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Sequential arabinosylcytosin with or without fludarabine in paracmastic patients with acute myeloid leukemia

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The purpose of this study is to assess how fludarabine (Fa) influences arabinosylcytosin's (Ara-C) mode of action. Plasma, cerebrospinal and urine samples were withdrawn from two study groups at specific time points and analyzed by HPLC. Group A was treated with Ara-C only whereas Group B was treated with Fa + Ara-C. The two study groups are all undergoing complete remission (CR). The Ara-C dose for Group A was 3 g/m^2 × 2, and the $AUC_{0–4}$ was 5.131 ± 0.936. The Ara-C dose for Group B was 2 g/m^2 × 2, and the $AUC_{0–4}$ was 12.245 ± 3.863. The $AUC_{0–4}$ for Group B is more than twice the $AUC_{0–4}$ for Group A, and these results indicate that Fa conduces a synergistic increase in the concentration and AUC of Ara-C in plasma and in cerebrospinal fluid. The pharmacokinetics between the different dose treatments was statistically different ($P = 0.016$). The differences in the ratios of $C_{Ara\text{-}u}$ to $C_{Ara\text{-}C}$, and in the T_{max} between Groups A and B could indicate whether or not Ara-C combined with Fa. Although Group B demonstrates a higher $AUC_{0\text{-}4}$ with lower doses of Ara-C (2 g/m^2 × 2), the adverse drug reaction (ADR) and bone inhibition were not more pronounced in Group B compared to Group A. These results are based on a limited number of case studies, hence, additional studies are necessary to support and prove this hypothesis.

1. Introduction

Sixty to seventy percent of patients with acute myeloid leukemia (AML) who are younger than 60 years old undergo complete remission (CR). However, relapse occurs in about 50% of the cases, and only 20–30% of the patients survive for more than 10 years. Therefore, despite the increasing CR rate due to induction chemotherapy over the past decade, the progression of remissions to complete cures has not been achieved. In an attempt to improve the recovery from acute myeloid leukemia (AML), many treatment strategies have been developed and tested. Arabinosylcytosin (Ara-C) is one of the most effective anti-leukemic agents that has a steep dose-response curve. Regimens consisting of a high dose of Ara-C alone are somewhat effective in the treatment of patients with low-risk acute leukemia, but remissions tend to be temporary. One treatment strategy for enhancing the anti-leukemic effect is to increase the accumulation of Ara-C in plasma, but this cannot be accomplished simply by giving higher doses of Ara-C because of serious adverse effects (SAE) (Miyazaki et al. 2004; Ek et al. 2005).

To increase the concentration of intracellular Ara-C, various modulations of Ara-C metabolism were attempted. One approach is to administer fludarabine (Fa), a purine analog with modest antileukemic activity, before Ara-C (Milligan et al. 2006; Malagola et al. 2007). Fa increases intracellular Ara-C accumulation in all phases of the cell cycle by enhancing the activity of the phosphorylating enzyme, deoxycytidine kinase, and by inhibiting ribonucleotide reductase to its metabolic 1-β-D-arabinofuranosyl-uracil (Ara-U), thus, decreasing the nucleoside pool. The increased Ara-C concentration results in greater leukemic cell mortality compared to treatments with either Ara-C or Fa alone. However, Fa does not always increase intracellular Ara-C accumulation in plasma during combined clinical therapy. This study presents the results of clinical studies wherein five adult patients were treated with Ara-C alone or in combination with Fa.

2. Investigations and results

2.1. Ara-C in plasma

The concentrations of Ara-C at each time point in the five patients are shown in Table 1 and Fig. 1. The concentration ratios of Ara-U/Ara-C are shown in Fig. 2.

2.2. Ara-C in cerebrospinal fluid

The concentrations of Ara-C in cerebrospinal fluid and in plasma were determined as discussed above, and the percentages of Ara-C in the five patients, based on its concentration in the cerebrospinal fluid relative to its concentration in plasma ($C_{\text{in cerebrospinal fluid}}/C_{\text{in plasma}}$) are shown in Table 2.

