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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).

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

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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.

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possible for Ara-U to accumulate in brain parenchyma and modulate Ara-C catabolism in nervous tissue, thus leading to clinically significant CNS injury. One must be cautious using THU with Ara-C since CSF levels of Ara-C might result which are toxic to nervous tissue, despite a presumed lack of CSF Ara-U accumulation in that treatment regimen. At present there is no evidence that the strategy of decreasing plasma Ara-U levels by coadministering THU with standard-dose Ara-C (100–200 mg/m^2) will alter the therapeutic index of Ara-C therapy (11). The use of intermediate dose rates of Ara-C (0.25 g/m^2/h) on an intermittent schedule produces Ara-C levels similar to those produced by the Ara-C/THU regimen without clinical neurotoxicity (21). This question can be resolved only by a prospective trial comparing standard-dose Ara-C/THU therapy to Ara-C dosing which generates similar Ara-C pharmacological characteristics.

Lloyd E. Damon, M.D.
Cancer Research Institute
University of California
San Francisco, California 94143

William Plunkett, Ph.D.
University of Texas
M. D. Anderson Cancer Center
Department of Medical Oncology
Houston, Texas 77030

Charles A. Linker, M.D.
Cancer Research Institute
University of California
San Francisco, California 94143

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