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ORIGINAL ARTICLE

Pharmacology of intracellular cytosine-arabinoside-5′-triphosphate in malignant cells of pediatric patients with initial or relapsed leukemia and in normal lymphocytes

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Abstract
Purpose The prodrug cytosinearabinoside (ara-C) is widely used in the treatment of acute leukemias. The active drug is the intracellular metabolite cytosine-arabinoside-5′-triphosphate (ara-CTP). The purpose of the present study was to investigate the relation between sensitivity and pharmacokinetic parameters C_{max}, $t_{1/2}$ and AUC of ara-CTP. The obtained results were compared to previous studies.

Experimental design C_{max}, $t_{1/2}$ and AUC of ara-CTP were assessed in leukemic cells of 17 pediatric patients with acute lymphoblastic leukemia (ALL) and in 6 lymphoblastic cell lines and compared with normal lymphocytes of 9 healthy donors by high pressure liquid chromatography (HPLC). The sensitivity of the cells against ara-C was determined by the MTT assay.
Results The intracellular accumulation of ara-CTP was significantly lower in normal lymphocytes (C_{max} 47.7–60.9 pmol/10^6 cells) compared to leukemic cell lines (C_{max} 11–1128 pmol/10^6 cells) and leukemic cells of our patients (C_{max} 85.9–631 pmol/10^6 cells). Similar results were found for the AUC. There was no significant difference between initial and relapsed leukemias in our small cohort. A correlation between sensitivity in terms of IC_{50} values and the intracellular ara-CTP accumulation was observed in cell lines, but not in leukemic cells and normal lymphocytes from healthy donors.
Conclusions Pharmacokinetic parameters varied tremendously in leukemic cells in contrast to normal lymphocytes without a difference in sensitivity. It is worthwhile to compare literature data to assess an optimal dosage of ara-C in pediatric patients.

Keywords Ara-C · Pharmacokinetics · Ara-CTP · High pressure liquid chromatography · Acute lymhoblastic leukemia

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Introduction

The prodrug cytosinearabinoside (ara-C) is one of the most effective agents in the treatment of acute lymphoblastic and myelogenous leukemia (ALL, AML)

[40, 54]. Ara-C is administered at different doses: low, conventional and high dose. Administration of a high dose of ara-C augmented remission rates but due to severe side effects no improvement in the overall survival could be obtained [4]. The ara-C therapy may be improved if the optimal dose is selected according to the pharmacokinetics of the intracellular active metabolite of ara-C, cytosinearabinoside 5′-triphosphate (ara-CTP).

The rate limiting step in ara-C metabolism is the phosphorylation to ara-C monophosphate which is catalysed by the deoxycytidine kinase (dCK) [32, 41]. The capacity of the cells to accumulate and retain ara-CTP seems to play a pivotal role for the cytotoxic effect of ara-C [14, 48]. Moreover, it has been shown that ara-C directly leads to apoptosis [26]. The amount of intracellular ara-CTP is influenced by the extracellular concentration of ara-C, the ara-C transport through the cell membrane, deamination and the rates of phosphorylation and dephosphorylation.

Miscellaneous mechanisms of cellular resistance to ara-C have been proposed based on in vitro and in vivo studies: low accumulation of the active metabolite ara-CTP due to reduced influx of ara-C by nucleoside transporter [19, 59], reduced phosphorylation by the dCK or even the abscence of the dCK. Furthermore, high intracellular levels of dNTPs, being competing metabolites, lead to an inhibition of ara-C phosphorylation [3]. To prevent high levels of dNTPs and, consequently, augment the accumulation of ara-CTP, protocols have been established using the sequential administration of a purine nucleoside analogue and ara-C [22]. Further drug combinations are under investigation in order to overcome or at least modulate ara-C resistance. Another approach to overcome resistance is to administer high dose ara-C (HIDAC) regimens. Several trials provide evidence of a dose-response effect [4, 14, 24].

There are investigations which indicate a relationship between accumulation and retention of ara-CTP on one the hand and clinical parameters like remission and response to ara-C on the other [42, 45, 48]. A study with leukemic cells taken from pediatric patients showed that ara-CTP retention was inversely correlated with the risk groups defined by the ALL-BFM treatment protocols [51]. However, the precise mechanisms by which ara-C induces a cytotoxic effect are still unknown. Comparisons between normal and malignant cells as well as between pediatric patients with initial and relapsed leukemia regarding pharmacokinetics and sensitivity are rare in the literature. The aim of the present study was to determine differences among these groups.

Materials and methods

Materials

Ara-CTP, tartaric acid and other nucleotides were obtained from Sigma (Deisenhofen, Germany). ACN was purchased from Zinsser (Frankfurt, Germany); KH_2PO_4 and H_3PO_4 from Baker (Gross-Gerau, Germany); Tetrabutyl-ammonium-phosphate from Waters (Eschborn, Germany) and MTT from Serva (Heidelberg, Germany).

Cell lines

Molt-4, a human T-cell leukemia cell line was obtained from the German collection of microorganisms and cell cultures (DSMZ). H9 is of cutaneous T-lymphocyte origin and was purchased at the American type culture collection (ATCC). The cell lines KFB-1 and KFB-2 are of B-lypmhoblastic (B-ALL), the others of T-lymphoblastic (T-ALL) origin, all derived from relapsed patients in our hospital [30]. Molt-4r has been selected for resistance to ara-C by exposure to stepwise increasing ara-C concentrations from 0.5 to 24.3 ng/ml (0.1 μM). All cells were cultured in RPMI 1640 medium (Biochrom, Berlin, Germany) supplemented with 10% fetal calf serum (Biopro, Karsruhe, Germany), 1% penicillin (Seromed, Berlin, Germany), 1% streptomycin (Grünenthal, Stolberg, Germany) and 1 mM L-glutamine (Seromed, Berlin, Germany) at 37°C, 5%CO_2 exponential growth phase.

Patients and healthy donors:

Pharmacokinetic investigations were performed in bone marrow blasts of 17 pediatric patients (age range 0.3–18 years) with ALL (initial $n = 10$ and relapsed $n = 7$ including cALL, pre-pre-B-ALL as well as T-ALL; Table 1) and in normal lymphocytes of healthy donors ($n = 9$) after informed consent of the parents or the patients. Patients with an initial ALL were treated according to the ALL-BFM95/2000 protocol; both including ara-C treatment at a concentration of 75–2,000 mg/m^2 depending on the risk group [34, 52, 53]. Patients with a relapse of leukemia received HIDAC therapy according to the ALL-REZ BFM95/2000 protocol. The leukemic cells of patients with an initial leukemia were still ara-C naïve, while leukemic cells of patients with a relapse may have acquired ara-C resistance. Normal lymphocytes from healthy donors were directly isolated (3/9) or from buffy coats (6/9). Mononuclear cells were separated by Ficoll density gradient centrifugation.