2.3. Pharmacodynamics and ADR

Table 3 shows the pharmacodynamic parameters, which consist of the hemoglobin (Hb), white blood cell count (WBC), platelet (Plt) and blast obtained by bone puncture. Table 3 also shows the ADR parameters, which consist of data on alanine

Table 1: Plasma concentrations and AUC_{0-4} in 5 self-control patients

	HAra-C-1	HAra-C-2	HAra-C-3	HAra-C-4	HAra-C-5	Fa-HAra-C-1	Fa-HAra-C-2	Fa-HAra-C-3	Fa-HAra-C-4	Fa-HAra-C-5
Time (h)			C (μg/mL)					C (μg/mL)		
0	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.5	1.232	2.108	0.746	0.896	0.644	6.522	4.534	2.668	4.324	3.512
1	1.872	2.040	2.035	1.004	1.958	7.543	3.414	1.834	4.033	4.262
2	1.923	2.007	2.364	0.347	2.102	7.489	2.759	0.989	5.609	7.075
3	2.057	0.171	0.939	2.615	0.589	1.051	3.187	2.236	0.371	1.473
4	0.000	0.000	2.096	0.000	0.000	0.000	3.077	1.709	0.000	0.000
AUC_{0-4}(mg/L·h)	6.000	4.762	6.250	4.164	4.481	17.457	12.312	6.789	11.167	13.501
$X \pm SD$			5.131 ± 0.936					12.245 ± 3.863		
statistics difference	$P = 0.016 (0.01 \leq P \leq 0.05)$									

Table 2: Percentage($C_{\text{in cerebrospinal fluid}}/C_{\text{in plasma}}$) of Ara-C in 5 self-control patients

	HAra-C-1	HAra-C-2	HAra-C-3	HAra-C-4	HAra-C-5	Fa-HAra-C-1	Fa-HAra-C-2	Fa-HAra-C-3	Fa-HAra-C-4	Fa-HAra-C-5
Time(h)	%							%		
1	0.0	0.0	0.0	0.0	0.0	5.7	4.1	0.0	0.0	13.8

Table 3: Pharmacodynamics and ADR parameters in 5 self-control patients

Hemogram	HAra-C-1		HAra-C-2		HAra-C-3		HAra-C-4		HAra-C-5	
Indicator	a	p	a	p	a	p	a	p	a	p
Hb	126	113	99↓	86↓	133	120↓	74↓	69↓	77↓	61↓
WBC	3.96	2.99	4.82	3.85	5.74	8.34	3.2↓	1.35↓	2.52↓	6.07
PLT	161	100	131	7	141	93	119	108	85↓	111
Blast	0	0	0	0	0	0	0	0	0	0
ALT	13	17	9	8	45	37	52↑	52↑	339↑	104↑
AST	12	15	16	13	23	20	32	40	106↑	46
rGT	18	27	25	28	32	22	65↑	74↑	111↑	75↑
BUN	5.7	4.5	4.93	5.22	5.9	3.9	4.6	5.9	2.1	2.6
Cr	41↓	48↓	40↓	56	65	66	72	68	64	57↓
Hemogram	Fa-HAra-C-1		Fa-HAra-C-2		Fa-HAra-C-3		Fa-HAra-C-4		Fa-HAra-C-5	
Indicator	a	p	a	p	a	p	a	p	a	p
Hb	123	123	98↓	88↓	105↓	111	87↓	78↓	94↓	101↓
WBC	3.42↓	2.5↓	1.2↓	14.72↑	4.25	1.95↓	4.8	3.7↓	3.80↓	2.81↓
PLT	158	80↓	50↓	98	359↑	79↓	391↑	152	140	63↓
Blast	0	0	0	0	0	0	0	0	0	0
ALT	20	17	18	13	81↑	14	15	43	13	10
AST	17	15	21	19	33	12	18	38	15	13
rGT	40	23	21	19	14	18	118↑	90↑	24	28
BUN	4.5	4.9	4.8	5.1	6.5↑	4.3	3.6	3.8	4.6	4.7
Cr	47	47	60	74	66	56	63	61↓	52↓	49↓

* a: ahead therapy, p: post therapy

aminotransferase (ALT), aspartate aminotransferase (AST), *r*-glutamyltransferase (*r*GT), blood urea nitrogen (BUN) and creatinine (Cr) in peripheral blood.

