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ORIGINAL ARTICLE

Plasma and cerebrospinal fluid pharmacokinetics of nelarabine in nonhuman primates

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Abstract
Introduction Nelarabine is a water-soluble prodrug of the cytotoxic deoxyguanosine analog ara-G, to which it is rapidly converted in vivo by adenosine deaminase. Nelarabine has shown activity in the treatment of T-cell malignancies, especially T-cell acute lymphoblastic leukemia. Preliminary data suggested that nelarabine might penetrate into the CSF. We therefore studied the CSF penetration of nelarabine and ara-G in a nonhuman primate model that has been highly predictive of anticancer drug distribution in humans.
Methods Nelarabine (35 mg/kg, ~700 mg/m^2) was administered over 1 h through a surgically implanted central venous catheter to four nonhuman primates. Blood (four animals) and ventricular CSF (three animals) samples were obtained at intervals for 24 h for determination of nelarabine concentrations, which were measured by HPLC-mass spectrometry.
Results The nelarabine plasma AUC (median ± s.d.) was 2,820 ± 1,140 μM min and the ara-G plasma AUC was 20,000 ± 8,100 μM min. The terminal half-life of nelarabine in plasma was 25 ± 5.2 min and clearance was 42 ± 61 ml/min/kg. The terminal half-life of ara-G in plasma was 182 ± 45 min. In CSF the terminal half-life of nelarabine was 77 ± 28 min and of ara-G was 232 ± 79 min. The AUC_{csf}:AUC_{plasma} was 29 ± 11% for nelarabine and 23 ± 12% for ara-G.
Conclusion The excellent CSF penetration of nelarabine and ara-G supports further study of the contribution of nelarabine to the prevention and treatment of CNS leukemia.

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Introduction

Administration of antimetabolites either systemically or by the intrathecal route is important in the prevention of central nervous system (CNS) leukemia [1, 2]. Although limited penetration into the CNS often favors intrathecal administration as a strategy for producing therapeutic drug concentrations in the cerebrospinal fluid (CSF), some nucleoside analogs penetrate relatively well into CSF after systemic administration. For example, the CSF penetration of both cytarabine and cladribine is approximately 20% [3–5]. Clofarabine also penetrates into the CSF at concentrations that may be therapeutic [6].

Nelarabine is a water-soluble prodrug of the cytotoxic deoxyguanosine analog ara-G, to which it is rapidly converted in vivo by adenosine deaminase [7]. Nelarabine has shown activity in the treatment of T-cell malignancies, especially T-cell acute lymphoblastic leukemia [8, 9]. Preliminary data suggested that

nelarabine might penetrate into the CSF [10]. We therefore studied the CSF penetration of nelarabine and ara-G in a nonhuman primate model that has been highly predictive of anticancer drug distribution in humans [11].

Materials and methods

Drug

Nelarabine was supplied by GlaxoSmithKline (Collegeville, PA, USA) in 50-ml vials containing 5 mg/ml of nelarabine. The appropriate dose of the drug was administered without further dilution.

Animals

The study was approved by the Animal Care and Use Committee. Four healthy adult male rhesus monkeys (*Maccaca mulatta*) weighing 10.5–16 kg were used in these experiments. The animals were fed Open Formula Extruded Non-Human Primate Diet twice daily and group-housed in accordance with Guide for the Care and Use of Laboratory Animals [12]. Drug was administered through a surgically implanted central venous catheter. Blood samples were drawn through a catheter placed in the contralateral femoral or saphenous vein. Ventricular CSF samples were obtained from a chronically indwelling fourth ventricular catheter attached to a subcutaneously implanted Ommaya reservoir [11]. The reservoir was pumped four times before and after each CSF sample collection to ensure adequate mixing with ventricular CSF.

