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ORIGINAL ARTICLE

High-dose idarubicin in combination with Ara-C in patients with relapsed or refractory acute lymphoblastic leukemia: a pharmacokinetic and clinical study

Alessandra Tedeschi · Marco Montillo · Elena Strocchi · Anna Maria Cafro · Elisabetta Tresoldi · Liliana Intropido · Michele Nichelatti · Laura Marbello · Claudia Baratè · Carlo Maurizio Camaggi · Enrica Morra

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Abstract
Objective High dose (HD) Ara-C combined with a single HD idarubicin dose (IDA) is an efficient and safe salvage regimen for patients with refractory or relapsed acute lymphoblastic leukemia as indicated by phase II studies. No data are available on the pharmacokinetics of IDA after a rapid HD intravenous infusion. An open phase II pharmacokinetic and clinical study was performed to evaluate antileukemic efficacy, IDA pharmacokinetics and to investigate the presence of IDA and its reduced metabolite idarubicinol (IDAol) in cerebrospinal fluid (CSF) of patients treated with HD-IDA.
Patients and methods Twenty-five patients with refractory or relapsed acute lymphoblastic leukemia received Ara-C 3 g/m^2 from days 1–5, idarubicin (HD-IDA) 40 mg/m^2 as rapid intravenous (i.v.) infusion on day 3 and subcutaneous G-CSF 5 μg/kg from day 7 until PMN recovery. Pharmacokinetics of IDA was evaluated after HD idarubicin administration in nine of these patients. CSF samples were collected in 15 patients at different times. IDA and IDAol concentrations were quantified by a validated HPLC assay described in detail elsewhere.
Results Eleven patients (44%, 95% CI: 23–65%) achieved complete remission with median disease free survival for 6 months. After administration of HD-IDA i.v. bolus of 40 mg/m^2, plasma level profiles of unchanged drug and IDAol were similar to those previously described after standard dose and measured with the same analytical method. The mean terminal half-life measured for IDA in this group of patients (14.9 h) was not significantly different from the mean value observed after standard dose (13.9 h, $P = 0.72$). IDAol $t_{1/2}$ was also similar after HD-IDA (46.2 h) and standard dose (39.4 h, $P = 0.79$). Pharmacokinetic data reveal that in our series of patients IDA and IDAol clearances are significantly higher than those observed in patients treated with 12 mg/m^2 of IDA but, although the administered dose (mg/m^2) of the drug is 3.3 times higher, IDA exposure (measured in terms of AUC) is only 2.3 times and IDAol exposition 2.1 times greater. Furthermore, HD infusion resulted in a ratio between the AUC of parent drug and idarubicinol not different from the value observed with the standard-dose. IDA and IDAol were measurable only in 3 of the 15 cerebrospinal fluid samples collected.
Conclusion Responses observed in our series are comparable to those reported with other salvage regimens. The IDA exposure lower than expected may explain the safety of the single i.v. administration of 40 mg/m^2 of IDA, combined with HD Ara-C, with a degree of myelosuppression equivalent to that reported with this agent administered in standard doses. Our data do not allow us to clearly attribute this behavior to a pharmacokinetic non-linearity since the

The authors declare that they have no potential conflict of interest.

A. Tedeschi (✉) · M. Montillo · A. M. Cafro · E. Tresoldi · L. Intropido · M. Nichelatti · L. Marbello · C. Baratè · E. Morra
Department of Oncology/Hematology, Division of Hematology, Niguarda Ca' Granda Hospital, Piazza Ospedale Maggiore, 3, 20162 Milan, Italy
e-mail: ale.ted@katamail.com

E. Strocchi · C. M. Camaggi
Department of Organic Chemistry, University of Bologna, Bologna, Italy

baseline creatinine clearance, even within normal values, and patient age are significantly different in the two groups. Cerebrospinal fluid penetration was poor, reaching levels not considered as cytotoxic.