3. Discussion

Antitumor antimetabolites have been used for many years for the treatment of different types of cancer. Commonly used antimetabolites include Ara-C and Fa. Although these agents demonstrate clinical efficacy, there are limitations for the use of these drugs due to resistance mechanisms. Resistance of some cells to Ara-C has been attributed to the decreased phosphorylation of Ara-C or the increased degradation of Ara-C by cytidine deaminase. Resistance mechanisms ultimately lead to decreased amounts of active drugs at the desired target site, thus, leading to lower survival rates of patients. Therefore, there is an urgent need for compounds that will overcome resistance mechanisms. Ara-C is a deoxycytidine analog that has been effective in treating several types of hematological cancers, such as leukemia and lymphoma. The drug is transported across cell membranes via a facilitated diffusion nucleoside transporter. Once inside the cell, Ara-C is phosphorylated by deoxycytidine kinase to yield Ara-C monophosphate (Ara-CMP), which is further phosphorylated to its active form, Ara-C triphosphate (Ara-CTP). The initial phosphorylation of Ara-C is the rate-limiting step in the activation of this drug. The active form of Ara-C is a potent inhibitor of DNA synthesis and acts by terminating the growing DNA chain and breaking the DNA strand (Lamba et al. 2007). One limitation in the use of Ara-C is its short plasma half-life because