Table 1 Pharmacokinetic parameters C_{max}, $t_{1/2}$ and AUC of ara-CTP

cell lines patients	Diagnosis rel (relapsed) init (initial)	C_{max} 10 µM + [pmol /10^6 cells]	$t_{1/2}$ • [h]	AUC* 0-4 [pmol·h/10^6cells]	AUC* 4-∞ [pmol·h/10^6cells]	AUC* total [pmol·h/10^6cells]
Molt-4	T-ALL	1128.2 ±23	3.0 $^{+0.1}_{-0.1}$	2875	4940	7815
H 9	T-ALL	225.8 ±12	1.9 $^{+0.1}_{-0.1}$	563	583	1146
KFB-1	B-ALL	86.5 ±9.6	1.6 $^{+0.2}_{-0.2}$	235	200	435
KFB-2	B-ALL	640.4 ±24	5.1 $^{+0.1}_{-0.1}$	1144	5012	6156
KFT-1	T-ALL	69.3 ±10				
Molt-4r	T-ALL resistant	10.7 ±3.8				
1	pre-pre-B-ALL init	105.1	7.3	210	1109	1319
2	pre-pre-B-ALL init	109.2 ± 17.9	6.7 $^{+0.5}_{-0.5}$	218	1059	1277
3	cALL init	237.0 ± 29.5				
4	cALL init	215.2 ± 44.7				
5	cALL init	181.7 ± 11.8	4.9 $^{+0.7}_{-0.5}$	363	1289	1652
6	cALL init	155.7 ± 7.8				
7	cALL init	318.5 ± 51.0				
8	cALL init	301.3 ± 42.1	2.8 $^{+1.1}_{-0.6}$	603	1238	1841
9	cALL init	133.9	3.2 $^{+2.5}_{-1.0}$	268	626	894
10	T-ALL init	136.9 ± 18.0				
11	pre-pre-B-ALL rel	185.3				
12	cALL rel	161.0 ± 2.5	4.7 $^{+1.1}_{-0.7}$	322	1099	1421
13	cALL rel	85.9 ± 11.8	1.3 $^{+0.9}_{-0.4}$	172	157	329
14	cALL rel	206.3 ± 20.5	8.9 $^{+1.4}_{-1.0}$	413	2640	3053
15	cALL rel	631.0 ± 93.7	2.3 $^{+0.6}_{-0.4}$	1262	2069	3331
16	T-ALL rel	90.8 ± 26.7				
17	T-ALL rel	591.1 ± 38.6	11.2 $^{+20}_{-4.4}$	1182	9533	10715
L1	normal lymphocytes	51.4 ± 1.4	2.6 $^{+0.3}_{-0.2}$	119	202	312
L2	normal lymphocytes	58.6 ± 3.3	2.1 $^{+0.2}_{-0.1}$	129	170	304
L3	normal lymphocytes	51.8 ± 2.1	2.9 $^{+0.5}_{-0.3}$	126	218	342
L4	normal lymphocytes	60.9				
L5	normal lymphocytes	50.8 ± 6.2				
L6	normal lymphocytes	59.3 ± 3.8				
L7	normal lymphocytes	47.7 ± 2.0				
L8	normal lymphocytes	54.2 ± 12.6				
L9	normal lymphocytes	59.6 ± 7.3				

1-17 Lymphatic bone marrow blasts of patients, L1-9 normal lymphocytes from healthy donors

+ Mean ± SD, to determine ara-CTP plateau level, 10 ml cell suspension ($1{\cdot}10^6$ cells/ml) was incubated for 4 h with 10 µM extra cellular ara-C

• To determine half-life of ara-CTP, at the end of the 4 h incubation period cells were washed with fresh medium to remove drug. Then the cells were reincubated in medium without ara-C and extracted after 1, (2), 3, (4), 16 and (18) h

* AUC_{total}, AUC_{0-4} and $AUC_{4-\infty}$ were calculated as described in "pharmacokinetics and statistical methods"

Each value is the mean of four experiments run in triplicate in cell lines and the mean of 1–2 experiments run in duplicate in lymphocytes and in leukemic cells of the patients

Formation and determination of ara-CTP

The intracellular formation of ara-CTP is saturated at a concentration of 10 µM ara-C after 4 h incubation as described previously [30]. Hence, all experiments were performed with 10 µM ara-C. 10 ml cell suspension was kept at a density of 5×10^5 or 1×10^6 cells/ml. Control cells were incubated without ara-C. At the end of the incubation time with ara-C, cells were washed twice with PBS in order to remove excess drug. All steps of extraction procedure were performed on ice. After the supernatant was discarded, the cell pellet was resuspended in 300 µl PBS containing 100 mM tartaric acid and gently vortexed. After 100 µl were removed

for cell counting, cells were extracted using 100 µl buffer solution. The extraction buffer was the mobile phase of the HPLC procedure, which was adjusted to pH 2.35 with H_3PO_4. Following vigorous vortex-mixing, centrifugation at 1,000 *g* for 10 min at 4°C, the supernatant was collected. Ara-CTP was stable after storage of the sample for 9 months at –20°C at a pH of 2.6–2.7. This pH was adjusted by adding 100 µl of the buffer (pH 2.35) to the cell pellet. The number of total cells was counted with an electronic blood cell counter.

HPLC determination of ara-CTP in cellular acid extracts

The ara-CTP concentration was quantified by a HPLC method (LDC/Milton Roy system) as described previously [5, 10, 30]. In brief, a reversed phase ODS2 C18-column, 5 µm, 4.6 mm × 25 cm (Grom, Herrenberg, Germany) was used with a solution of 0.1 M KH_2PO_4, 5 mM tetrabutylammoniumphosphate and 0.5% acetonitrile as the mobile phase, which was adjusted to pH 2.6–2.7 with H_3PO_4. During the initial 16 min the flow rate was 0.8 ml/min and increased linearly to 1.8 ml/min until the 18th min and maintained until the end of separation at the 25th min. The detection limit of ara-CTP was 5 pmol using a UV-detector LDC spectro monitor D (LDC, Darmstadt, Germany) with a wavelength of 280 nm. The amounts of ara-CTP in the cell extracts were measured by integrating the area under the peak in the cell lysate compared to known concentrations of external standards. The AUC was calculated by the trapezoid method. The concentration of ara-CTP in cells was calculated by dividing the amount of ara-CTP in each sample analysed by the number of cells in the extract. Values were expressed in pmol/10^6 cells. The intra assay variation coefficient was <3% (n = 15), and the day to day variation was <5% (n = 13) for a 25 µl injection of 1 µg/ml ara-CTP-standard. The precision of ara-CTP determination in cell extracts of all studied cell lines was <10% for an incubation with 10 µM ara-C for 4 h (n = 10).

To compare the intracellular ara-CTP concentrations expressed in pmol/10^6 cells with values expressed in mol/l, the average volume of a lymphoblast/myeloblast must be considered. Leukemic lymphoblasts in children have a mean volume of 200 ±50 µm^3 [58], hence 1×10^6 leukemic cells have a volume of 2×10^{-4}ml. It follows: 1 pmol/10^6 cells = 5×10^3 pmol/ml = 5 µM. Leukemic myeloblasts in adults have a mean volume of 275 ± 125 µm^3 [46], hence 1 pmol/10^6 cells = 3.63×10^3 pmol/ml = 3.63 µM.

