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Isao Takahashi, *Kochi Municipal Central Hospital*
Naoki Hayashi, *Okayama University*
Toru Nakamura, *Kochi Municipal Central Hospital*
Toshiro Matuzaki, *Okayama University*
Toshio Murase, *Okayama University*
Ken Osada, *Okayama University*
Makoto Takeuchi, *Okayama University*
Yasufumi Ueki, *Okayama University*
Hiroshi Nakada, *Kochi Municipal Central Hospital*
Seiichi Yorimitsu, *Kochi Municipal Central Hospital*
Ikuro Kimura, *Kochi Municipal Central Hospital*

Increased accumulation of cytosine arabinoside in human leukemic cells and enhancement of its cell-killing activity by uridine.*

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Abstract

The effects of uridine(UR) on the cell-killing activity of cytosine arabinoside(ara-C) against human leukemic cells, MOLT-4, and on ara-C accumulation in cells were studied. The 50% lethal dose(LD50) of ara-C as determined by clonogenic assay was decreased to 5.0 x 10(-8) mol from 9.0 x 10(-7) mol after 3 days exposure to 10(-3) mol of UR. The accumulation of 3H-ara-C at 24 and 48 h was significantly increased in culture medium containing 10(-8) mol of 3H-ara-C and 10(-3) mol of UR (5,129 +/- 123.5 vs 2,554 +/- 115.5 cpm/10(5) cells at 24 h, p less than 0.01, and 5,772 +/- 123.2 vs 1,372 +/- 51.8 cpm/10(5) cells at 48 h, p less than 0.01). It is noteworthy that cell-killing activity of ara-C against human leukemic cells was enhanced by the combination with a nucleoside(UR), but not with antileukemic agents.

KEYWORDS: cytosine arabinosids, uridine, antileukemic effect, accumulation of cytosine arabinoside

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— Brief Note —

Increased Accumulation of Cytosine Arabinoside in Human Leukemic Cells and Enhancement of Its Cell-Killing Activity by Uridine

Isao Takahashi[a,b,*] Naoki Hayashi[b], Toru Nakamura[a,b], Toshiro Matuzaki[b], Toshio Murase[b], Ken Osada[b], Makoto Takeuchi[b], Yasufumi Ueki[b], Hiroshi Nakada[a,b], Seiichi Yorimitsu[a] and Ikuro Kimura[b]

[a]Department of Medicine and Cancer Institute, Kochi Municipal Central Hospital, Sakurai-Cho, Kochi 780, and [b]Department of Medicine, Okayama University Medical School, Okayama 700, Japan

The effects of uridine(UR) on the cell-killing activity of cytosine arabinoside(ara-C) against human leukemic cells, MOLT-4, and on ara-C accumulation in cells were studied. The 50% lethal dose(LD_{50}) of ara-C as determined by clonogenic assay was decreased to 5.0×10^{-8} mol from 9.0×10^{-7} mol after 3 days exposure to 10^{-3} mol of UR. The accumulation of ^{3}H-ara-C at 24 and 48 h was significantly increased in culture medium containing 10^{-8} mol of ^{3}H-ara-C and 10^{-3} mol of UR ($5,129 \pm 123.5$ *vs* $2,554 \pm 115.5$ cpm/10^5 cells at 24 h, $p < 0.01$, and $5,772 \pm 123.2$ *vs* $1,372 \pm 51.8$ cpm/10^5 cells at 48 h, $p < 0.01$). It is noteworthy that cell-killing activity of ara-C against human leukemic cells was enhanced by the combination with a nucleoside(UR), but not with antileukemic agents.