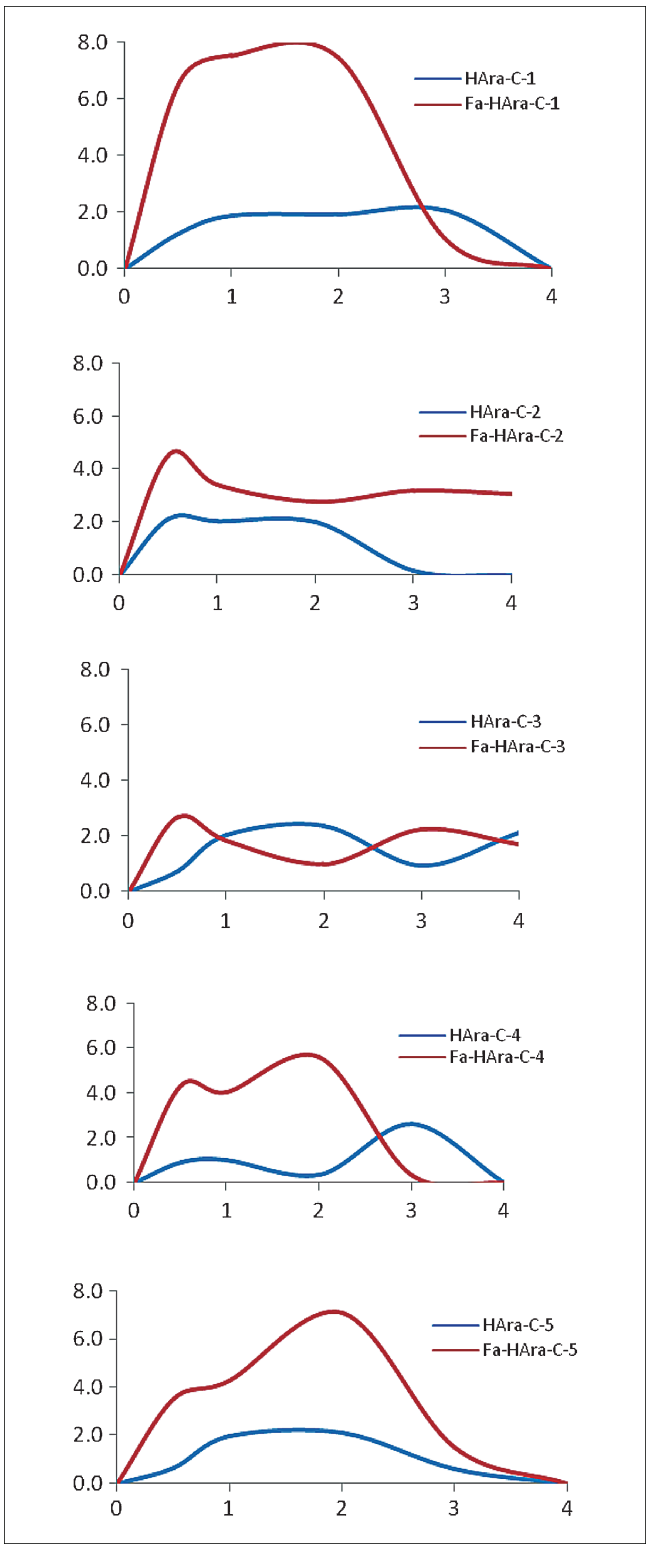

Fig. 1: Ara-C plasma concentration-time profile in 5 self-control patients

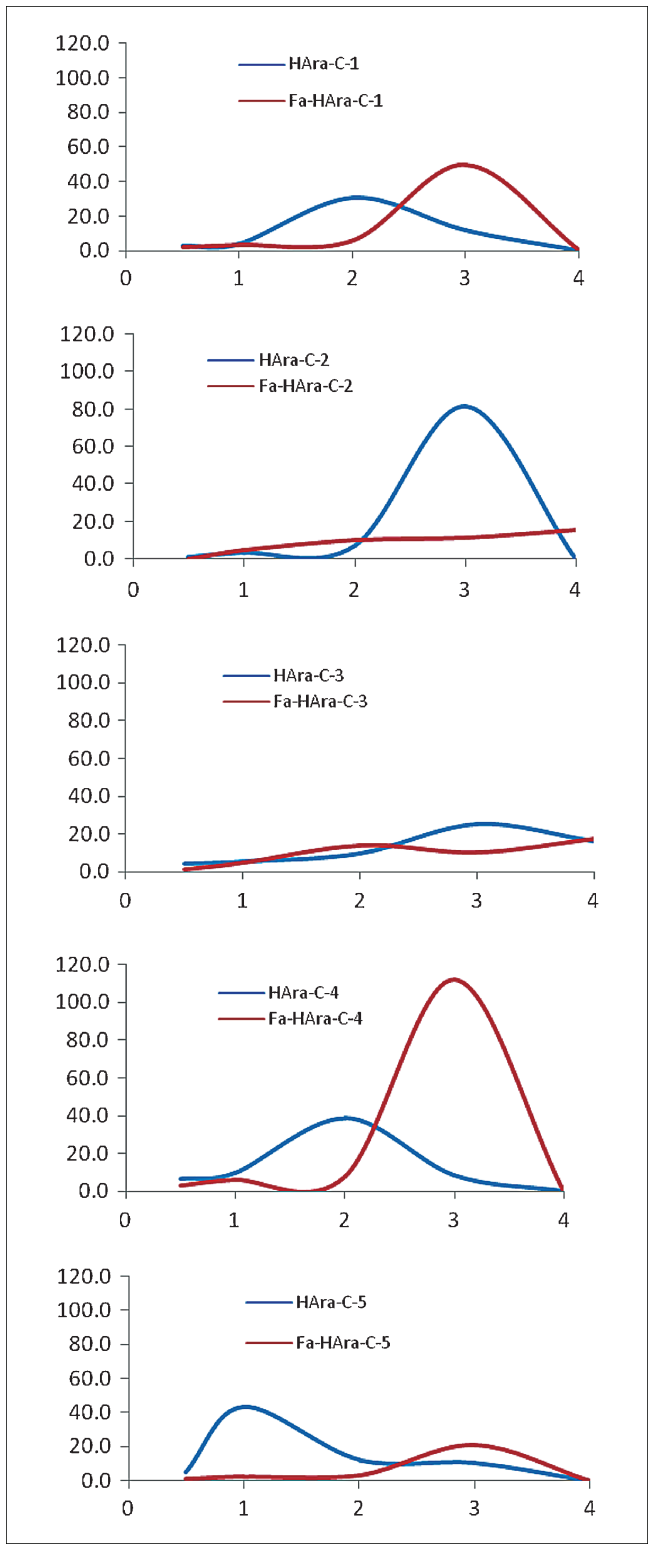

Fig. 2: The ratio of $C_{Ara\text{-}U}$ and $C_{Ara\text{-}C}$ in plasma-time profile in 5 self-control patients

it is deaminated to uracil arabinoside by cytidine deaminase, which is found in high concentrations in the intestine, liver, and kidneys. Cytidine deaminase inhibits the oral bioavailability of Ara-C, thus, Ara-C must be administered intravenously.