MTT-assay

The sensitivity of the cells to ara-C was determined in terms of the IC_{50} by the MTT-assay as described previously [30]. Briefly, cells were incubated on 96 well plates with different concentrations of ara-C for 4 days (density of cell lines 2.5–5 × 10^5/ml; of leukemic cells and lymphocytes 2×10^6/ml). The outer wells were filled with medium only and used for blanking the reader. For control, cells were cultured drug-free. After the end of incubation with ara-C 10 µl of the MTT-solution (5 mg/ml in PBS) was added to each well followed by another incubation period of 4 h. Finally, the enzyme reaction was stopped by the addition of 150 µl 0.04 M HCl in isopropanol and the extinction was measured on a multiscan ELISA plate spectrophotometer using a test wavelength of 540 nm and a reference wavelength of 630 nm. At the beginning and the end of the experiment viability was checked by trypan blue exclusion.

Pharmacokinetics and statistical methods

During the in vitro incubation of the cell lines with 10 µM ara-C the maximum of ara-CTP formation was reached within 3–4 h. For that reason C_{max} of ara-CTP was determined after 4 h incubation period with the saturation dose of 10 µM ara-C. The half-life $t_{1/2}$ of ara-CTP elimination was estimated according to the method of semilogarithmic regression. The dispersion of $t_{1/2}$ was assessed with the standard deviation of the elimination constant as follows:

$$\Delta t_{up} = \frac{\ln 2}{k_{el} - s_{k_{el}}} - t_{1/2}$$

$$\Delta t_{down} = \frac{\ln 2}{k_{el} + s_{k_{el}}} - t_{1/2}$$

Δt_{up} = upper dispersion of the half-life
Δt_{down} = inferior dispersion of the half-life

$t_{1/2}$ = half-life of elimination
k_{el} = elimination constant
s_{kel} = standard deviation of the elimination constant

For the calculation of the area under the concentration curve, AUC_{total} was subdivided into the area under the curve from 0 to 4 h (AUC_{0-4}) and the area under the curve during ara-CTP elimination ($AUC_{4-\infty}$). The AUC_{0-4} for the accumulation of ara-CTP was calculated according to the trapezoid method. The $AUC_{4-\infty}$

for the elimination of ara-CTP was determined by $t_{1/2}$ and C_{max} as well as by applying the trapezoid method. If $AUC_{4-\infty}$ was calculated with the trapezoid method, $t_{1/2}$ was used for the extrapolation to ∞. AUC_{total} was the sum of AUC_{0-4} and $AUC_{4-\infty}$.

Differences in the pharmacokinetics of ara-CTP between leukemic cells and normal lymphocytes, and between leukemic cells of patients with an initial and with a relapsed leukemia were analysed using the Mann–Whitney *U*-test and the rank dispersion test of Siegel and Tukey [48].

Results

Differences in the pharmacokinetics of ara-CTP in lymphoblasts and in normal lymphocytes

Ara-CTP maximum concentration

The patient with the lowest C_{max} of ara-CTP accumulated 85.9 ± 11.8 pmol/10^6 cells (mean ± SD). The highest accumulation in leukemic bone marrow cells of patients was 631.0 ± 93.7 pmol/10^6 cells (Table 1). The median of all ALL patients was 181.7 pmol/10^6 cells after 4 h in vitro incubation with 10 μM ara-C. Similar ara-CTP concentrations were found in lymphoblastic cell lines ranging from 10.7 ± 3.8 (Molt-4r) up to 1128.2 ± 22.7 pmol/10^6 cells (Molt-4). Hence, the ara-CTP formation in the resistant cell line Molt-4r was about 100 times lower than in the sensitive cell line Molt-4. In contrast to the great variability in the amount of ara-CTP formation in malignant cells, ara-CTP accumulation in lymphocytes of the nine healthy donors was rather similar (Fig. 1a). Moreover, the median of ara-CTP formation was significantly (3 times) lower in lymphocytes than in malignant bone marrow cells of patients as determined by the Mann–Whitney *U*-test ($U_1 = 0, U_2 = 153, U_{17;9:0.002} = 21$). In normal lymphocytes the lowest C_{max} was 47.7 ± 2.0 and the highest 60.9 ± 3.3 pmol/10^6 cells (the median of the 9 healthy donors was 54.2 pmol/10^6 cells). Anyhow, in leukemic cells of three patients with a relapsed leukemia and in two cell lines the ara-CTP maximum concentration was only 1.3–1.6 times higher than in normal lymphocytes.

Neither was a significant difference found in ara-CTP formation between normal lymphocytes of buffy coats and those directly isolated from healthy donors, nor between lymphoblasts of patients with initial leukemia and relapsed leukemia. Albeit the range of C_{max} in patients with initial leukemia appeared to be smaller than in patients with relapsed leukemia (Fig. 1a), no significant difference was found for the dispersion of C_{max} in the two groups determined by the rank dispersion test of Siegel and Tukey. Comparison of C_{max} in patients and in cell lines did not show a statistically significant difference either.

In two patients the ara-CTP concentration was determined in both leukemic peripheral and bone marrow cells. The results were very similar (patient 1: 161 and 173 pmol/10^6 cells; patient 2: 105 and 110 pmol/10^6 cells).

Half-life of ara-CTP

The elimination kinetics of ara-CTP was monoexponential with a $t_{1/2}$ of 1.3–11.2 h in leukemic cells of patients, 1.6–5.1 h in cell lines and 2.1–2.9 h in normal lymphocytes (Table 1). There was neither a significant difference between normal lymphocytes and patients, nor between patients and cell lines. Moreover, no significant difference was found in the $t_{1/2}$ values between patients with an initial and a relapsed leukemia.

Area under the concentration curve

The AUC values of ara-CTP were small and varied very little in normal lymphocytes (Table 1 and Fig. 1b). Patients with initial leukemia had higher AUC values with some variations. In contrast, AUC of ara-CTP in relapsed patients and in cell lines showed great variations with a range of 329–10,715 pmol h/10^6 cells and 435–7,815 pmol h/10^6 cells, respectively. AUC values in normal lymphocytes were significantly different from AUC values in lymphoblastic cells of patients ($U_1 = 0, U_2 = 30, U_{10;3:0.01} = 0$). But there was no significant difference between AUC values in patients with an initial and with a relapsed leukemia. Nor was there a significant difference between these two groups regarding the dispersion of AUC values in these two groups determined by the rank dispersion test of Siegel and Tukey, even though Fig. 1b shows a smaller spreading of AUC values in lymphoblasts of patients with an initial leukemia than with a relapsed leukemia.