Keywords High-dose idarubicin · Pharmacokinetics · Acute lymphoblastic leukemia · Salvage regimen

Introduction

In adults with acute lymphoblastic leukemia (ALL) complete remission rates (CR) with induction chemotherapy programs range from 60 to 90%. In most studies the disease in 10–25% of patients is resistant to a standard four or five drug regimen based on vincristine, prednisone, L-asparaginase and anthracycline. Among patients achieving CR the long-term disease-free survival (DFS) rates are only 20–40% [1]. Therefore, the majority of adult patients will require treatment in relapsed or refractory disease. Outcome after salvage therapy in adult ALL remains unsatisfactory and is worse than that of newly diagnosed patients with a median DFS of only 2–7.5 months. For these patients the only realistic chance of cure lies in achieving remission followed by a successful transplant strategy [2]. In the salvage setting the use of chemotherapeutic agents similar to those administered during initial therapy may induce CR but the likelihood of a second CR is less than 50% [3]. Additionally, this approach is limited particularly in patients with primary resistant disease. A great deal of experience with Ara-C including high dose Ara-C (HD Ara-C) as reinduction regimen for relapsed or refractory patients with ALL appears in the published literature [4]. As a single agent HD Ara-C can induce CR in approximately 30–60% of relapsed/refractory patients, but generally leading to a short median CR duration; the main alternative is to utilize HD Ara-C combined with L-asparaginase, fludarabine, anthracyclines (idarubicin, doxorubicin) or non-anthracycline intercalators (mitoxantrone and m-AMSA) [5–11]. Most studies have combined HD Ara-C with what is considered to be a standard dose of an anthracycline. Complete remission rates in these studies ranged from 30 to 87%, but in most series disappointing CR durations (2–5.5 months) have been reported [2–5].

Approaches to further improve the efficacy of antileukemic therapy are often based on the introduction of new agents or the application of new dosages. Arlin et al. obtained encouraging results in adult ALL using HD of mitoxantrone associated with HD Ara-C. Other studies have been performed to evaluate the therapeutic activity of this drug combination [12]. However, the clinical benefit of HD mitoxantrone in comparison to standard doses has not yet been established [13]. Escalating doses of idarubicin (IDA) with a fixed high dose of Ara-C were used by Weiss et al. [14] in a phase I study in patients with resistant or refractory ALL. These Authors identified the dosage of 40 mg/m^2 of IDA, in combination treatment with Ara-C, as the maximum tolerated single dose, since an unacceptable toxicity was observed when IDA was administered at a dose of 50 mg/m^2 with one death secondary to cardiotoxicity.

We report here the results of our trial carried out to evaluate the efficacy of a single HD-IDA at 40 mg/m^2 combined with HD Ara-C as salvage therapy in patients with recurrent or refractory ALL.

Many studies have been reported in the literature regarding the pharmacokinetic behavior of IDA administered at a standard dosage (8–12 mg/m^2) after a single intravenous 5–15 min or oral administration [15–17]. To our knowledge there are no data regarding the bioavailability of the drug and its main metabolite, IDAol, after the administration of IDA at a high dosage (40 mg/m^2) following a rapid intravenous infusion. To assess the activity of HD-IDA this study was complemented by pharmacokinetic analysis of IDA including the evaluation of the drug levels and its main metabolite IDAol in the cerebrospinal fluid (CSF).

Patients and methods

Patients and treatment

Twenty-five consecutive patients over 18 years with refractory or relapsed ALL, and meeting the entry criteria were enrolled into the current study after informed consent was obtained according to institutional guidelines. The diagnosis of ALL was based on morphologic evaluation, histochemical and flow cytometric immunophenotypic analyses of the bone marrow aspirate. In addition, cytogenetic and RT–PCR for bcr/abl detection were performed. Patients with mature B-ALL and patients with isolated extramedullary relapse were excluded. Adequate hepatic and renal function (creatinine and bilirubin $\leq$ 2 mg/dl and creatinine clearance $\geq$ 80 ml/min) as well as left ventricular ejection fraction (LVEF) $\geq$ 50% were required.

Therapy consisted of Ara-C 3 g/m^2/day intravenously (i.v.) over 3 h infusion on days 1–5 and one dose

of IDA 40 mg/m^2 given as rapid i.v. infusion over 5 min on day 3. G-CSF was subcutaneously administered at a dose of 5 μg/kg/day starting on day 7 and continuing until the granulocyte count exceeded 0.5×10^9/l for three consecutive days.

Therapeutic intrathecal methotrexate 12 mg plus prednisone was planned weekly only for patients with CNS leukemia and continued until the clearance of blasts of the cerebrospinal fluid.

Patients achieving CR were further treated at their physician's discretion most commonly with regimens including HD methotrexate. Responsive patients with HLA identical donors were recommended for allogeneic transplant.

