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*Letter to the Editor*

# Correspondence re: D. H. W. Ho, C. J. Carter, N. S. Brown, J. Hester, K. McCredie, R. S. Benjamin, E. J Freireich, and G. P. Bodey. Effects of Tetrahydrouridine on the Uptake and Metabolism of 1-β-D-Arabinofuranosylcytosine in Human Normal and Leukemic Cells. Cancer Res., *40:* 2441–2446, 1980.

## 1-β-D-Arabinofuranosylcytosine (ara-C) Deamination in Human Leukemic Myeloblasts is not a Mechanism of Resistance to ara-C Therapy

We have studied the effect of THU[1] on ara-C metabolism in the bone marrow myeloblasts (3) of 20 patients with newly diagnosed untreated adult acute myeloid leukemia, and our conclusions are rather different from those of Ho *et al.* (6).

Washed marrow myeloblasts (concentration, 0.5 to 10 × $10^6$ cells/ml) were incubated in a final volume of 125 μl Eagle's minimal essential medium with [5-$^3$H]ara-C (14 to 24 Ci/mmol) for 45 min at 37°. Ten nM, 100 nM, and 1 μM ara-C was used, representative of the concentration range found *in vivo* with current dose schedules (4). ara-U in the medium was measured on Ag 50W 1X4 columns (3) and intracellular ara-CTP was measured by thin-layer chromatography on PEI cellulose plates (2). THU was used at a final concentration of 1 mM.

Our results for 1 μM ara-C are shown in Table 1. The ratio of ara-U to ara-CTP ranged from 0.32 to 19.11. In a further 9 patients, not studied as intensively, ratios were 0.13, 0.53, 1.03, 1.87, 2.48, 4.2, 4.3, 5.57, and 6.19 [median, 2.7; mean, 4.2 ± 4.49 (S.D.); $n = 20$). This is much less than the median deaminase/kinase ratio found in lysed cells (1, 6). This may be because lysing the cells increases the enzyme activity and the measurement of the actual products of ara-C metabolism in intact cells is therefore probably more representative of the *in vivo* metabolism.

Ho *et al.* (6) measured the deaminase activity using 5 times the substrate concentration used to measure the kinase activity (300 and 60 nmol ara-C per 0.3 ml, respectively), yet this difference does not seem to have been taken into account when measuring enzyme activity. Obviously, *in vivo* and in intact cells, one may expect both pathways to be exposed to similar ara-C concentrations, unless the deaminase diverted ara-C intracellularly from the kinase pathway.

Using 1 mM THU, we were able to completely prevent deamination. Although ara-U production exceeded ara-CTP production in most of the marrows (Table 1), the effect of THU on ara-CTP production was not proportional to the ratio of ara-U to ara-CTP. The increase in ara-CTP produced by THU was proportional to the change in ara-C concentration in the medium (Table 1, Columns D and E; $r = 0.897$; $p < 0.001$).

Our conclusion was that deaminase does not exert its effect by competing intracellularly with deoxycytidine kinase for ara-C but rather by depleting the medium of ara-C. The results of Ho *et al.* (6) can be similarly interpreted, *e.g.*, Table 2, AML [acute myelocytic leukemia], 24-hr incubation. A 5.4-fold increase in ara-C in the medium at 24 hr, produced by THU, is associated with a 7.6-fold increase in ara-CTP; *i.e.*, the increase is roughly proportional. However, the deaminase/kinase ratio is 30- or 65-fold (Ref. 6, Table 3). If ara-C was given as a constant infusion, as recommended by Ho *et al.* (6), or was continually replaced in the medium, then this effect is probably not relevant. It is an artifact of a closed system.

Because the measurements of ara-CTP might not be representative of those in S-phase cells, we also measured the effects of THU and ara-C on DNA synthesis, measured under conditions identical to those already described (2, 3). THU did not increase the inhibition of DNA synthesis produced by ara-C at concentrations of 10 nM to 10 μM. This again suggests that the intracellular and extracellular ara-C concentrations are very similar, in spite of a very active deaminase pathway, and that the extracellular concentration determines the amount available for both the kinase and deaminase pathways.

In mice, THU did not enhance the effect of ara-C in 4 tumors, with high deaminase/kinase ratios (sarcoma T241, adenocarcinoma E0771, Lewis Lung carcinoma, solid Sarcoma 180 Japan) (7). Only with the ascitic form of Sarcoma 180 Japan and i.p. drug was there any enhancement, and an effective

Table 1

*ara-CTP and ara-U production by marrow myeloblasts in vitro*

| Patient | A ara-CTP production (pmol/$10^6$ cells/45 min at 1 μM ara-C) | B ara-U production (pmol/$10^6$ cells/45 min at 1 μM ara-C) | C Ratio of ara-U/ara-CTP | D % of deamination[a] | E % of increase in ara-CTP[b] |
|---|---|---|---|---|---|
| 1 | 2.07 | 3.47 | 1.67 | 1.00 | 2.80 |
| 2 | 17.62 | 5.56 | 0.32 | 1.16 | 3.29 |
| 3 | 20.50 | 12.85 | 0.63 | 2.68 | 6.50 |
| 4 | 31.40 | 12.70 | 0.40 | 3.68 | 7.60 |
| 5 | 7.99 | 152.70 | 19.11 | 11.5 | 17 |
| 6 | 7.61 | 34.20 | 4.49 | 12.3 | 9 |
| 7 | 15.03 | 98.80 | 6.57 | 12.9 | 22.9 |
| 8 | 14.40 | 128.60 | 8.43 | 14.8 | 15.2 |
| 9 | 9.84 | 35.54 | 3.61 | 20.2 | 16.5 |
| 10 | 6.50 | 64.60 | 9.93 | 22 | 23 |
| 11 | 22.70 | 70.75 | 3.11 | 29.8 | 26.6 |