Relationship of sensitivity to pharmacokinetics of ara-C

Ara-CTP plateau level C_{max} and sensitivity of the cells to ara-C correlated well in 5/6 cell lines (Fig. 2a). The correlation coefficient was $r = -0.967$ without the value of KFB-1 and $r = -0.697$ including the value of KFB-1. In the second case r was not significantly different from zero—the value of KFB-1 was out of the 95% confi-

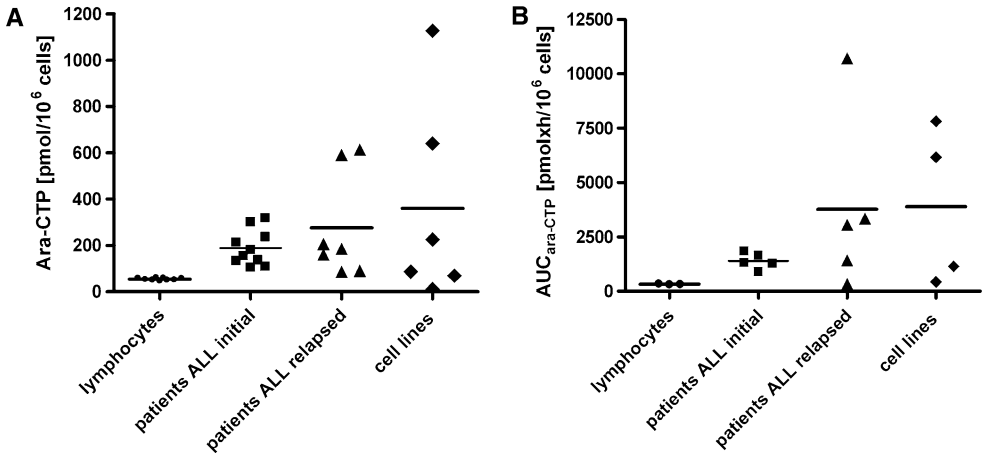

Fig. 1 Ara-CTP and AUC formation in leukemic cells and normal lymphocytes. The spreading in ara-CTP accumulation is shown for each cell type after a 4 h incubation with 10 μM ara-C (**a**). Ara-CTP accumulation in normal lymphocytes was significant lower and very uniform compared to higher values and a great variability in leukemic cell lines and leukemic cells of pediatric patients. No significant difference was found in leukemic cells of initial leukemia compared to relapsed disease. Similar results could be shown for the AUC of ara-CTP (**b**)

dence interval. As described previously, omitting the outlier KFB-1 may be justified on the grounds of an increased activity of unspecific phosphatases in this cell line [30].

There was no correlation between ara-CTP plateau levels and sensitivity of the cells to ara-C in lymphoblastic bone marrow cells of patients and in normal lymphocytes (Fig. 2b). The correlation coefficient was $r = -0.292$ for normal lymphocytes and $r = -0.608$ for leukemic bone marrow cells of patients. Similar results were found for sensitivity and AUC values. The correlation in cell lines reached $r = -0.999$ without the outlier KFB-1 ($r = -0.521$ including KFB-1). In normal lymphocytes the correlation coefficient was $r = -0.564$ and in leukemic bone marrow cells $r = -0.744$ (data not shown).

There was no correlation between half-life of ara-CTP and sensitivity of the cells to ara-C in cell lines, normal lymphocytes and leukemic bone marrow cells of patients (data not shown).

Discussion

In this study the pharmacokinetics of ara-CTP in normal lymphocytes and leukemic cells of pediatric patients were compared. To investigate differences, an extracellular concentration of 10 μM ara-C was selected at which formation of ara-CTP was saturated in all cells. This is in accordance with our previous findings and other studies, in vivo and in vitro [29, 30, 43, 48]. The fact that the activity of the ara-C phosphory-

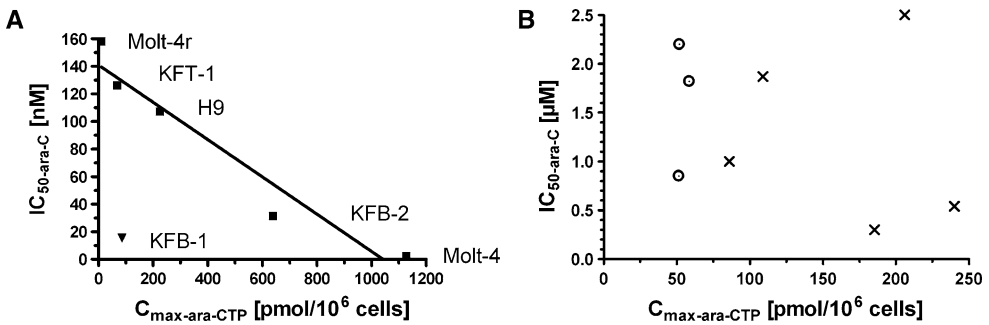

Fig. 2 Relation between ara-CTP accumulation and drug sensitivity against ara-C. There is a high correlation between intracellular accumulation of ara-CTP and sensitivity against ara-C in leukemic cell lines when the outlier KFB-1 is omitted (**a**). In contrast, in leukemic cells of patients (×) and in normal lymphocytes (*open circle*) there was no correlation between ara-CTP accumulation and sensitivity against ara-C (**b**)

lating enzyme dCK is saturated within a range of 7–10 μM ara-C might be considered as an explanation. Because of our short incubation period of 4 h, deamination as the overlying inactivation mechanism of ara-C to arabinosyluracil should not have affected the results. Besides, the enzyme cytidine deaminase is only present at low levels in plasma. The major part of deamination occurs in the liver, spleen and kidney [8, 9].

The present work exhibited great interpatient variations in the pharmacokinetic parameters C_{max}, $t_{1/2}$ and AUC of ara-CTP. The ratio between the highest and the lowest maximum concentration as well as between the highest and lowest half-time value was nine. The largest AUC value was 30 times higher than the lowest. Analogous results were reported in the literature by various studies with a focus on leukemic cells of adult patients (Table 2). To give a review for comparison of the clinical and experimental studies about the pharmacokinetics of ara-CTP, we translated all values into the unit of pmol/10^6 cells as described in Materials and methods.

In the small cohort used in the present study, the range of the pharmacokinetic parameters of ara-CTP seemed to be lower in the lymphoblastic leukemic cells of patients with an initial than with a relapsed leukemia but no statistical significance was reached. We also investigated the accumulation of ara-CTP in myelogenous cells but only of two patients (in relapse) since AML is less common in children compared to ALL. C_{max} values were small (71.5 ± 15.8 and 240.2 ± 109 pmol/10^6 cells) but due to the small sample size and the observed great variability in patients hardly interpretable (data not shown).