Response criteria

CR was defined as a normal peripheral blood count (granulocyte count $\geq 1.5 \times 10^9$/l, platelet count $\geq 100 \times 10^9$/l) with less than 5% blast cells in the bone marrow for at least 4 weeks duration. Aplastic death occurred when a patient died after the introduction of treatment with hypocellular bone marrow.

Non-hematological toxicity was assessed according to the National Cancer Institute common toxicity criteria [18].

Survival was measured from the initiation of protocol treatment. Disease free survival was calculated from the date of CR until the end of follow-up, relapse or death while in CR.

Survival and disease free survival were analyzed according to the methods of Kaplan and Meier.

Sampling collection

Plasma samples to determine IDA and IDAol plasma levels were collected in nine patients all showing normal liver and renal function and creatinine clearance ≥ 80 ml/min (mean value 136 ml/min). Peripheral blood samples were obtained and immediately centrifuged before the start of IDA, at time 0 (immediately after the end of i.v. infusion), 5, 15, 30, 60 min and at 2, 4, 8, 24, 48, 72 and 96 h after drug administration. Samples were obtained from a peripheral venous access of the arm as in all patients chemotherapy was infused through a central line. Plasma samples, which were separated after centrifugation, were kept frozen at −20°C in light-protected tubes until the analysis.

After HD-IDA administration a CSF sample was collected in 15 patients at respectively: 24 (7 patients), 48 (5 patients) and 72 h (3 patients). Samples were stored at −20°C until analysis.

Analytical procedure

IDA and IDAol levels were measured by a validated reversed-phase high-performance liquid chromatography method with internal standard (daunorubicin) and fluorescence detection as previously described [17].

Plasma and CSF samples (1–2 ml), after the addition of the internal standard (daunomycin), were solid-phase extracted with C18 reversed phase cartridges (Isolute, Mid-Glamorgan, UK) and analyzed by HPLC. The HPLC instrumentation consisted of a Merck–Hitachi system (L-4200) equipped with a Perkin–Elmer 650–10LC fluorescence detector (excitation wavelength: 470 nm, emission: 580 nm). Complete separation of unchanged drug and main metabolites was achieved with a cyanopropyl chromatographic column (Supelcosil LC-CN, 25 cm × 4.6 mm id, particle size: 5 μm). Routine analyses were performed using a mobile phase containing KH2PO4 10 mM and CH3CN (pH 2.5).

Pre-study and in-study validations were carried out according to FDA guidelines. For this batch of analyses, LOD and LOQ were respectively 0.3 and 1.0 ng/ml for IDA and 0.4 and 1.1 ng/ml for IDAol. The method was linear ($r^2 > 0.995$, linear regression analysis) over the analytical concentration range 1.05–350 and 1.22–51 ng/ml respectively for IDA and IDAol. Mean inter- and intra-assay precision of the method (coefficient of variation) were respectively 8 and 7.3% (IDA) and 11 and 9% (IDAol). Overall accuracy was over the concentration interval in the range −9.2 to 11.9% for IDA and −11.3 to 15.1% for IDAol. Mean recovery was 84% for IDA and 79% for IDAol.

Pharmacokinetic analysis

Plasma concentration data were elaborated with the Pharsight WinNonlin 4.01 package (WinNonlin Pro v.4.01, SCI Software, Pharsight Corp, Cary NC, 2002) using both compartmental and non-compartmental procedures, taking into account the infusion time in both cases (IDA, compartmental: Pharsight compiled model 19, i.v. infusion, three compartments, macro constants, no lag time, first order elimination; compiled model 10, i.v. infusion, two compartments, macro constants, no lag time, first order elimination. IDA, non-compartmental: model 202, constant infusion. IDAol, non-compartmental: model 200, extravascular input). The best compartmental mode was chosen according to the Akaike information criterion [19].

For comparison with the present data, raw data relative to nine patients with advanced solid tumors and creatinine clearance ≥ 80 ml/min, previously treated

with IDA (12 mg/m^2, rapid intravenous infusion over 5 min), were re-analyzed with WinNonlin 4.01 package following the same pharmacokinetic procedure. Plasma concentration data for these patients were obtained in the same laboratory following the same certified analytical procedure used for the HD samples [17].

For each patient, IDA area under curve (AUC_{0-inf}), terminal half-life ($t_{1/2}$ gamma), plasma clearance (PlCl), mean residence time (MRT) and volume of distribution at steady-state (Vss), IDAol $AUC_{0-inf,}$ terminal half-life, dose-corrected AUC (F. clearance, IDA dose/IDAol AUC) and MRT were computed using both the compartmental approach and the non-compartmental one. Data obtained from both algorithms were identical within the experimental error. In the following, data obtained from the non-compartmental procedure will be discussed.