Experiments

Four animals received 35 mg/kg (~700 mg/m^2) of nelarabine administered intravenously over 1 h. Blood and ventricular CSF samples were collected into EDTA-containing tubes with 15 µl of 0.5 mM deoxycoformycin added to prevent ex vivo adenosine deaminase activity. Plasma samples were obtained from the four animals before the dose, at 30 min during the infusion, at the end of the infusion, and at 15 min, 30 min, and 1, 2, 4, 6, and 8 h after the infusion. Three animals also had plasma sampling at 24 h after the infusion. CSF samples were obtained from three animals before the dose, at 30 min during the infusion, at the end of the infusion, and at 30 min and 1, 2, 4, 6, and 8 h after the infusion. Two animals also had CSF sampling at 24 h after the infusion. Plasma was separated immediately by centrifugation at 1,500 rpm for 10 min. Plasma and CSF were frozen immediately after collection. Clinical laboratory studies including complete blood counts, electrolytes, liver function tests, and renal function tests were obtained on a weekly basis for a minimum of 3 weeks after the nelarabine infusion. Animals were also observed on a daily basis for a minimum of 3 weeks postinfusion for any evidence of clinical toxicity.

Sample analysis

Plasma and CSF of nelarabine and ara-G were measured by a validated HPLC-MS/MS bioanalytical method with a dynamic range of 5–5,000 ng/ml for nelarabine and 50–50,000 ng/ml for ara-G. The coefficient of variation was <10%. Nelarabine and ara-G were extracted from 50 µl of stabilized monkey plasma by protein precipitation using acetonitrile containing stable isotopically labeled internal standards ([$^{13}C^{15}N_3$]-GI262250 and [$^{13}C^{15}N_3$]-GI186898). CSF samples for nelarabine and ara-G analysis were prepared by adding 50 µl of sample to water containing stable isotopically labeled internal standards. The HPLC-MS/MS system consisted of an Applied Biosystems API–4000 mass spectrometer (Applied Biosystems/MDS Sciex, Canada) coupled to a Shimadzu SCL–10A VP HPLC system (Shimadzu, Columbia, MD). For the plasma extracts, separation was achieved on a 50 × 3.0 mm i.d. Genesis C18, 3 µm column using an isocratic HPLC method with 8% acetonitrile and a 1.8-min cycle time. For CSF samples, a 3-min gradient elution consisting of 5 mM ammonium acetate, pH 4 (mobile phase A) and 100% acetonitrile (mobile phase B) and a 50 × 3.0 mm i.d. Genesis C18, 3 µm column were used to achieve the desired separation. The mass spectrometer was equipped with a TurboIonSpray™ interface. Multiple reaction monitoring was used to monitor characteristic precursor $[M + H]^+$ to product ions transitions of nelarabine (*m/z* 298/166) and ara-G (*m/z* 284/152) and the stable isotopically labeled internal standards [$^{13}C^{15}N_3$]-GI262250 (*m/z* 302/170) and [$^{13}C^{15}N_3$]-GI186898 (*m/z* 288/156).

Pharmacokinetic analysis

Nelarabine plasma and CSF clearance, area under the concentration time curve (AUC), half life, and volume of distribution at steady state (Vd_{ss}) were determined using noncompartmental methods. The AUC was calculated using the trapezoidal method and the clearance was calculated from the equation clearance = dose/AUC. The steady-state volume of distribution (Vd_{ss}) was calculated from the area under the moment curve

[13]. Subsequently, a compartmental model was built that could be used to predict plasma and CSF concentrations after various infusion regimens. For this model, first, one and two compartment open models were fit to the individual animals' plasma concentration-time data using ADAPT II [14]. The best fit was determined using Akaike's information criterion [15]. Subsequently, concentrations of nelarabine and ara-G in plasma and CSF were modeled simultaneously for the three animals having both plasma and CSF sampling using a four-compartment model (Fig. 1) where V_1 and V_2 represent the systemic volumes for nelarabine and ara-G, V_3 and V_4 represent the CSF volumes for nelarabine and ara-G, and k with a subscript represents the rate constant for transfer between the indicated compartments. We assumed that the conversion of nelarabine to ara-G takes place primarily in the systemic compartment and fixed the CSF volumes at approximately 15 ml (the approximate CSF volume of the rhesus monkey). Finally, the parameters derived from this model were used to simulate plasma and CSF concentrations after the administration of 650 mg/m^2 of nelarabine daily for 5 consecutive days (the recommended dose for T-cell acute lymphoblastic leukemia).