In contrast to the considerable variability of ara-CTP pharmacokinetics in leukemic cells, C_{max}, AUC and $t_{1/2}$ values of normal lymphocytes have a small margin of deviation. Furthermore, the median of ara-CTP maximum concentration is significantly lower (3 times) in normal lymphocytes than in leukemic cells. Simultaneously, there were two cell lines and three patients with ALL exhibiting similar C_{max} and AUC values as normal lymphocytes. In addition, we found a similar small C_{max} of ara-CTP in 1 of 2 pediatric patients suffering from a relapsed AML (data not shown). It should be noted that even though we could show a reduced accumulation of ara-CTP in normal lymphocytes they were similarly sensitive as leukemic cells of patients expressed in IC_{50} values. Normal lymphocytes as being mature cells in contrast to immature leukemic cells might contain less dCK since they have lost their self-renewal capacity. This would result in reduced accumulation of ara-CTP. Simultaneously, mature lymphocytes may have not acquired mechanisms for escape from DNA repair and apoptosis. Anyhow, a recent study by Hubeek et al. showed that normal bone marrow cells were significantly more resistant to ara-C than leukemic blasts from patients [28].

In cell lines we could only demonstrate a correlation between sensitivity and C_{max} when KFB-1 was considered as an outlier. If KFB-1 is included there will be no correlation in cell lines as there is no correlation in leukemic cells of patients and in normal lymphocytes. KFB-1 is highly susceptible to ara-C without strongly accumulating ara-CTP. But may be ara-CTP was only quickly dephosphorylated and, therefore, impossible to detect even though a sufficiently cytotoxic amount was already incorporated into the DNA. To become susceptible to ara-C, cells need a certain amount of intracellular ara-CTP. Beyond that other cellular mechanisms (phosphatases, DNA incorporation, repair of DNA, induction of apoptosis, etc.) play an important role influencing laboratory and clinical results.

At an extracellular concentration of 10 μM ara-C, which correlates with an intermediate and high-dose ara-C therapy regimen the formation of ara-CTP was saturated. It is striking that some studies [24] underline the enhanced efficacy of HIDAC, although we showed a saturation generally reached with intermediate doses of 250 mg/m^2 [43, 44, 47], alluding there may be additive effects above saturation. On the other hand it has been shown that an intensification of ara-C doses do not necessarily correlate with a therapeutic gain but only with increased toxicity [4, 49]. In most studies the investigation was confined to a dose-response effect without measuring the intracellular accumulation of ara-CTP or the intracellular ara-CTP levels were monitored without analysing the therapeutic outcome [7, 31, 60]. Hence it is hard to judge from these data if HIDAC augments the wanted cytotoxic effects by simply raising ara-C plasma levels or if there really is a correlation between intracellular ara-CTP levels and response of the patients. Furthermore, the doses that are administered vary widely from protocol to protocol (Table 2). The term intermediate dose of ara-C has been used in a variety of different meanings and there is no clear definition with regard to dose or dose rate [31].

In order to elevate intracellular ara-CTP levels, a combination of ara-C with purine nucleoside analogues has been introduced. Suitable drugs are fludarabine (FLA regimen), cladribine (CLA regimen) and, more lately, clofarabine. Even though the data are conflicting several in vitro and in vivo studies by Gandhi et al. demonstrated the feasibility of augmenting ara-CTP

Table 2 Pharmacokinetic parameters of ara-CTP

Cells	Adult (A) child (C)	PB/BM	Incubation/ infusion time* [h]	Drugs	*n*	C_{max} ara-CTP range (m:median) (x: mean) [pmol/10^6 cells]	$t_{1/2}$ ara-CTP range (m: median) (x: mean) [h]	AUC ara-CTP range (m: median) (x: mean) [pmol × h/10^6 cells]	Source
Cell lines									
ALL, AML	–	–	1.5	1 μM ara-C	12	2–11	–	–	Tanaka [57]
ALL, AML	–	–	4	1 μM ara-C	3	20–90	0.5–2.6	–	Momparler [33]
ALL, AML	–	-	4	0.1 μM ara-C	3	3.6- 35.5	0.5–3.4	–	Abe [1]
ALL, AML	–	–	3	10 μM ara-C	3	250–1400	–	–	Zittoun [61]
AML	–	–	24	1 μM ara-C 10 μM ara-C	2	(x: 36) (x: 194)	–	–	Noordhuis [37]
AML	–	–	1	10 μM ara-C	60	(x: 38.8)	–	–	Freund [17]
ALL	–	–	4	10 μM ara-C	6	11–1128 (m:152.6)	1.6–5.1 (x:2.9)	435–7815 (x:3888)	present work
Patients in vivo									
AML	A	PB	2 (96)	3(6) g/m^2 ara-C	147	12–348 (m:92) #	1.3–13.3 (m:2.8)	59–4336 (m:484) #	Estey [15]
AML	A	BM	96	3(6) g/m^2 ara-C	7	9.9–92 (x:30.3)	–	–	Rustum [50]
AML	A	PB	24	0.02 g/m^2 ara-C	4	(x:0.28) #	–	(x:6.6) #	Yamauchi [60]
AML	A	PB	2	0.07 g/m^2 ara-C	4	(x:0.85) #	(x:4.5)	(x:4.6) #	Yamauchi [60]
AML	A	PB	24	0.07 g/m^2 ara-C	3	(x:1.0) #	–	(x:25.4) #	Yamauchi [60]
AML	A	PB	3	3 g/m^2 ara-C	10	41.4–300 (m:140.1)	1.6–7.6 (m:2.4)	231–1820 (m:975)	Hiddemann [25]
AML	A	PB	4 (2)	0.5 g/m^2/h ara-C	6	63–187 (x: 111)	–	–	Gandhi [23]
ALL	C	PB	3	3 g/m^2 ara-C	8	52–234 (m:108) #	2.9–10.8 (m:7.4)	215–2950 (x:1129) #	Avramis [2]
ALL	C	PB	96	3.5(5) g/m^2 ara-C	6	13–380	–	–	Ochs [38]
AML, MDS	A	BM	4	2 g/m^2 ara-C + 5 μg/kg/m^2 G-CSF	6	6.1–87.5	–	–	Ossenkoppele [39]
AML, MDS	A	BM	4	2 g/m^2 ara-C + 5 μg/kg/m^2 G-CSF + 25 mg/m^2 fludara	6	107–337	–	–	Ossenkoppele [39]
AML	A	PB	4	2 g/m^2 ara-C	5	91–258 #	1.8–4.0 (x:2.8) #	430–1909 (x:905) #	Gandhi [22]
AML	A	PB	4	2 g/m^2 ara-C + 30 mg/m^2 fludara	5	172–381 #	2–4.3 (x:3) #	1022–2959 (x:1580) #	Gandhi [22]
AML	C	BM	2	0.5 g/m^2 ara-C	17	(m: 25)	–	–	Crews [12]
AML	C	BM	2	0.5 g/m^2 ara-C + 9 mg/m^2 2-CdA	17	(m: 36)	–	–	Crews [12]
AML	A	PB	2	1 g/m^2 ara-C	9	41–212 (m: 69) #	–	–	Gandhi [21]
AML	A	PB	2	1 g/m^2 ara-C + 12 mg/m^2 2-CdA	9	61–220 (m: 121) #	–	–	Gandhi [21]
AML, ALL	A	PB	2	1 g/m^2 ara-C	6	29–103 (x: 52) #	–	–	Faderl [16]
AML, ALL	A	PB	2	1 g/m^2 ara-C + 15–40 mg /m^2 clofarab	6	29–90 (x: 62) #	–	–	Faderl [16]
Patients in vitro									
AML, ALL	A	BM	0.5	10 μM ara-C	69	12–125	0.9–3.5	–	Muus [35]
AML	A	PB	1	1 μM ara-C	11	10–166	–	–	Jamieson [29]
ALL	A	PB	1	1 μM ara-C	9	20–110	–	–	Jamieson [29]

