Cranial Irradiation and Permeability of Blood-Brain Barrier to Cytosine Arabinoside in Children with Acute Leukemia

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ABSTRACT
Cranial Irradiation (CI) is an effective way to prevent central nervous system (CNS) leukemia in children with acute leukemia (AL). However, it is still unclear whether the antileukemic effect of CI is mediated by alteration of blood-brain barrier (BBB) permeability and consequent increased levels of systemically administered drugs or whether it simply results from a direct cytolytic effect on leukemic cells in the meninges at diagnosis. We evaluated the influence of CI on BBB permeability to 1-β-D-arabinofuranosylcytosine (ara-C) in 23 children with AL undergoing CI for CNS leukemia prophylaxis. CI was administered at 18 Gy (16 patients) and 24 Gy (7 patients). ara-C levels were measured in cerebrospinal fluid (CSF) and plasma before, during, and after CI. Two doses were evaluated: 75 mg/m²/day (12 patients) and 480 mg/m²/day (11 patients). CSF and plasma ara-C levels were measured when steady state was achieved. CSF:plasma ratios, obtained before, during, and after CI, were compared by an ANOVA model for repeated measures and by Tukey’s test. At the 75-mg/m²/day dose, the mean values of ara-C CSF:plasma ratios before, during, and after CI were 0.20, 0.27, and 0.27, respectively. At the 480-mg/m²/day dose, the mean CSF:plasma ratios before, during, and after CI were 0.09, 0.12, and 0.13, respectively. No significant differences were observed when CSF:plasma ratios were compared before and during, during and after, and before and after CI. Our results indicate that CI at the doses used for CNS prophylaxis in AL does not significantly alter the BBB as far as ara-C is concerned.

INTRODUCTION
To date, an overall cure rate of about 65% is obtained in children with ALL (1). These therapeutic results can be achieved only if some form of CNS prophylaxis is added to an intensive multidrug protocol (1, 2). In addition, a recent report suggests that, in AML, CNS prophylaxis may be of benefit, at least in standard-risk patients (3). CNS preventive therapy or CNS “prophylaxis,” based on the combined use of CI and multiple i.t. injections of MTX, was pioneered in ALL by Pinkel et al. (4) in the early 1970s. In these initial studies, the dose of CI that was combined with i.t. MTX was progressively escalated from 5 to 24 Gy. The 24-Gy dose plus six i.t. injections of MTX appeared to be the most effective way to decrease the incidence of CNS relapse, which, at that time, was observed in more than 50% of patients achieving remission with a multidrug chemotherapeutic regimen. The dose of 24 Gy has been subsequently reduced to 18 Gy, with the goal of decreasing the incidence of possible neurotoxicity while effectively preventing the occurrence of CNS leukemia. In young children, the 18-Gy dose has been shown to be less neurotoxic and equally effective in preventing CNS leukemia (5). However, the complete omission of RT, at least as attempted by one study from BFM group, led to an unacceptably high CNS relapse rate (6). RT appears to be the most effective way to prevent the occurrence of CNS leukemia in high-risk patients, whereas high-dose systemic therapy with MTX and i.t. chemotherapy may be useful to treat less aggressive diseases, such as standard-risk leukemia.

The pathogenesis of CNS leukemia and the mechanism of action of CI in preventing CNS leukemia remains unclear. It has been suggested that CI may prevent CNS leukemia by killing leukemic cells that are already present in the meninges at the time of diagnosis (4); however, it is also possible that RT may alter the BBB in such a way as to facilitate penetration of systemically administered antileukemic drugs into the CSF. This hypothesis was proposed by the clinical observation of unexpected neurotoxicity in patients receiving systemic MTX after CI (7). Recently, it has been demonstrated, in children with standard-risk AML, that CI and i.t. chemotherapy significantly reduced CNS recurrence and also the risk for bone marrow relapse (3). Assuming that leukemic blasts are already present at the time of diagnosis, it is conceivable that radiation-induced alteration of BBB may increase ara-C concentration up to cytotoxic levels within the CNS, thus preventing reseeding of bone marrow by leukemic blasts that are present in the CNS.