Results

Animals did not show overt signs of neurologic toxicity. The noncompartmentally determined pharmacokinetic parameters of nelarabine and ara-G in plasma and CSF are shown in Tables 1 and 2, respectively. The nelarabine plasma AUC (median ± SD) was 2,820 ± 1,140 μM min and the ara-G plasma AUC was 20,000 ± 8,100 μM min. The terminal half-life of nelarabine in plasma was 25 ± 5.2 min, clearance was 42 ± 61 ml/min/kg, and central volume of distribution was 1.1 ± 3.1 l/kg. The terminal half-life of ara-G in plasma was 182 ± 45 min and the central volume of distribution was 1.4 ± 3.2 l/kg. In CSF the terminal half-life of nelarabine was 77 ± 28 min and of ara-G was 232 ± 79 min. The AUC_{csf}:AUC_{plasma} was 29 ± 11% for nelarabine and 23 ± 12% for ara-G.

Figure 2 shows the concentrations of nelarabine and ara-G in plasma and CSF predicted by the parameters estimated for the four-compartment model shown in Fig. 1 compared with measured values in the three animals that had both plasma and CSF sampling. The estimated parameter values are: $k_{10} = 0.00008$ min^{-1}, $k_{12} = 0.04$ min^{-1}, $k_{13} = 0.000004$ min^{-1}, $k_{20} = 0.0044$ min^{-1}, $k_{24} = 0.0000019$ min^{-1}, $k_{31} = 0.0093$ min^{-1}, $k_{42} = 0.0061$ min^{-1}; $V_1 = 1.0$ l/kg, $V_2 = 1.0$ l/kg.

This model predicts that the maximum concentration (C_{max}) and time to maximum concentration (T_{max}) is 52 μM at 1 h for nelarabine in plasma; 98 μM at 1.7 h for ara-G in plasma; 5.1 μM at 1.5 h for nelarabine in CSF; and 17.1 μM at 4.2 h for ara-G in CSF. The same model was then used to simulate plasma and CSF nelarabine and ara-G concentrations after infusion of 650 mg/m^2/day of nelarabine daily × 5 days, the recommended dose and schedule in children with leukemia. The simulation predicts that nelarabine and ara-G do not accumulate with daily infusions because of their relatively short half-lives (data not shown).

Discussion

The plasma clearance and half-life of nelarabine and ara-G in the nonhuman primate are similar to those

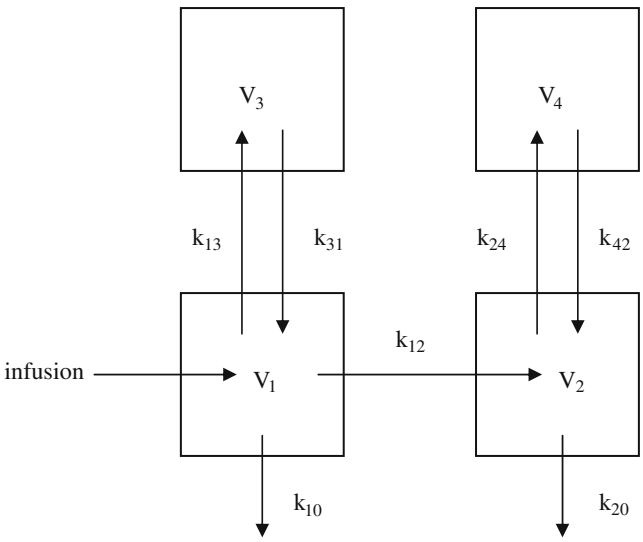

Fig. 1 Compartmental model for nelarabine and ara-G in plasma and CSF, where V_1 and V_2 represent the systemic volumes for nelarabine and ara-G, V_3 and V_4 represent the CSF volumes for nelarabine and ara-G, and k with a subscript represents the rate constant for transfer between the indicated compartments or out of the body. k_{12} represents the metabolic transformation of nelarabine to ara-G

Table 1 Pharmacokinetic parameters of nelarabine and ara-G in plasma after administration of 35 mg/kg of nelarabine IV over 1 h

Animal	Nelarabine AUC (μM min)	Nelarabine half-life (min)	Nelarabine clearance (ml/min/kg)	Nelarabine Vdss (l/kg)	Ara-G AUC (μM min)	Ara-G half-life (min)
1	2,940	19.5	40	0.9	19,330	115
2	3,260	27.3	36	1.3	20,670	180
3	2,700	22.6	44	2.3	25,620	183
4	730	31.4	162	7.9	6,600	223
Median	2,820	25.0	42	1.3	20,000	182
SD	1,140	5.2	61	3.3	8,100	45