Table 2 continued

Cells	Adult (A) child (C)	PB/BM	Incubation/ infusion time* [h]	Drugs	*n*	C_{max} ara-CTP range (m:median) (x: mean) [pmol/10^6 cells]	$t_{1/2}$ ara-CTP range (m: median) (x: mean) [h]	AUC ara-CTP range (m: median) (x: mean) [pmol × h/10^6 cells]	Source
ALL	C < 12 m	PB/	24	1 μM ara-C	15	20–156 (m: 67)	–	–	Stam [55]
	C > 12 m	BM			8	< 30–140 (m: 28)			
AML	A	PB/	20	10 μM ara-C	5	57–412 (m: 172)	Low retention	–	Elgie [13]
		BM			11	80–412 (m: 191)	High retention		
AML	A	PB	24	1 μM ara-C	10	ND–32	–	–	Noordhuis [37]
				10 μM ara-C	10	5–246			
AML	A	BM	18	4 μM ara-C	18	(x: 20)	–	–	Braess [6]
				20 μM ara-C	18	(x: 40)			
ALL	C	BM	4	10 μM ara-C	17	86–631 (181.7)	1.3–11.2 (m:4.8)	329–10715 (m:1604)	present work

Conversion formula for leukemic lymphoblasts 1 pmol/10^6 cells = 5×10^3 pmol/ml = 5 μM [58], leukemic for myeloblasts 1 pmol/10^6 cells = 3.63×10^3 pmol/ml = 3.63 μM [46]; values have been converted as described in "Materials and methods"

* Incubation or infusion time, *ND* not detectable

accumulation [11, 12, 16, 21, 22, 54, 56]. The rationale for the development of such a combination chemotherapy is the inhibition of the ribonucleotide reductase by purine nucleoside analogues resulting in lower levels of deoxynucleotides. This includes a decline in the intracellular deoxycytidine triphosphate (dCTP) pool. Due to a feedback mechanism dCTP inhibits the dCK, thus preventing phosphorylation of ara-C. Vice versa low levels dCTP prevents inhibition and promotes accumulation of ara-CTP [18].

Regarding clinical parameters, especially overall survival, results were not as promising as expected and similar to results previously achieved with HIDAC. Therefore, another agent, the growth factor G-CSF, has been introduced the FLAG and CLAG regimen for fludarabine and cladribine, respectively in order to make leukemic cells more susceptible. The mechanism of action of G-CSF is not elucidated completely but it is assumed that it pushes cells to the S phase of the cell cycle where they are most vulnerable to ara-C.

A study using AML cells of children in vitro could not show an increased cytotoxicity with the pre-exposure of G-CSF before incubation with fludarabine and ara-C although the results indicate a benefit of using the combinations G-CSF with ara-C and fludarabine with ara-C [27]. Recent clinical trials comparing FLA versus FLAG demonstrate the capacity of FLAG to enhance ara-CTP accumulation but clinical outcome in terms of complete remission, overall survival, event and disease free survival was not significantly improved [36, 39].

In summary, there was no significant difference observable between leukemic cells of patients (initial and relapsed leukemia) and cell lines with regard to maximum concentration, area under the curve and half-life. In contrast, AUC and C_{max} in normal lymphocytes were significantly smaller than in patient leukemic cells. Nevertheless, normal lymphocytes did not show reduced sensitivity. Further studies are desirable to reveal differences between normal lymphocytes and malignant cells in more recent therapy regimens as FLA/CLA and FLAG/CLAG in order to reduce toxicity while preserving efficacy.

References

1. Abe I, Saito S, Hori K, Suzuki M,Sato H (1982) Role of dephosphorylation in accumulation of 1-beta-D-arabinofuranosylcytosine 5′-triphosphate in human lymphoblastic cell lines with reference to their drug sensitivity. Cancer Res 42:2846–2851
2. Avramis VI, Biener R, Krailo M, Finklestein J, Ettinger L, Willoughby M, Siegel SE, Holcenberg JS (1987) Bio-