Statistical analysis

Statistical analysis was carried out with the NCSS 2001 package (Hintze J. NCSS 2001, Statistical Systems, Kaysville, Utah, 2003). Data were divided into two groups: "standard-dose" comprising of nine patients treated with 12 mg/m^2 of IDA and "high-dose" comprising of eight patients treated with 40 mg/m^2 dose.

Univariate tests (*t* test and non-parametric Mann–Whitney *U* or Wilcoxon rank-sum test for difference in medians) and Hotelling t^2 test with randomization (100,000 Monte–Carlo samples) were used to investigate the influence of dose level on the response variables (IDA terminal half-life, plasma clearance, mean residence time and volume of distribution at steady-state, IDAol terminal half-life, dose-corrected AUC and MRT). Linear regression and ANOVA were also applied to investigate the correlation between the response variables and creatinine clearance. Analysis of covariance (ANCOVA) was then used to investigate the sources of variation.

Results

Clinical results

Twenty-five patients, 14 males and 11 females, were enrolled in this study. Patients' characteristics are summarized in Table 1. The median age was 31 years (range 18–55). The majority of patients had a B cell immunophenotype ALL (22 of 25 patients, 88%). On cytogenetical analysis nine patients exhibited poor-risk karyotype, eight of them being positive for the Philadelphia chromosome and one carrying *t*(4;11).

Table 1 Characteristics of the 25 patients with refractory or relapsed ALL

Characteristic	Number of patients
Age, median (range; years)	31 (18–55)
Male/female	14/11
Disease status	
Refractory disease	12
Recurrent disease	13
Early relapse: complete remission duration < 6 months	6
Late relapse: complete remission duration ≥ 6 months	7
Cytogenetics	
Normal	11
Philadelphia chromosome positive	8
t(4; 11)	1
T(12; 21)	1
Hypodiploid	1
Hyperdiploid	1
Failed	2
Anthracyclines pre-treatment	
Daunorubicin 270 mg/m^2	16
Daunorubicin 420 mg/m^2	3
Daunorubicin 270 mg/m^2 + mitoxantrone 30 mg/m^2	2
Daunorubicin 100 mg/m^2 + doxorubicin 150 mg/m^2	3
Mitoxantrone 75 mg/m^2	1

Twelve patients showed refractory disease to induction standard therapy while 13 patients were in first relapse after a median duration of CR of 8 months (range 2–39); 4 of these patients experienced a recurrence of disease after completing maintenance treatment. Only one patient presented CNS involvement.

All but one patient had received as front-therapy a four-drug regimen consisting of prednisone, vincristine, daunorubicin and L-asparaginase followed by either consolidation or II reinduction therapy and maintenance according to various trials (GIMEMA: ALL-0288, ALL-0496 and GMALL 04/89) [20–22]. The median total cumulative dose of daunorubicin administered in the 24 patients was 270 mg/m^2 (range 100–420); three patients receiving a lower dosage of 100 mg/m^2 of daunorubicin had also received 150 mg/m^2 of doxorubicin. One patient received mitoxantrone as induction treatment instead of daunorubicin at a total dosage of 75 mg/m^2 according to the GIMEMA ALL-059722 trial [23]. Three patients had already received, as consolidation regimen, HD chemotherapy with mitoxantrone and Ara-C (HAM) [11] followed by autologous peripheral blood stem cell transplantation in one case. Previous anthracyclines treatment and relative cumulative dosages are summarized in Table 1.

After the administration of the single HD-IDA associated to HD Ara-C, 11 of the 25 patients achieved

CR (44%, 95% CI: 23.1–64.9%); whereas in 14 cases leukemia recovered after the aplastic phase; none of the patients died during marrow aplasia.

Analysis according to the status of the disease at salvage treatment revealed no difference between the two subgroups: in particular, 38% CR was observed in patients treated at relapse, 50% in patients showing refractory disease (Table 2). Four of the seven patients (57%) with the first remission ≥ 6 months achieved CR. A CR rate of 44% was observed in both groups of patients divided according to cytogenetic risk, high versus intermediate/low-risk.

From the 11 patients achieving CR, 5 underwent an allogeneic bone marrow transplantation; 3 of them relapsed after 6, 10, 11 months, while 2 are in ongoing remission after 38 and 40 months, respectively.