Key worde: cytosine arabinosids, uridine, antileukemic effect, accumulation of cytosine arabinoside

Cytosine arabinoside(ara-C), one of the most active drugs for treating patients with acute nonlymphocytic leukemia, is phosphorylated to ara-C triphosphate(ara-CTP), which inhibits DNA polymerase by competitive inhibition of deoxycytidine triphosphate(dCTP), through three sequential phosphorylations (1-3). It is rapidly inactivated to arabinosyl uracil(ara-U) by cytidine deaminase (4). Because some metabolic pathways are common to endogenous nucleosides as well as ara-C, the effect of the combination of ara-C and natural nucleosides has been investigated. For example, tetrahydrouridine(THU), an inhibitor of cytidine deaminase, was capable of increasing the biological activity of ara-C (5). Thymidine enhanced the cytotoxicity of ara-C subsequently to a reduction in dCTP pools (6).

The concurrent administration of suboptimal dose of ara-C and uridine(UR) also resulted in longer survival of mice bearing leukemia L 1210 than the administration of ara-C alone (7).

These findings led us to investigate the effect of UR on the cell-killing activity of ara-C against human leukemic cells, MOLT-4, and on ara-C accumulation in cells *in vitro*.

* To whom correspondence should be addressed.

Materials and Methods

The human lymphoblastic cell line, MOLT-4, was maintained in suspension in culture medium containing RPMI 1640 medium with 10 % heat-inactivated fetal calf serum(FCS) and fed with fresh medium three times a week. Cells in the exponential growth phase were used. Cell-killing activity of the combination of ara-C and UR was determined by the clonogenic assay. Ten thousand cells were obtained after 3 days of culture at 37°C in medium containing different concentrations of ara-C with or without 10^{-3} mol of UR, which was non-toxic and the highest dose in this experimental system, and then they were incubated in soft agar. Seven days after incubation at 37°C in a 5 % CO_2 atomosphere, percents of colonies containing 50 or more cells at different concentrations of ara-C were determined.

The accumulation of ^{3}H-ara-C in cells was also examined. Cells, 1.5×10^5/ml, were cultured in medium containing 10^{-8} mol of ^{3}H-ara-C with or without 10^{-3} mol of UR at 37°C in a 5 % CO_2 atomosphere for 48 h. The growth rate of cells in medium with UR was not different from that in medium without UR. The radioactivity of 200 μl of the cell suspension was determined in a scintillation counter 6, 24 and 48 h after incubation. ^{3}H-ara-C accumulation with cells was expressed by the radioactivity per 1.0×10^5 viable cells. The experiments were carried out in triplicate.

Results and Discussion

The effect of UR on the cell-killing activity of ara-C is shown in Fig. 1A. The 50 % lethal dose(LD_{50}) of ara-C, which was 9.0×10^{-7} mol in the control, decreased to 5.0×10^{-8} mol after