In order to increase the concentration of intracellular Ara-C, various modulations of Ara-C metabolism include administering Fa, a purine analog with modest antileukemic activity, before Ara-C. Fa increases intracellular Ara-C accumulation in all phases of the cell cycle by enhancing the activity of the phosphorylating enzyme, deoxycytidine kinase, and by decreasing the nucleoside pool through inhibition of ribonucleotide reductase. The increased Ara-C concentration results in greater leukemic cell mortality compared to administering either Ara-C or Fa alone.

This study compared the data from two groups of five patients. The clinical protocol consisted of Group A being administered with Ara-C only, whereas Group B was administered with Fa and Ara-C. As shown in Fig. 1 and Table 1, the $C_{Ara\text{-}C}$ in patients Fa-HAra-C-1, Fa-HAra-C-2, Fa-HAra-C-4 and Fa-HAra-C-5 were higher than in HAra-C-1, HAra-C-2, HAra-C-4 and HAra-C-5.

An exception is patient No. 3, who did not exhibit a discernible difference between Fa-HAra-C-3 and HAra-C-3. The results also demonstrated that the AUC_{0-4} of Group B and Group A were statistically different ($P = 0.016$). The AUC_{0-4} of Group B was 12.245 ± 3.863 whereas the AUC_{0-4} of Group A was 5.131 ± 0.936. Although the dose of Ara-C in Group B was $2\ g/m^2 \times 2$, its AUC_{0-4} was more than twice that of Group A ($3\ g/m^2 \times 2$).

Figure 2 shows that in plasma, the ratios of $C_{Ara\text{-}u}$ and $C_{Ara\text{-}C}$ in Fa-HAra-C-2 were much lower than in HAra-C-2, except in patient No. 3. The AUC_{0-4} was clearly different between the two groups except for patient No. 3, where there was no observed difference in the ratio curve between Fa-HAra-C-3 and HAra-C-3. The lower the ratio of $C_{Ara\text{-}u}$ to $C_{Ara\text{-}C}$, the longer it takes to transform the active $C_{Ara\text{-}C}$ to the inactive $C_{Ara\text{-}u}$. The data also show that the T_{max} of the ratios in Fa-HAra-C-1, Fa-HAra-C-4 and Fa-HAra-C-5 were lower than in HAra-C-1, HAra-C-4 and HAra-C-5, except for the Fa-HAra-C-2 and HAra-C-2. In patient No. 3, however, the T_{max} of the ratio in Fa-HAra-C-3 was ahead of HAra-C-3. The longer the T_{max} of the ratio of $C_{Ara\text{-}u}$ to $C_{Ara\text{-}C}$, the longer it takes for $C_{Ara\text{-}u}$ to reach the peak concentration. Correspondingly, this means that the transformation of the active $C_{Ara\text{-}C}$ to the inactive $C_{Ara\text{-}u}$ takes longer. Therefore, more of the active form of $C_{Ara\text{-}C}$ is present. Therefore, a change in the T_{max} of the ratio of $C_{Ara\text{-}u}$ to $C_{Ara\text{-}C}$ could be an indicator for evaluating if Ara-C can be combined with Fa.

Table 2 shows that the $C_{Ara\text{-}C}$ in the cerebrospinal fluid was below detection limits within 1 h after the last intravenous treatment in Group A, whereas 5.7%, 4.1%, and 13.8% of $C_{Ara\text{-}C}$ in the plasma collected from Group B permeated into the cerebrospinal fluid. This phenomenon would be beneficial to AML patients, hence, Fa might conduce increased concentrations of $C_{Ara\text{-}C}$ in cerebrospinal fluid.

Table 3 shows that the two study groups are all under the CR process. Although Group B demonstrates a higher AUC_{0-4} with lower doses of Ara-C ($2\ g/m^2 \times 2$), the ADR and bone inhibition in Group B were not more pronounced than in Group A.