Of the remaining six patients in CR after salvage treatment one died while in CR due to thoracic aortic dissection secondary to disseminated aspergillosis, the remaining five showed a recurrence of disease after a median of 4 months (range 3–7); three patients having received high dose methotrexate-based consolidation treatment and two ph + patients having received Imatinib mesilate-based regimens.

Eleven of the 12 patients refractory to HD-IDA and HD Ara-C treatment received a second salvage regimen, either with HD Methotrexate or HD VP-16 associated to HD cyclophosphamide; none of them obtained a response.

The median overall survival was 8 months (95% CI: 5–9) for all patients (Fig. 1), while the median DFS was 6 months (95% CI: 0–11) (Fig. 2).

All patients experienced aplasia showing grade 4 neutropenia and thrombocytopenia. In patients achieving CR, recovery to PLT $\geq 20 \times 10^9$/l occurred after a median of 17 days (range 0–29) from the beginning of therapy, 17 days also being (range 9–27) the median time for PMN recovery to $\geq 0.5 \times 10^9$/l.

As regards non-hematological toxicity of grade 3/4 according to NCI criteria, severe nausea/vomiting, diarrhea and mucositis occurred in 4 (16%), 2 (8%) and 3 (12%) patients, respectively. Major grade 3 hepatic toxicity was observed in one case and a grade 3 bladder hemorrhage in one case. None of the patients developed cardiac toxicity even of grade 1/2.

All but two patients experienced an episode of fever ($t > 38$°C) lasting for a median of 7 days (range 1–30); fever of unknown origin remained in ten cases. Seven patients presented an episode of sepsis sustained by *Pseudomonas Aeruginosa* in one case and by gram positive in the remaining cases. Six pneumonia cases were radiologically documented. Two fungal infections were recorded: candidemia in one case and disseminated Aspergillosis in the other case.

Table 2 Complete Remission rate according to disease status and cytogenetic pattern

Disease	No	CR (%)
All patients	25	11 (44)
Refractory	12	6 (50)
Relapsed	13	5 (38)
Early relapse: CR duration < 6 months	6	1 (17)
Late relapse: CR duration ≥ 6 months	7	4 (57)
High-risk karyotype	9	4 (44)
Intermediate/low-risk karyotype	16	7 (44)

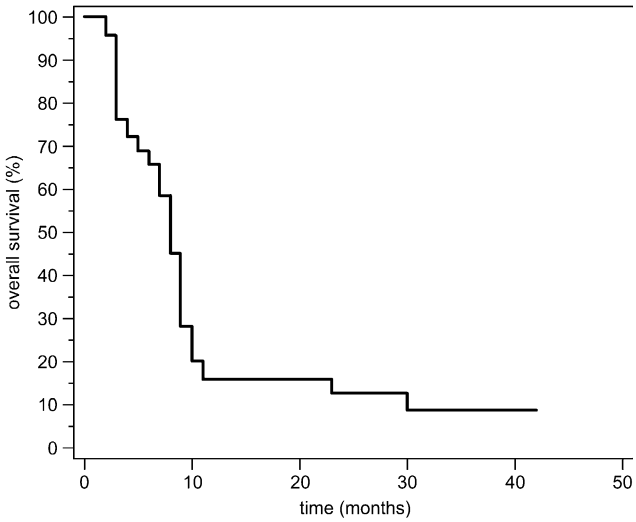

Fig. 1 Overall survival of 25 refractory and relapsed ALL patients after HD idarubicin and HD Ara-C

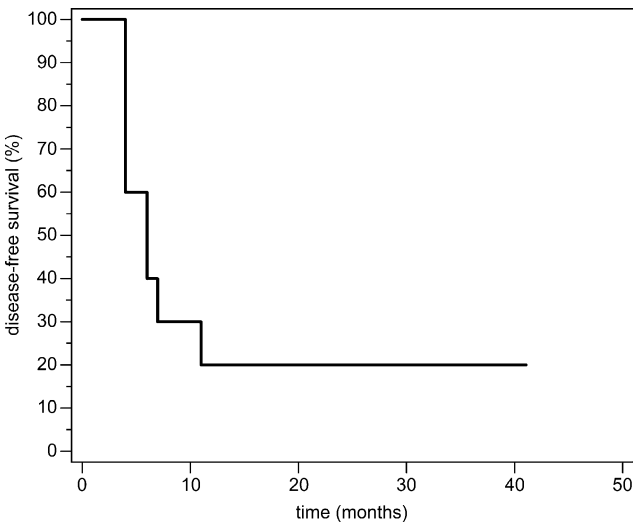

Fig. 2 Disease free survival of 11 patients in CR after HD idarubicin and HD Ara-C

