M in a partially purified human liver deaminase preparation (13). This compound has been evaluated extensively, both experimentally and clinically (14–19), and THU plasma (and possibly tissue levels) necessary for effective deaminase inhibition can easily be achieved by bolus and s.c. and even p.o. administration (18, 19).

In experimental tumors, THU increased V_{max} for kinase activity in tumors possessing deaminase activity (20). *In vitro*, addition of THU to Ara-C significantly increased intracellular Ara-CTP in human leukemia cells (21–23) with demonstrated high amounts of cytidine deaminase (20).

From the studies mentioned above, one may conclude that the data presented in the paper by Damon *et al.* indicate the need for effective deaminase inhibition to increase the effectiveness of Ara-C and to possibly reduce central nervous system toxicity by the putative central nervous system-toxic compound Ara-U (24). These goals could be achieved by a combination of Ara-C and THU (19).

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[2] The abbreviations used are: Ara-C, 1-β-D-arabinofuranosylcytosine; Ara-U, 1-β-D-arabinofuranosyluracil; THU, tetrahydrouridine.

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Reply

Drs. Kreis and Budman have written a cogent argument in favor of modulating Ara-C[1] cytotoxicity through the inhibition of cytidine deaminase by THU. Some authors have put forth the proposal that Ara-C cytotoxicity can be modulated by the inhibitory effects of Ara-U on cytidine deaminase, resulting in Ara-C "self-potentiation" (1, 2). Kreis and Budman point out that high levels of Ara-U might be detrimental because of its inhibition of Ara-C cellular uptake (3). Furthermore, the maximal plasma and CSF levels of Ara-U in our study do not approach the Ki of Ara-U for cytidine deaminase inhibition, making it unlikely that Ara-U would effectively inhibit that enzyme. However, as Capizzi *et al.* argue (1), when Ara-C levels fall after an infusion of Ara-C, the ratio of Ara-U to Ara-C rises substantially, and inhibition by Ara-U of cytidine deaminase might occur, resulting in a prolonged γ phase of Ara-C elimination. In our study, the trough plasma levels of patients receiving 3 g/m^2/dose of Ara-C showed an Ara-U:Ara-C ratio of 1000:1, suggesting cytidine deaminase inhibition by Ara-U is possible.

Kreis and Budman also note that high doses of Ara-C might not achieve improved cytotoxicity, since Ara-C induces cytidine deaminase and would lead to reduced intracellular Ara-CTP (4–6). This problem, plus the negative effects of Ara-U, could be avoided by inhibiting cytidine deaminase with THU in the setting of standard-dose Ara-C administration. THU has been shown to increase intracellular Ara-CTP in some human leukemia cells *in vitro* (7–9) but not in murine L1210/0 cells despite the fact that THU increased plasma Ara-C levels in mice bearing the L1210/0 leukemia (10). In humans with acute leukemia, THU has been shown to modulate Ara-C pharmacokinetics and generate Ara-C plasma levels of 3305 ± 1901 ng/ml when used in conjunction with Ara-C (200 mg/m^2) administered i.v. over 3 h (11). These Ara-C levels would be expected following the administration of 0.5–1 g/m^2 of Ara-C i.v. over 3 h. Also, the plasma Ara-U levels generated in this study were lower than would be expected following the administration of high doses of Ara-C. However, the fears of negative effects of high levels of Ara-U generated following the administration of high doses of Ara-C are probably unfounded. High plasma Ara-U concentrations produced by one dose of 3 g/m^2 Ara-C did not alter the plasma or CSF pharmacokinetics of a second 3 g/m^2 Ara-C dose 24 h later in humans with primary CNS lymphoma (12). Likewise, Ara-CTP pharmacokinetics in circulating human leukemic blasts were unaltered by the high plasma Ara-U levels generated by high dose Ara-C administration (13).

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[1] The abbreviations used are: Ara-C, 1-β-D-arabinofuranosylcytosine; THU, tetrahydrouridine; Ara-U, 1-β-D-arabinofuranosyluracil; CSF, cerebrospinal fluid; CNS, central nervous system; Ara-CTP, 1-β-D-arabinofuranosylcytosine-5′-triphosphate; Ara-UTP, 1-β-D-arabinofuranosyluracil-5′-triphosphate.

Finally, Kreis and Budman claim one further benefit when using THU with Ara-C: a reduction in CNS toxicity due to the "putative CNS toxic compound Ara-U." There is no evidence that Ara-U is a neurotoxin. Kreis and Budman refer to a paper by Muller and Zahn as evidence that Ara-U is cytotoxic (14). In that paper, Ara-U was shown to be cytostatic against murine L51718Y cells. The assay used was not designed to assess whether Ara-U was cytotoxic against L51718Y cells. If Ara-U is cytotoxic against L51718Y cells, it would not be possible to extrapolate that Ara-U is also toxic to postmitotic neurons. Kreis and Budman also reference a report by Lopez and Agarwal (15) as evidence that Ara-U is a neurotoxin. That report showed persistent cerebrospinal fluid levels of Ara-U 7 days after the administration of high-dose Ara-C and cisplatin to a patient who developed renal failure and cerebellar toxicity. These authors speculated that the cause of neurotoxicity was either Ara-U, Ara-C, or cisplatin. In addition, a paper by Lopez *et al.* (16) showed slowing of electroencephalogram wave patterns in nonhuman primates following the intraventricular administration of Ara-U. This did not occur after the intraventricular administration of Ara-C. Slowing of electroencephalogram waves is not proof that Ara-U is neurotoxic. Neither Ara-U or Ara-C resulted in clinical neurotoxicity in these animals.

On the other hand, there is evidence that Ara-C is a neurotoxin. Martin *et al.* (17, 18) showed that Ara-C kills postmitotic rat sympathetic neurons in a manner resembling nerve growth factor deprivation. However, other antimetabolites, such as 1-β-D-arabinofuranosyladenine, 1-β-D-arabinofuranosylthymine, 5-fluorodeoxyuridine, and hydroxyurea, did not kill neurons. The Ara-C neurotoxic effect could be blocked by 2′-deoxycytidine. It appears that Ara-C kills postmitotic neurons by interfering with a 2′-deoxycytidine-dependent neurotrophic signal transduction mechanism which is independent of DNA synthesis.

New data demonstrate that Ara-UTP resembles Ara-CTP and is falsely incorporated in DNA strand templates and terminates strand prolongation in the presence of DNA polymerase α (19). Ara-UTP, therefore, is potentially cytotoxic. Human acute myeloid leukemia cells incubated *in vitro* with [^{3}H]Ara-U did not accumulate [^{3}H]Ara-UTP, but did when incubated with [^{3}H]Ara-C (19). Although 2′-deoxytetrahydrouridine (an inhibitor of cytidine-5′-monophosphate deaminase) blocked the production of [^{3}H]Ara-UTP from [^{3}H]Ara-C, THU did not. Thus, Ara-UTP production does not occur via exogenous Ara-U. It is unknown whether Ara-UTP is a neurotoxin, but if it is, CNS toxicity from Ara-C would not be prevented by the concomitant use of THU.

Our study showed that Ara-U accumulated in CSF after repeated i.v. administrations of high- and intermediate-dose Ara-C (20). Ara-C did not accumulate in CSF. We believe it is