Conclusions: The results of this study show that there is a synergistic action between Fa and Ara-C. Fa conduces increased concentrations of Ara-C and AUC in plasma, and increased Ara-C concentrations in cerebrospinal fluid. The change in the T_{max} of the ratio of $C_{Ara\text{-}u}$ to $C_{Ara\text{-}C}$ could indicate whether or not Ara-C combined with Fa. However, these five cases comprise a limited study, hence, more studies are needed to support and prove this hypothesis.

4. Experimental

4.1. Chemicals, reagents, drugs and equipment

Ara-C (No. 77K5050), Ara-U (No. 44K7064), Cortisol (purity > 98%, No. 046K1300), 6β-hydroxycortisol (purity > 98%, No. 113K0631), and dexamethasone (purity > 98% HPLC, No. 077K1050) standards were purchased from SIGMA (USA). Methanol and acetonitrile, HPLC grade, were purchased from Shanghai Chemical Reagent Research Institute (China). Analytical grade sodium dehydrogenate phosphate, trichloracetic acid, ethyl acetate, ether, ammonium sulphate, acetic acid and sodium hydroxide were purchased from Shanghai Chemical Reagent Research Institute (China). Double-distilled water was used for mobile-phase preparation.

Cytosar^{TM} (Ara-C Injection, 0.5 g) was supplied by Pharmacia Italia S.P.A, and Fludara (fludarabine phosphate for injection, 50 mg) was supplied by Intendis Manufacturing SPA.

HPLC was performed with Agilent (Germany) 1100 series HPLC system equipped with G1311A Quatpump, G1379A Degasser, G1329A ALS Injector, G1315B diode-array detector (DAD), G1316A Colcom, and Agilent 1100 ChemStation Software (Software Products, Rev. A.10.02, 2004). Other equipment included a model N-EVAP111 nitrogen evaporation system (Organomation Associates Inc., U.S.A.), a model BS210S electronic balance (Sartorius, P.R.China), a model Vibrax VXR basic (IKA, Germany), a model XW-80A whirl mixer (Shanghai Medical University Instruments Factory, P.R. China, a model 80–2 centrifuge (Shanghai Medical Instruments Surgical Instruments Factory, P.R. China), a model TGL-16G centrifuge (Shanghai Anting Scientific Instruments Factory, P.R.China), a pH meter (Rex Instruments Factory, China), a model DW-HL328 ultra-low temperature refrigerator (–80 °C) (China-tech Meiling, P.R.China), an ordinary refrigerator (Haier Group, P.R.China), and a Pipetman (Gilson Inc., France).

4.2. Clinical protocol

This clinical study was conducted at the Department of Clinical Pharmacology and Hematology, Tongji Hospital, Shanghai, China. The study was approved by the Independent Ethics Committee of Tongji Hospital of Tongji University (Shanghai, China), and was in full compliance with the principles of the 'Declaration of Helsinki'(current revision) and the 'Good Clinical Practice' guidelines. Witten informed consent was obtained from the subjects before the study.

Five patients (3 males, 2 females) diagnosed with AML were in the CR process as a result of two standard protocol therapies. The patients were divided into two groups: Group A was treated intravenously with Ara-C ($3\ g/m^2 \times 2$) for three days. About 1 month later, Group B was treated initially with Fa ($40\ mg/m^2 \times 1$), followed by an intravenous treatment of Ara-C ($2\ g/m^2 \times 2$) four hours later. Treatment with Fa and Ara-C was given for three days.

4.3. Preparation and analysis of blood, cerebrospinal fluid, and urine samples

Blood samples were withdrawn at 0 h, 0.5 h, 1 h, 2 h, 3 h and 4 h after the last infusion of Ara-C. Cerebrospinal and urine samples were withdrawn 1 h and 2 h, respectively, after the last infusion of Ara-C. The heparinized blood was centrifuged to obtain the plasma. The plasma, cerebrospinal fluid and urine samples were all stored at –80 °C until analysis was performed.

The HPLC analysis of Ara-C and Ara-U has been reported previously (Zhang et al. 2006).

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