chemical pharmacology of high dose 1-beta-D-arabinofuranosylcytosine in childhood acute leukemia. Cancer Res 47:6786–6792
3. Bhalla K, MacLaughlin W, Cole J, Arlin Z, Baker M, Graham G, Grant S (1987) Deoxycytidine preferentially protects normal versus leukemic myeloid progenitor cells from cytosine arabinoside-mediated cytotoxicity. Blood 70:568–571
4. Bishop JF, Matthews JP, Young GA, Szer J, Gillett A, Joshua D, Bradstock K, Enno A, Wolf MM, Fox R, Cobcroft R, Herrmann R, Van Der Weyden M, Lowenthal RM, Page F, Garson OM, Juneja S (1996) A randomized study of high-dose cytarabine in induction in acute myeloid leukemia. Blood 87:1710–1717
5. Boos J (1991) A simple isocratic ion-pair high-performance liquid chromatographic determination of 1-beta-D-arabinofuranosylcytosine 5′-triphosphate for intracellular drug-monitoring and in vitro incubation assays. J Pharm Biomed Anal 9:47–52
6. Braess J, Wegendt C, Feuring-Buske M, Riggert J, Kern W, Hiddemann W, Schleyer E (1999) Leukaemic blasts differ from normal bone marrow mononuclear cells and $CD34^+$ haemopoietic stem cells in their metabolism of cytosine arabinoside. Br J Haematol 105:388–393
7. Buchner T, Hiddemann W, Berdel W, Wormann B, Loffler H, Schoch C, Haferlach T, Ludwig WD, Maschmeyer G, Staib P, Andreesen R, Balleisen L, Haase D, Eimermacher H, Aul C, Rasche H, Uhlig J, Gruneisen A, Reis HE, Hartlapp J, Hirschmann WD, Weh HJ, Pielken HJ, Gassmann W, Sauerland MC, Heinecke A (2001) Remission induction therapy: the more intensive the better? Cancer Chemother Pharmacol 48(Suppl 1):S41–S44
8. Camiener GW, Smith CG (1965) Studies of the enzymatic deamination of cytosine arabinoside. I. Enzyme distribution and species specificity. Biochem Pharmacol 14:1405–1416
9. Chabner BA, Johns DG, Coleman CN, Drake JC, Evans WH (1974) Purification and properties of cytidine deaminase from normal and leukemic granulocytes. J Clin Invest 53:922–931
10. Chen TR (1977) In situ detection of mycoplasma contamination in cell cultures by fluorescent Hoechst 33258 stain. Exp Cell Res 104:255–262
11. Cooper T, Ayres M, Nowak B, Gandhi V (2005) Biochemical modulation of cytarabine triphosphate by clofarabine. Cancer Chemother Pharmacol 55:361–368
12. Crews KR, Gandhi V, Srivastava DK, Razzouk BI, Tong X, Behm FG, Plunkett W, Raimondi SC, Pui CH, Rubnitz JE, Stewart CF, Ribeiro RC (2002) Interim comparison of a continuous infusion versus a short daily infusion of cytarabine given in combination with cladribine for pediatric acute myeloid leukemia. J Clin Oncol 20:4217–4224
13. Elgie AW, Sargent JM, Alton P, Peters GJ, Noordhuis P, Williamson CJ, Taylor CG (1998) Modulation of resistance to ara-C by bryostatin in fresh blast cells from patients with AML. Leuk Res 22:373–378
14. Estey E, Plunkett W, Dixon D, Keating M, McCredie K, Freireich EJ (1987) Variables predicting response to high dose cytosine arabinoside therapy in patients with refractory acute leukemia. Leukemia 1:580–583
15. Estey EH, Keating MJ, McCredie KB, Freireich EJ, Plunkett W (1990) Cellular ara-CTP pharmacokinetics, response, and karyotype in newly diagnosed acute myelogenous leukemia. Leukemia 4:95–99
16. Faderl S, Gandhi V, O'Brien S, Bonate P, Cortes J, Estey E, Beran M, Wierda W, Garcia-Manero G, Ferrajoli A, Estrov Z, Giles FJ, Du M, Kwari M, Keating M, Plunkett W, Kantarjian H (2005) Results of a phase 1–2 study of clofarabine in combination with cytarabine (ara-C) in relapsed and refractory acute leukemias. Blood 105:940–947
17. Freund A, Rossig C, Lanvers C, Gescher A, Hohenlochter B, Jurgens H, Boos J (1999) All-trans-retinoic acid increases cytosine arabinoside cytotoxicity in HL-60 human leukemia cells in spite of decreased cellular ara-CTP accumulation. Ann Oncol 10:335–338
18. Frewin RJ,Johnson SA (2001) The role of purine analogue combinations in the management of acute leukemias. Hematol Oncol 19:151–157
19. Galmarini CM, Thomas X, Calvo F, Rousselot P, Rabilloud M, El Jaffari A, Cros E, Dumontet C (2002) In vivo mechanisms of resistance to cytarabine in acute myeloid leukaemia. Br J Haematol 117:860–868
20. Gandhi V, Estey E, Keating MJ, Plunkett W (1993) Fludarabine potentiates metabolism of cytarabine in patients with acute myelogenous leukemia during therapy. J Clin Oncol 11:116–124
21. Gandhi V, Estey E, Keating MJ, Chucrallah A,Plunkett W (1996) Chlorodeoxyadenosine and arabinosylcytosine in patients with acute myelogenous leukemia: pharmacokinetic, pharmacodynamic, and molecular interactions. Blood 87:256–264
22. Gandhi V, Estey E, Du M, Keating MJ, Plunkett W (1997) Minimum dose of fludarabine for the maximal modulation of 1-beta-D-arabinofuranosylcytosine triphosphate in human leukemia blasts during therapy. Clin Cancer Res 3:1539–1545
23. Gandhi V, Xu YZ, Estey E (1998) Accumulation of arabinosyluracil 5′-triphosphate during arabinosylcytosine therapy in circulating blasts of patients with acute myelogenous leukemia. Clin Cancer Res 4:1719–1726
24. Herzig RH (1996) High-dose ara-C in older adults with acute leukemia. Leukemia 10(Suppl 1):S10–11
25. Hiddemann W, Schleyer E, Unterhalt M, Zuhlsdorf M, Rolf C, Reuter C, Kewer U, Uhrmeister C, Wormann B, Buchner T (1992) Differences in the intracellular pharmacokinetics of cytosine arabinoside (Ara-C) between circulating leukemic blasts and normal mononuclear blood cells. Leukemia 6:1273–1280
26. Huang P, Plunkett W (1995) Fludarabine- and gemcitabine-induced apoptosis: incorporation of analogs into DNA is a critical event. Cancer Chemother Pharmacol 36:181–188
27. Hubeek I, Litvinova E, Peters GJ, Broekhuizen R, Haarman EG, Huismans DR, Cloos J, Zwaan CM, Fleischhack G, Creutzig U, Kaspers GJ (2004) The effect of G-CSF on the in vitro cytotoxicity of cytarabine and fludarabine in the FLAG combination in pediatric acute myeloid leukemia. Int J Oncol 25:1823–1829
28. Hubeek I, Peters GJ, Broekhuizen R, Zwaan CM, Kaaijk P, van Wering ES, Gibson BE, Creutzig U, Janka-Schaub GE, den Boer ML, Pieters R, Kaspers GJ (2006) In vitro sensitivity and cross-resistance to deoxynucleoside analogs in childhood acute leukemia. Haematologica 91:17–23
29. Jamieson GP, Snook MB, Wiley JS (1990) Saturation of intracellular cytosine arabinoside triphosphate accumulation in human leukemic blast cells. Leuk Res 14:475–479
30. Kohl U, Schwabe D, Montag E, Bauer S, Mieth B, Cinatl J, Cinatl J Jr, Rohrbach E, Mainke M, Weissflog A et al. (1995) Formation of cytosine arabinoside-5′-triphosphate in different cultured lymphoblastic leukaemic cells with reference to their drug sensitivity. Eur J Cancer 31A:209–214
31. Mantovani L, Hasenclever D, Krahl R, Ponisch W, Herold M, Pasold R, Fiedler F, Dolken G, Kampfe D, Schmoll HJ, Subert R, Kubel M, Niederwieser D, Helbig W (2002) Intermediate-dose cytarabine treatment delivered at mod-