Pharmacokinetic results

The time course of IDA and IDAol concentrations after patients received HD-IDA are reported in Fig. 3 and compared with mean drug and metabolite levels observed after the administration of 12 mg/m^2 of the drug previously measured in our laboratory [17]. IDA plasma levels rapidly decreased and followed a well-defined triphasic decay, quite similar to that observed in the nine patients who received the standard dose. Plasma concentration of the active metabolite IDAol increased rapidly after IDA infusion, and exceeded the IDA concentration 2–8 h after administration. In each patient, IDAol terminal half-life was significantly longer than IDA $t_{1/2}$ gamma (46.2 ± 19.01 h, IDAol; 14.9 ± 7.07 h, IDA), and IDAol was the only fluorescent compound detectable in plasma 24–72 h after treatment. On average AUC_{0-inf} of IDAol was 3.7 times higher than that of parent drug after HD-IDA, not so different from the value of 4.0 times observed after standard-dose treatment.

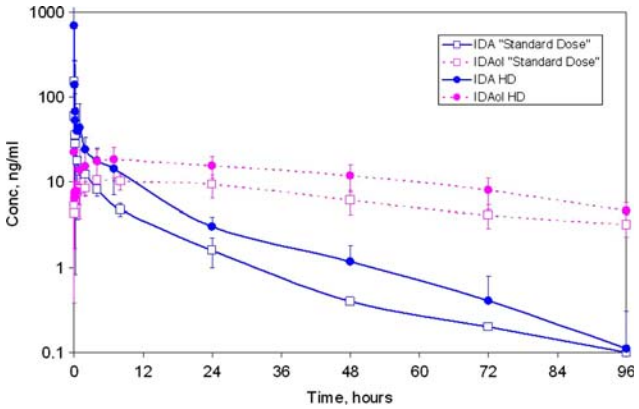

Fig. 3 Mean idarubicin (IDA) and idarubicinol (IDAol) plasma levels (ng/ml) after i.v. bolus administration of IDA "standard dose" (12 mg/m^2; *open square*) and IDA HD (40 mg/m^2; *filled circle*). Results show the mean ± SD plasma levels of IDA (*solid line*) and IDAol (*dashed line*) measured up to 96 h after administration

Main pharmacokinetic parameters after high-dose IDA administration are reported in Table 3 and compared with the standard dose administration. Both parametric- and non-parametric tests showed a statistically significant difference in the pharmacokinetic parameters IDA AUC_{0-inf}, IDA plasma clearance and IDAol apparent clearance measured after high and standard dose treatment.

Linear regression and analysis of variance (ANOVA) showed, however, a significant correlation between the response variables IDA plasma clearance, IDA Vss, IDAol apparent clearance and the creatinine clearance of the patients. Mean creatinine clearance was actually significantly different in the two dose groups of patients: 100.2 ± 12.1 ml/min (standard dose) versus 138.5 ± 30.42 ml/min (high dose), $P = 0.008$, and the question arises whether the disposition differences between the two dose levels depend on a baseline disparity in creatinine clearance or on IDA non-linear kinetics. The analysis of covariance (ANCOVA) might be useful in these cases to investigate the sources of variation, but it does have the (highly) restrictive assumption that the slope of the linear regression between response variable and covariates is constant over all the groups involved. The validity of this assumption was tested by comparing the confidence intervals of the slopes in the two dose groups by linear regression and bootstrapping (5,000 bootstrap samples). ANCOVA procedure confirmed creatinine clearance as a significant factor in determining IDA plasma clearance, IDA Vss and IDAol F. clearance, but the power of the test was not sufficient to define the role of the dose level.