To evaluate the possible effect of CI on the permeability of

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The abbreviations used are: ALL, acute lymphoblastic leukemia; CNS, central nervous system; AML, acute myelogenous leukemia; CI, cranial irradiation; i.t., intrathecal; MTX, methotrexate; RT, radiotherapy; BFM, Berlin-Frankfurt-Munster; BBB, blood-brain barrier; CSF, cerebrospinal fluid; Lp, lumbar puncture.
BBB to ara-C, we have measured CSF and plasma ara-C levels during i.v. continuous infusion before, during, and after CI. We chose to measure ara-C because it is one of the few drugs that is active in the treatment of both ALL and AML and, as a part of ALL BFM protocols, is administered in conjunction with CI and i.t. MTX injections (1).

**PATIENTS AND METHODS**

**Patients.** Twenty-three children with acute leukemia (19 with ALL and 4 with AML) were included in this study. The median age was 7 years and 2 months, ranging from 2 to 14 years. Eight patients were male, and 15 were female. All patients were in complete remission following induction therapy. Informed consent was obtained from patients or their families.

The ALL patients were treated according to ALL BFM-81 protocol (8); patients were studied during the second part of BFM protocol I, when CI was delivered together with four weekly i.t. injections of MTX and ara-C was administered as a 4-day continuous infusion (75 mg/m²/day). AML patients were treated with a slightly modified AML BFM-78 protocol (9) and studied during consolidation phase, when CI was administered together with ara-C continuous infusion. All patients were in continuous complete remission and had normal renal and hepatic function. In ALL patients, CI of the entire skull was administered at the dose of either 18 Gy (12 patients) or 24 Gy (7 patients), depending on prognostic risk factors. AML patients received 18 Gy. Radiation doses were divided into daily doses administered for 2 weeks (18 Gy) or 3 weeks (24 Gy).

**Drug Administration and Doses.** ara-C, dissolved in saline solution, was administered i.v. at a constant rate using an infusion pump; we initially studied 12 patients who were treated with the standard protocol dose of 75 mg/m²/day. At this dose, in some CSF samples, ara-C levels were below the assay limit of sensitivity. To achieve baseline CSF detectable levels in the subsequent 11 patients, we administered a higher dose at the beginning of the 4-day continuous infusion: 20 mg/m²/h over the first 2 h, equivalent to 480 mg/m²/day.

**Sample Collection.** Plasma and CSF samples, obtained by Lps, were taken at steady state: during day 2 of infusion for the 75-mg/m²/day infusion and at the end of hour 2 of infusion for the 20-mg/m²/h dose. The latter time point ensured ara-C steady state, as demonstrated by preliminary tests performed in our laboratory and by De Angelis et al. (10). Lps were performed at the weekly time interval, as indicated by the treatment protocol. One CSF sample was collected before RT was started, and one was collected during RT, with dose administered varying from 6 to 24 Gy. In patients who reached the total planned RT dose, CSF samples were taken no later than 24 h from the last RT dose. Subsequent Lps were performed at least 2 months from the end of RT. CSF and plasma samples were collected in heparinized tubes containing 10⁻⁴ M tetrahydrouridine to prevent in vitro ara-C deamination. Tubes were kept on ice, and plasma was separated by centrifugation at 4°C. Samples were stored frozen at -20°C until analysis.

**ara-C Analysis.** ara-C levels were measured according to a previously described high-performance liquid chromatography method that was specific for ara-C and its major metabolite, uracil arabinoside (11). The sensitivity limit of the method was 5 ng/ml.