erate infusion rates for de novo acute myeloid leukemia-results of a phase I-II study. Leuk Lymphoma 43:265–274
32. Momparler RL, Fischer GA (1968) Mammalian deoxynucleoside kinase. I. Deoxycytidine kinase: purification, properties, and kinetic studies with cytosine arabinoside. J Biol Chem 243:4298–4304
33. Momparler RL, Onetto-Pothier N, Bouffard DY, Momparler LF (1990) Cellular pharmacology of 1-beta-D-arabinofuranosylcytosine in human myeloid, B-lymphoid and T-lymphoid leukemic cells. Cancer Chemother Pharmacol 27:141–146
34. Moricke A, Zimmermann M, Reiter A, Gadner H, Odenwald E, Harbott J, Ludwig WD, Riehm H, Schrappe M (2005) Prognostic impact of age in children and adolescents with acute lymphoblastic leukemia: data from the trials ALL-BFM 86, 90, and 95. Klin Padiatr 217:310–320
35. Muus P, Drenthe-Schonk A, Haanen C, Wessels H, Linssen P (1987) In-vitro studies on phosphorylation and dephosphorylation of cytosine arabinoside in human leukemic cells. Leuk Res 11:319–325
36. Naito K, Ohnishi K (2005) [Current and new therapeutic strategies in acute myeloid leukemia]. Gan To Kagaku Ryoho 32:292–296
37. Noordhuis P, Kazemier KM, Kasperrs GJ, Peters GJ (1996) Modulation of metabolism and cytotoxicity of cytosine arabinoside with N-(phosphon)-acetyl-L-aspartate in human leukemic blast cells and cell lines. Leuk Res 20:127–134
38. Ochs J, Sinkule JA, Danks MK, Look AT, Bowman WP, Rivera G (1984) Continuous infusion high-dose cytosine arabinoside in refractory childhood leukemia. J Clin Oncol 2:1092–1097
39. Ossenkoppele GJ, Graveland WJ, Sonneveld P, Daenen SM, Biesma DH, Verdonck LF, Schaafsma MR, Westveer PH, Peters GJ, Noordhuis P, Muus P, Selleslag D, van der Holt B, Delforge M, Lowenberg B, Verhoef GE (2004) The value of fludarabine in addition to ARA-C and G-CSF in the treatment of patients with high-risk myelodysplastic syndromes and AML in elderly patients. Blood 103:2908–2913
40. Peters WG, Colly LP, Willemze R (1988) High-dose cytosine arabinoside: pharmacological and clinical aspects. Blut 56:1–11
41. Plagemann PG, Marz R, Wohlhueter RM (1978) Transport and metabolism of deoxycytidine and 1-beta-D-arabinofuranosylcytosine into cultured Novikoff rat hepatoma cells, relationship to phosphorylation, and regulation of triphosphate synthesis. Cancer Res 38:978–989
42. Plunkett W, Iacoboni S, Estey E, Danhauser L, Liliemark JO, Keating MJ (1985) Pharmacologically directed ara-C therapy for refractory leukemia. Semin Oncol 12:20–30
43. Plunkett W, Liliemark JO, Adams TM, Nowak B, Estey E, Kantarjian H, Keating MJ (1987) Saturation of 1-beta-D-arabinofuranosylcytosine 5′-triphosphate accumulation in leukemia cells during high-dose 1-beta-D-arabinofuranosylcytosine therapy. Cancer Res 47:3005–3011
44. Plunkett W, Liliemark JO, Estey E, Keating MJ (1987) Saturation of ara-CTP accumulation during high-dose ara-C therapy: pharmacologic rationale for intermediate-dose ara-C. Semin Oncol 14:159–166
45. Preisler HD, Rustum YM, Azarnia N, Priore R (1987) Abrogation of the prognostic significance of low leukemic cell retention of cytosine arabinoside triphosphate by intensification of therapy and by alteration in the dose and schedule of administration of cytosine arabinoside. Cancer Chemother Pharmacol 19:69–74
46. Rayappa C, McCulloch EA (1993) A cell culture model for the treatment of acute myeloblastic leukemia with fludarabine and cytosine arabinoside. Leukemia 7:992–999
47. Riva CM, Rustum YM, Preisler HD (1985) Pharmacokinetics and cellular determinants of response to 1-beta-arabinofuranosylcytosine (ara-C). Semin Oncol 12:1–8
48. Rustum YM,Preisler HD (1979) Correlation between leukemic cell retention of 1-beta-D-arabinofuranosylcytosine 5′-triphosphate and response to therapy. Cancer Res 39:42–49
49. Rustum YM, Raymakers RA (1992) 1-Beta-arabinofuranosylcytosine in therapy of leukemia: preclinical and clinical overview. Pharmacol Ther 56:307–321
50. Rustum YM, Slocum HK, Wang G, Bakshi D, Kelly E, Buscaglia D, Wrzosek C, Early AP, Preisler H (1982) Relationship between plasma Ara-C and intracellular Ara-CTP pools under conditions of continuous infusion and high-dose Ara-C treatment. Med Pediatr Oncol 10(Suppl 1):33–43
51. Schiller M, Hohenlochter B, Schulze-Westhoff P, Zimmermann M, Ritter J, Jurgens H, Boos J (1996) [Intracellular retention of cytarabine-triphosphate (Ara-CTP) in blasts of children with acute lymphoblastic leukemia. Correlation with clinical course parameters]. Klin Padiatr 208:151–159
52. Schrappe M (2004) Evolution of BFM trials for childhood ALL. Ann Hematol 83(Suppl 1):S121–123
53. Schrappe M, Reiter A, Zimmermann M, Harbott J, Ludwig WD, Henze G, Gadner H, Odenwald E, Riehm H (2000) Long-term results of four consecutive trials in childhood ALL performed by the ALL-BFM study group from 1981 to 1995. Berlin-Frankfurt-Munster. Leukemia 14:2205–2222
54. Seymour JF, Huang P, Plunkett W, Gandhi V (1996) Influence of fludarabine on pharmacokinetics and pharmacodynamics of cytarabine: implications for a continuous infusion schedule. Clin Cancer Res 2:653–658
55. Stam RW, den Boer ML, Meijerink JP, Ebus ME, Peters GJ, Noordhuis P, Janka-Schaub GE, Armstrong SA, Korsmeyer SJ, Pieters R (2003) Differential mRNA expression of Ara-C-metabolizing enzymes explains Ara-C sensitivity in MLL gene-rearranged infant acute lymphoblastic leukemia. Blood 101:1270–1276
56. Suki S, Kantarjian H, Gandhi V, Estey E, O'Brien S, Beran M, Rios MB, Plunkett W, Keating M (1993) Fludarabine and cytosine arabinoside in the treatment of refractory or relapsed acute lymphocytic leukemia. Cancer 72:2155–2160
57. Tanaka M, Yoshida S (1987) Formation of cytosine arabinoside-5′-triphosphate in cultured human leukemic cell lines correlates with nucleoside transport capacity. Jpn J Cancer Res 78:851–857
58. Tausch W, Thom R, Odenwald E, Riehm H (1977) [Differential diagnosis of acute leukemia in childhood by means of electronic cell-volume determination]. Monatsschr Kinderheilkd 125:563–565
59. Wiley JS, Taupin J, Jamieson GP, Snook M, Sawyer WH, Finch LR (1985) Cytosine arabinoside transport and metabolism in acute leukemias and T cell lymphoblastic lymphoma. J Clin Invest 75:632–642
60. Yamauchi T, Kawai Y, Kishi S, Goto N, Urasaki Y, Imamura S, Fukushima T, Yoshida A, Iwasaki H, Tsutani H, Masada M, Ueda T (2001) Monitoring of intracellular 1-beta-D-arabinofuranosylcytosine 5′-triphosphate in 1-beta-D-arabinofuranosylcytosine therapy at low and conventional doses. Jpn J Cancer Res 92:546–553
61. Zittoun J, Marquet J, David JC, Maniey D, Zittoun R (1989) A study of the mechanisms of cytotoxicity of Ara-C on three human leukemic cell lines. Cancer Chemother Pharmacol 24:251–255