IDA and IDAol levels were detected in three of the seven patients in which the CSF samples were collected 24 h after anthracycline administration (IDA:<0.01, 8.79 and 0.16 ng/ml; IDAol: 0.16, 0.83 and 0.37 ng/ml) with a ratio to plasma significantly lower than 1 (IDA: 0.85 and 0.034; IDAol: 0.022, 0.001 and 0.013). No drug or metabolite levels were measured in CSF sample

Table 3 Pharmacokinetic parameters after high (40 mg/m^2) and standard (12 mg/m^2) idarubicin dose

	High dose	SD	Standard dose	SD	*P*
IDA terminal half-life (h)	14.9	7.07	13.9	3.79	0.724
IDA AUC_{0-inf} (ng h/ml)	397.8	103.0	186	49.4	0.0008
IDA plasma clearance, PlCl (l/h)	197.2	70.84	120.69	35.64	0.005
IDA mean residence time, MRT (h)	13.3	6.65	14.9	3.00	0.251
IDA volume of distribution, Vss (l/kg)	37.1	22.01	24.2	5.28	0.216
IDAol terminal half-life (h)	46.2	19.01	39.4	7.90	0.791
IDAol AUC_{0-inf} (ng h/ml)	1,359	336	727	174.1	0.0014
IDAol F. clearance (l/h)	54.71	21.07	30.95	10.75	0.0047
IDAol mean residence time (h)	62.2	21.25	63.1	9.26	0.289
AUC_IDAol/AUC_IDA	3.5	1.08	4.0	0.62	0.289

AUC Area under the concentration–time curve, *SD* standard deviation, *P* probability level (Mann–Whitney *U* test)

collected in the remaining eight patients, neither at 48 (5 patients) nor at 72 h (3 patients) after IDA administration.

Discussion

The efficacy of regimens including anthracyclines associated to intermediate or high dose Ara-C has been demonstrated in the setting of patients with refractory or relapsed ALL. The combination of standard doses of IDA (12 mg/m^2/day for 3 days or 5 mg/m^2/day for 6 days) with HD Ara-C, both in children and in adults with pre-treated ALL, leads to CR rates ranging from 50 to 64% [5, 24].

Weiss et al. [14] in their phase I study demonstrated that the escalation of IDA beyond the conventional dose and schedule may be possible obtaining a CR rate of 58% after an infusion of a single dose of IDA at 40 mg/m^2 associated to Ara-C 3 g/m^2 daily for 5 days. According to the authors the rationale for administering HD-IDA is that the active metabolite idarubicinol (IDAol) has a very long half-life (>45 h). Therefore, giving a single high dose of the drug would maximize the peak level enhancing the cytotoxic effect on leukemic cells and achieving active therapeutic levels persisting for several days. The promising results obtained in the phase I study were not confirmed in the phase II trial using the same single dose of IDA. An overall CR rate of only 38% was reached in the same setting of patients, and this prompted the authors to conclude that this regimen has moderate activity in patients with recurrent or refractory ALL [25].

The CR rate (44%) obtained in our cohort of patients is similar to that obtained by Weiss et al. and to the one observed in a larger trial conducted by the GIMEMA group (GIMEMA ALL-rescue 97 trial) [26] including 135 patients treated with the same dosages of drugs. Significantly higher CR rates (69%) have been obtained by Testi et al. with the same combination regimen in a cohort of 26 patients; however, patients considered in the latter study had a more favorable prognosis, their median age being 20 years (range: 15.5–44) [27]. The importance of age as a prognostic factor in this setting has been established by Thomas et al. [28]: 40% CR in patients aged < 40 versus 18% in patients of 40–59 years ($P < 0.01$).

In our study the median duration of response was brief even if all patients, except the one with disseminate aspergillosis, promptly underwent consolidation treatment and allogeneic bone marrow transplant, when feasible, immediately upon achieving CR. The GIMEMA ALL-rescue 97 trial led to a 7.4% rate of living and disease free patients (all but one transplanted), the outcome of patients considered in our study is comparable, as only two patients (8%), both of them transplanted with an unrelated donor, are alive and disease free. These data confirm that the CR obtained after salvage treatment should be followed by allogeneic stem cell transplantation owing to the paucity of long-term survivors after chemotherapy alone.

The regimen was well tolerated with extra-hematological toxicities of grade 3–4 that may be considered acceptable in this patient population and comparable with those observed after other salvage regimens. The use of high-dose anthracyclines and mitoxantrone raises important issues with regard to cardiac toxicity [29]. Clinical cardiac toxicity was absent in this study despite the fact that all but one patient had significant prior exposure to daunorubicin, in many cases the cumulative dose administered being $\geq$ 270 mg/m^2. In none of the 22 patients in which echocardiography was performed before starting subsequent salvage or consolidation treatment, approximately 1 month after IDA administration, a significative decrease of the left ventricular ejection fraction was recorded. In accordance with other studies, in which one infusion of IDA at the dosage of 40 mg/m^2 was associated to HD Ara-C, our results show that it is perfectly safe to use high-dose (40 mg/m^2, i.v.) IDA, at least in patients with creatinine clearance greater than 80 ml/min.