**Statistical Analysis.** The one-way, within-subject ANOVA for repeated measures (ANOVA model) and Tukey’s post hoc comparison test (12) were used to compare CSF and plasma levels and CSF:plasma ratios obtained before, during, and after CI. Samples with ara-C levels below the assay’s sensitivity limit (5 ng/ml) were taken to be at a 2 ng/ml concentration.

**RESULTS**

ara-C plasma and CSF levels and ara-C CSF:plasma ratios obtained at the dose of 75 mg/m²/day (n = 12) before, during, and after CI are shown in Table 1. Before, during, and after CI,
mean (± SD) ara-C CSF levels were 6.3 ± 3.9, 8.8 ± 6.5, and 10.2 ± 9.7 ng/ml, respectively; mean (± SD) ara-C plasma levels were 36.3 ± 14.7, 32.8 ± 9.4, and 35.8 ± 19.3 ng/ml, respectively; and mean (± SD) CSF:plasma ratios were 0.20 ± 0.13, 0.27 ± 0.20, and 0.27 ± 0.12, respectively. There were no significant differences between the CSF:plasma ratios when they were compared before and during (P = 0.48), during and after (P = 0.99), and before and after (P = 0.50) CI (Tukey’s test). Table 2 shows ara-C plasma and CSF levels and ara-C CSF:plasma ratios achieved before, during, and after CI at the dosage of 20 mg/m²/h (n = 11), administered over 2 h, equivalent to 480 mg/m²/day. Before, during, and after CI, mean (± SD) ara-C CSF concentrations were 14.5 ± 6.7, 19.6 ± 8, and 19.8 ± 7.1 ng/ml, respectively; mean (± SD) ara-C plasma levels were 154.4 ± 34.5, 168.4 ± 35.5, and 158.5 ± 46.5 ng/ml, respectively; and CSF:plasma ratios (± SD) were 0.09 ± 0.05, 0.12 ± 0.05, and 0.13 ± 0.04, before, during, and after CI. No statistically significant differences were observed between the levels reached in CSF or in CSF:plasma ratios at this dose before and during, during and after, and before and after CI (P = 0.35, 0.86, and 0.15, respectively). In addition, no significant differences were found in CSF:plasma ratios after irradiation within patients treated with 18 Gy or 24 Gy. It appears that increasing the ara-C dose causes a smaller fraction of the drug to cross the BBB (0.12 ± 0.5 versus 0.27 ± 0.2).

**DISCUSSION**

The introduction in the treatment program of CI combined with i.t. MTX drastically reduced the incidence of CNS leukaemia, an invariably fatal complication of ALL (4). Although CI is capable of preventing CNS leukaemia, the exact mechanism remains unclear. It has been suggested that CI may act by killing the leukemic cells that are already present in CNS at the time of diagnosis (4). Another hypothesis is that CI may alter the BBB and allow a higher fraction of systematically administered drugs to cross into the CNS. Price and Jamieson (7) proposed that BBB permeability was increased following CI, thus facilitating the delivery of MTX to the brain. This mechanism was proposed after the occurrence of an unexpectedly high incidence of severe neurotoxicity in patients receiving systemic MTX after CI with 20 Gy or more (7). BBB is a highly selective barrier between blood and brain. Under normal circumstances, only a few drugs cross the BBB and reach therapeutic concentrations in the CSF (13). Ionizing radiation may, however, alter the BBB, the dose and fractionalization that are capable of determining a barrier alteration are, as yet, unclear. To evaluate the possible influence of CI on BBB in acute leukemia, we measured ara-C CSF levels that were obtained by Lps performed throughout the treatment plan. We chose to measure ara-C because this drug is administered by continuous infusion during CI. In addition, a CSF:plasma ratio between 0.2 and 0.4 has been reported with intact BBB (10, 14).

In experimental studies, after irradiation with doses higher than 30 Gy in a single fraction, all of the data show early BBB damage that is independent from the method of investigation and from the experimental model (15–17). At doses lower than 30 Gy, in single or fractionated doses, available data are less in favor of an alteration of BBB.