[a] % of deamination

$$= \frac{\text{ara-U concentration in medium at end of incubation}}{\text{ara-C concentration in medium at beginning of incubation}} \times 100$$

[b] % of increase in ara-CTP

$$= \frac{(\text{ara-CTP production with THU}) - (\text{ara-CTP production without THU})}{\text{ara-CTP production without THU}} \times 100$$

[1] The abbreviations used are: THU, tetrahydrouridine; ara-C, 1-β-D-arabinofuranosylcytosine; ara-U, 1-β-D-arabinofuranosyluracil; ara-CTP, 1-β-D-arabinofuranosylcytosine 5′-triphosphate.

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dose of ara-C was not determined.

Thus, deaminase activity in leukemic cells is not likely to be a mechanism of resistance to ara-C, and THU does not offer a therapeutic advantage in overcoming cellular ara-C resistance. However, because it is deaminated in the liver in humans, THU may be useful to produce a higher ara-C plasma concentration, an effect that could be achieved by increasing the ara-C dose. Dosage of ara-C could be individualized by use of an immunoassay for rapid measurement of plasma levels. It seems unlikely that there will be any differences in toxicity or therapeutic effect if ara-C is given by infusion and similar levels are maintained by either increasing the ara-C dose or using THU.

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## Correspondence re: D. H. W. Ho *et al.*, Cancer Res., *40:* 2441–2446, 1980 (cont'd.).

After carefully reading Dr. Harris' letter and our paper (5), we find for the following reasons that neither Dr. Harris nor we have presented data dealing with the mechanism of resistance to ara-C[1] in human leukemic myeloblasts. (*a*) Our work was strictly an *in vitro* cell uptake study which cannot possibly be used to draw a conclusion regarding patient response *in vivo*. And (*b*) Dr. Harris has not identified responses to ara-C treatment in his patient population, nor were the effects of THU on ara-CTP and ara-U formation in leukemic cells correlated with patient responses to ara-C. Therefore, we would like to know how Dr. Harris derived his conclusion, "ara-C deamination in human leukemic myeloblasts is not a mechanism of resistance to ara-C therapy." If he does have data to support his statement, it would indeed be an important although controversial finding, since Steuart and Burke (6) have demonstrated that "initial therapeutic responses to ara-C treatment in human leukemia are correlated with lower intracellular concentrations of deaminase than those present in non-responders. . . ."

In our studies, we did not identify the responses of the patients to ara-C treatment because those patients were on combination chemotherapy of ara-C with several other drugs which made the evaluation impossible. We made no conclusion about the response to ara-C therapy and its correlation to ara-C deamination. Nor did we make the conclusion that "deaminase activity in leukemic cells is likely to be a mechanism of resistance to ara-C and THU offers a therapeutic advantage in overcoming cellular ara-C resistance." In fact, our feelings on the mechanism of ara-C resistance are expressed in the following quote, "At present, the mechanism of clinical resistance to ara-C has not been defined with certainty" (Ref. 5, p. 2445, Paragraph 2, Line 1).

Dr. Harris found, in Paragraph 6, that "the increase in ara-CTP of human leukemic cells produced by THU was proportional to the change in ara-C concentration in the medium." His results support our findings also. Furthermore, his results substantiate our speculations that "THU may spare ara-C from degradation and permit target cells to be exposed to the agent" (Ref. 5, p. 2441, Paragraph 4, Line 17).

We have certainly made no conclusion that "deaminase exerts its effect by competing intracellularly with deoxycytidine kinase for ara-C." Our work was to show and to explain, in part, the *in vitro* differential THU effect on the formation of ara-CTP and ara-U among acute myelocytic leukemia, chronic myelocytic leukemia, and chronic lymphocytic leukemia cells (5).

Measuring the deaminase activity, we have indeed used 5 times the substrate concentration to measure the kinase activity. These concentrations were used because the reported $K_m$'s for the deaminase and kinase activities of human leukemic cells have an approximate 4-fold difference (1, 4). Using ara-C as a substrate, the $K_m$ for deaminase of human leukemic cells is $9 \times 10^{-5}$ M (1) and for kinase is $25 \times 10^{-6}$ M (4). Conventionally, it is necessary to measure enzyme activities under optimum conditions, *e.g.*, pH, buffer, cofactors, substrate concentration, etc. One could, of course, argue that these conditions are not the same as in intact cells and *in vivo*. In fact, the kinase reaction has always been measured with the addition of $10^{-3}$ M THU to prevent substrate consumption by the deaminase (3).

We totally agree with Dr. Harris that measuring actual products of ara-C metabolism in intact cells [as Dr. Harris, Chou *et al.* (2), and we (5) have done], rather than using the enzyme activity, may be more representative of the *in vivo* metabolism. However, measuring enzyme activities is not totally uninformative. Without this *in vitro* technique, we would not know that human liver is the major site of ara-C inactivation and why ara-C is rapidly deaminated to ara-U *in vivo*.

[1] The abbreviations used are: ara-C, 1-β-D-arabinofuranosylcytosine; THU, tetrahydrouridine; ara-CTP, 1-β-D-arabinofuranosylcytosine 5′-triphosphate; ara-U, 1-β-D-arabinofuranosyluracil.

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