In accordance with the phase II MSKCC study in the current study, we did not find an improvement of antileukemic activity after administration of a single high dose of IDA (40 mg/m^2), as the overall CR rate of 44% achieved in our patients is comparable to the results obtained after standard doses of IDA [5, 24]. Moreover, comparable results have been obtained by Karbasian-Esfahani et al. [30] (CR 30%, PR 25%), administering a standard dose of IDA associated with a continuous 7 day Ara-C 100 mg/m^2 daily infusion schedule commonly used in the treatment of acute myeloid leukemia. Pharmacokinetic data reveal that in our series of patients IDA and IDAol clearances are significantly higher than those observed in patients treated with 12 mg/m^2 of IDA but, although the administered dose (mg/m^2) of the drug is 3.3 times higher, IDA exposure (measured in terms of AUC) is only 2.3 times and IDAol exposition 2.1 times greater. These results may explain the safety of the single i.v. administration of 40 mg/m^2 of IDA, combined with HD Ara-C, with a degree of myelosuppression equivalent to that reported with this agent administered in standard doses. On the other hand, AUC and IDAol exposition ratios observed in this study could explain why higher doses of IDA did not translate an improved antileukemic activity.

Our data do not allow us to clearly attribute this behavior to a pharmacokinetic non-linearity or to an effect of baseline creatinine clearance. In fact, the median values were significantly different in the two patient groups even if in all patients creatinine clearance was $\geq$ 80 ml/min.

The plasmatic decay shows the same shape and does not indicate non-linearity. The significant correlation between renal functionality and IDA clearance has been previously observed in a study involving patients with impaired renal function treated with 12 mg/m^2 [16]. Whichever is the case, pharmacokinetic non-linearity or variability due to creatinine clearance level, even within normal values, a proportionally higher IDA and idarubicinol availability was not reached, in our study, with the administration of a higher dose of IDA given as a single i.v. infusion.

An improvement of the efficacy of IDA may be obtained not only by increasing the dosage but also by varying the schedule of drug administration. In our study the high dose short i.v. infusion of IDA resulted in a ratio between the AUC of parent drug and IDAol equal to 3.7, not so different with respect to the value observed with standard-dose IDA by i.v. short infusion. In contrast, Bengala et al. [31] administering the drug at a high dosage by continuous i.v. infusion of 48 h reported a higher ratio of 6.1.

Few studies have appeared in literature addressing the issue of anthracyclines penetration into CNS. Pharmacokinetic data obtained in our study provide evidence that penetration of IDA and IDAol into CSF is almost absent or really poor as only in three patients, out of seven, were the two drugs detectable in the sample obtained 24 h after drug administration. Furthermore, in none of the patients in which samples were obtained 48 or 72 h after IDA administration were the two drugs detectable. Our results are comparable to the data reported by Berg et al. [32] in a study performed in non-human primates; in this study the authors revealed a poor CSF penetration of both daunorubicin (30 mg/m^2) and IDA (8 mg/m^2) and their respective alcohol metabolites after an intravenous administration of 15 min. In contrast, Reid et al. [33] in a series of 21 pediatric leukemia patients demonstrated that while IDA was rarely detectable in CSF 18–30 h after the administration of 10–15 mg/m^2, IDAol was detectable in 20 patients. However, the IDAol concentrations reported in this study are low (mean value of 0.51 ng/ml; range: 0.22–1.05 ng/ml) and not to be considered cytotoxic as the IC_{50} value for a 72-h exposure for both IDA and IDAol in CCRF-CEM lymphoblastic leukemia cells is 2–5 nmol/l (1–2.5 ng/ml).

Moreover, Bengala et al. [31] administering IDA at the dosage of 60 mg/m^2 by 48 h i.v. continuous infusion, did observe higher CSF drug/metabolite even if lower levels were reached in patients treated with 70 mg/m^2.

Even if our data and data reported in literature are not comparable, not only for the schedule of IDA administration but also for the different timing of CSF sampling, it seems that the CSF levels of the drugs are not influenced by the administered drug dose or by the time of sample collection. The CSF levels of IDA and IDAol detected in the CSF are too low and erratic to sustain CNS antileukemic activity although from this data CNS tissue levels cannot be predicted.

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