Experimental studies performed by Griffin et al. (18) in rats showed an increased uptake of MTX (M, 454), up to 6 days after whole-brain irradiation of a single dose of 20 Gy, but no effect after 5–10 and 15 Gy. Furthermore, dose levels ranging from 2 to 30 Gy, given in a single fraction or with daily fractions of 2 Gy (19), did not alter in rat the BBB permeability for bleomycine (M, 1100) and for [3H]VM26 (M, 820). Similarly, Holenberg’s group showed that fractionated dose CI does not increase MTX brain concentrations in rats (20).

In humans, the influence of CI on BBB has been investigated by several different methods. In patients undergoing treatment for brain tumors, the influence of radiation on the BBB using emission computed tomography showed a mean increase of BBB permeability of approximately 24% in the normal brain following irradiation of 30–40 Gy (21). Stephani et al. (22) studied CSF albumin concentrations, as an indicator of blood-

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**Table 2** ara-C plasma and CSF levels and CSF:plasma ratios before, during, and after CI in 11 children treated with ara-C at 480 mg/m²/day.

The last two columns indicate the dose of irradiation reached when samples collected during RT were obtained and the total planned dose, respectively. Values obtained in the different conditions are not statistically different (Tukey’s test).

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<th>Patient no.</th>
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Mean values before and after irradiation were not significantly different.

Mean values before and during irradiation were not significantly different.

Mean values during and after irradiation were not significantly different.
brain integrity, in children undergoing treatment for ALL. Patients were randomized to receive (or not) CI with 18 Gy. High CSF albumin was noted in all irradiated patients, whereas no changes were apparent in patients treated with chemotherapy alone, suggesting a temporary alteration of BBB. No measurement of antineoplastic agents in the CSF was performed.

There are few studies with radiation doses that are similar to those used here. Following administration of 18–24 Gy with daily fractions of 2 Gy, 5 days a week, the BBB permeability in 128 children with leukemia or non-Hodgkin’s lymphoma was evaluated by Korinthenberg (23). Changes in the CSF were noted at the end of the irradiation, as were increases in albumin, α-macroglobulin, lactate dehydrogenase, and amino acid levels. No pharmacokinetic studies of antineoplastic agents were conducted in this study.

Seshandri et al. (24) studied the effect of CI on BBB permeability in 14 patients with ALL and non-Hodgkin’s lymphoma. In eight patients without CNS leukemia, no significant change in MTX CSF levels was noted following CI at the dose of 24 Gy. We found no significant variation of 6-mercaptopurine (M, 152) CSF levels in 15 children with ALL during or after a course of 18–24 Gy whole-brain irradiation for CNS prophy-laxis (25). Here, ara-C levels in CSF following the doses of RT used for CNS prophylaxis remain unchanged, suggesting that no alteration of BBB occurs as a consequence of CI. The BBB is a complex structure that acts as a highly selective barrier between blood and brain. The development and the maintenance of the BBB derives from a complex interaction between endothelial cells and trophic factors produced by astrocytes. In addition, it appears that multidrug resistance p-glycoprotein (MDR1) plays a central role in the maintenance of the BBB (26, 27). ara-C possesses physicochemical characteristics that are quite different from those of other anticancer drugs, for which BBB may play an important role in preventing adequate penetration of the CNS. ara-C is a small molecule (M, 341), is hydrophilic, and is not a substrate for p170 glycoprotein encoded by MDR1 gene. Therefore, our results cannot be extended to other antineoplastic agents. However, animal data and clinical studies appear to suggest that, following the dose of 18–24 Gy administered by standard schedules, no major changes of BBB permeability should occur. The beneficial therapeutic effect of CI clearly observed in ALL and, more recently, in AML (3) does not appear to be related to an alteration of BBB but, rather, to a direct effect on leukemic blasts.

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REFERENCES


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