Table 2 Pharmacokinetic parameters of nelarabine and ara-G in CSF after administration of 35 mg/kg of nelarabine IV over 1 h

Animal	Nelarabine AUC (μM min)	Nelarabine half-life (min)	Ara-G AUC (μM min)	Ara-G half-life (min)	Nelarabine AUC_{csf}:AUC_{plasma} (%)	Ara-G AUC_{csf}:AUC_{plasma} (%)
1	870	100	4,400	366	29	23
2	1,210	77	2,440	232	37	12
3	430	45	9,160	225	16	36
Median	870	77	4,400	232	29	23
SD	390	28	3,460	79	11	12

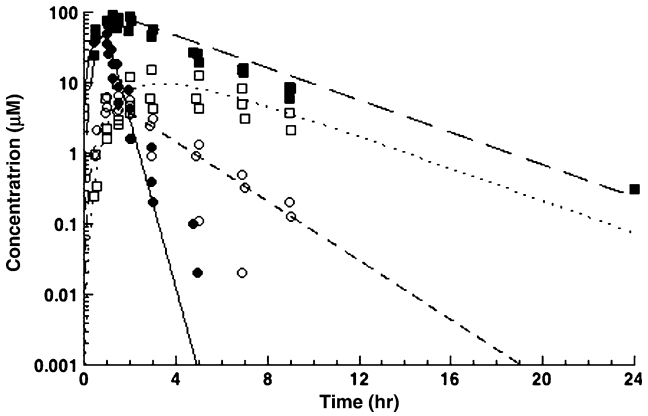

Fig. 2 Model-predicted versus measured concentrations of nelarabine and ara-G in plasma and CSF of three animals following a 1-h IV infusion of 35 mg/kg of nelarabine. *Solid line*, predicted plasma nelarabine concentration; *long dashed line* predicted plasma ara-G concentration; *short dashed line* predicted CSF nelarabine concentration; *dotted line* predicted CSF ara-G concentration; *filled circles* measured plasma nelarabine concentration; *filled squares* measured plasma ara-G concentration; *open circles* measured CSF nelarabine concentration; *open squares* measured CSF ara-G concentration

reported in adults and children [16]. Nelarabine is rapidly cleared from plasma, but exposure to ara-G is more prolonged, consistent with nelarabine's function as a prodrug for ara-G. The half-lives in CSF are slightly longer than those in plasma, probably due to ongoing distribution from the systemic compartment to the CSF compartment. It is unclear whether the high CSF penetration of ara-G may be related to the neurologic toxicity observed in some patients after nelarabine administration; the mechanism of this toxicity remains unknown.

The results of this study are consistent with the clinical observation that nelarabine appears to have at least some activity in clearing CSF lymphoblasts when administered as a single systemic agent. In children with T-cell malignancies, 8 of 22 patients who had positive CSF cytology pretreatment had negative CSF cytology 1 week later, after five daily infusions of 400 mg/m^2 of nelarabine, without intrathecal chemotherapy [9]. Our study shows that both nelarabine and ara-G penetrate well into the CSF, with an AUC_{csf} to AUC_{plasma} ratio of >20% for each. This is similar to the CSF penetration of high-dose cytarabine [3]. The IC_{50} (concentration required to inhibit 50% of the growth) of ara-G in MOLT-4 T-lymphoblasts during a 3-day exposure is 0.15 μM [17]. Concentrations tenfold higher than this were present in nonhuman primate CSF for 8 h after the dose of 35 mg/kg (~700 mg/m^2), and the simulations predict that concentrations above 0.15 μM persist for approximately 22 h after a 650 mg/m^2 dose. Thus, it is quite likely that cytotoxic ara-G exposure in CSF is prolonged in humans, where similar doses (650–1,500 mg/m^2) are used on daily for 5 days or every other day for 5-day schedules.

Strategies to prevent the development of overt CNS disease form an important component of leukemia therapy in childhood. Since the blood–CSF and blood–brain barriers limit the penetration of many agents into the CNS, it is important to evaluate the CSF penetration of new agents. The excellent CSF penetration of nelarabine and ara-G supports further study of the

contribution of nelarabine to the prevention and treatment of CNS leukemia.

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