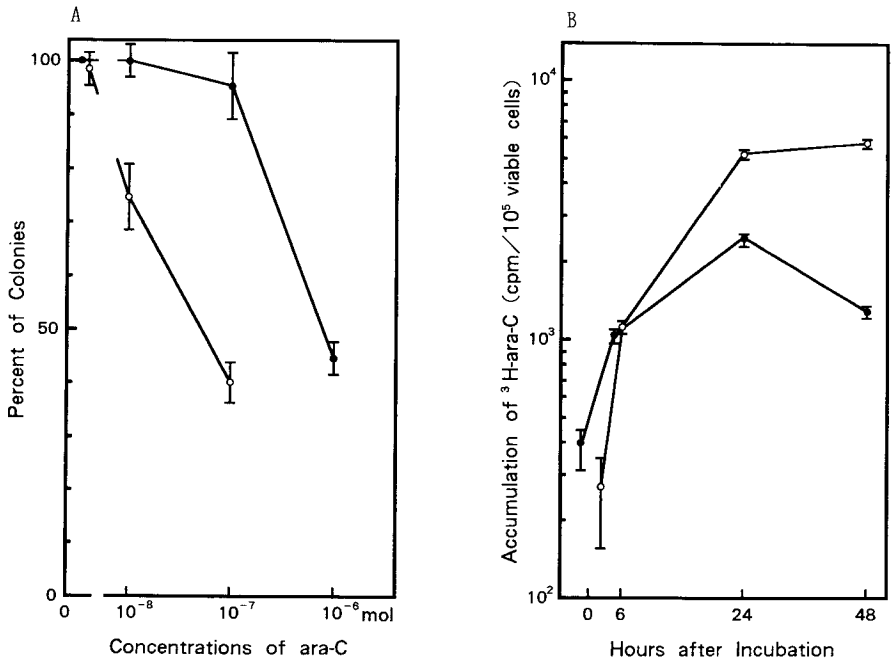

Fig. 1 Effects of uridine (UR) on cell-killing activity and accumulation of cytosine arabinoside (ara-C) *in vitro*.
The 50 % lethal dose (LD_{50}) of ara-C against MOLT-4 cells was 9.0×10^{-7} mol in medium without 10^{-3} mol of UR. On the other hand, it decreased to 5.0×10^{-8} mol in the presence of UR (Fig. 1A). Cells, 1.5×10^5/ml, were incubated in culture medium containing 10^{-8} mol of ^{3}H-ara-C with or without 10^{-3} mol of UR. ^{3}H-ara-C accumulation 24 and 48 h after incubation were accelerated in the presence of UR ($5{,}129 \pm 123.5$ *vs* $2{,}554 \pm 115.5$ cpm/10^5 viable cells at 24 h, $p < 0.01$ and $5{,}772 \pm 123.2$ *vs* $1{,}372 \pm 51.8$ cpm/10^5 viable cells at 48 h. $p < 0.01$, Fig. 1B). Closed circle: UR (−), Open circle: UR (+), Bar: Mean ± SD.

exposure to 10^{-3} mol of UR. Time courses of the accumulation of ^{3}H-ara-C are demonstrated in Fig. 1B. The accumulation of ^{3}H-ara-C at 24 h ($5,129 \pm 123.5$ *vs* $2,554 \pm 115.5$ cpm/10^{5} viable cells, $p < 0.01$) and 48 h ($5,772 \pm 123.2$ *vs* $1,372 + 51.8$ cpm/10^{5} viable cells, $p < 0.01$) were significantly accelerated by the presence of 10^{-3} mol of UR. Ara-C is widely used in combination with other antileukemic agents for the treatment of acute myelocytic leukemia. Because of the similarity of the metabolic pathways of ara-C to endogenous nucleosides, the combined effects of the above two have been investigated (5–7). Saslow *et al.*(7) found the life span of L 1210 leukemia-bearing mice to be prolonged by treatment with the conjugated suboptimal dose of are-C and UR. They also found greater radioactivity of the spleen after the concurrent administration of UR and ^{3}H-ara-C than after ^{3}H-ara-C alone. Our studies demonstrated that UR enhanced the cell-killing activity of ara-C against a human lymphoid leukemia cell line *in vitro* and also demonstrated that UR enhanced the accumulation of ^{3}H-ara-C in leukemic cells at the concentration of 10^{-8} mol of ara-C.

These findings indicate that one possible cause of the action of UR on the activity of ara-C *in vivo* is the enhancement of the cell-killing activity of ara-C. The amount of phosphorylated products are correlate with the response of experimental tumor systems as well as human leukemia to ara-C (8,9). Enzymatic studies with dialyzed splenic supernatant from BDF1 mice bearing advanced leukemia L 1210 disclosed that ^{3}H-ara-C was phosphorylated with uridine 5′-triphosphate(UTP) at twice the initial rate as compared to adenosine 5′-triphosphate(ATP) at equimolar concentrations (10). Therefore, the enhancement of the cell-killing activity of ara-C by UR may owe to an increased phosphorylation of ara-C in the presence of UTP derived from UR. The mechanism of the enhancement of ara-C accumulation in leukemic cells by UR is not clearly understood, but the increased cell-killing activity of ara-C against human leukemia cells could be achieved by the nucleoside, UR.

The combined efficacy of ara -C and UR on leukemia progenitors obtained from patients with acute nonlymphocytic leukemia is currently under study.

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