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Clinical study of an organic arsenical, melarsoprol, in patients with advanced leukemia

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Abstract Inorganic arsenic trioxide (As_2O_3) induces a high proportion of complete remissions in relapsed patients with acute promyelocytic leukemia (APL). Previously, we have shown that both As_2O_3 and melarsoprol, an organic arsenical used for the treatment of trypanosomiasis, exhibit broad antileukemic activity against both chronic and acute myeloid and lymphoid leukemia cell lines. Given the breadth of this activity, we initiated a clinical study to evaluate the pharmacokinetics, safety, and potential efficacy of melarsoprol in patients with refractory or resistant leukemia. Using the antitrypanosomal dose and schedule, patients received escalating intravenous doses daily for 3 days, repeated weekly for 3 weeks. Doses were 1 mg/kg on day 1, 2 mg/kg on day 2, and 3.6 mg/kg on day 3 and on all days thereafter, up to a maximum daily dose of 200 mg. Eight patients [6 AML (2 morphologic APL), 1 CML, 1 CLL] were treated. Mean peak plasma concentrations of the parent drug were obtained immediately after injection, ranged from 1.2 μg/ml on day 1 to 2.4 μg/ml on day 3, were dose proportional, and decayed with a $t_{1/2\beta} \cong 15$ min. A minor clinical response (regression of splenomegaly and lymphadenopathy) was observed in a patient with chronic lymphocytic leukemia. Central nervous system (CNS) toxicity proved limiting on this dose and schedule. Three patients experienced generalized grand mal seizures during the second week of therapy. We concluded that this dose and schedule of melarsoprol is associated with excessive CNS toxicity and that verification of the striking preclinical activity in patients with leukemia will require developing an alternative dose and schedule.

Key words Organic arsenical · Melarsoprol · Advanced leukemia · As_2O_3 · Inorganic arsenic trioxide

Introduction

Since the introduction of all-*trans* retinoic acid for remission induction, survival of patients with acute promyelocytic luekemia (APL) has increased more than twofold compared to patients treated with chemotherapy alone [1–3]. However, approximately 20% to 30% of patients still relapse despite having received state-of-the-art therapy. Such patients are frequently resistant to standard treatment, and salvage therapy is both highly toxic and rarely curative. Recently, two groups from China have reported that inorganic arsenic trioxide (As_2O_3) induces complete remissions in a high proportion of heavily pretreated patients with APL [4, 5]. Coincident with these reports, we evaluated both As_2O_3 and melarsoprol, an organic arsenical used for the treatment of African trypanosomiasis (due to *Trypanosoma brucei*) for possible antileukemic activity in vitro. In these studies melarsoprol exhibited broad antileukemic activity against both myeloid and lymphoid cells [6, 7]. In view of these striking antileukemic effects, we initiated a pilot study to evaluate the pharmacokinetics,

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safety, and efficacy of melarsoprol in patients with relapsed or refractory leukemia.

Methods

Study design

Patients with relapsed or refractory acute or chronic leukemia of any type were eligible for entry. Melarsoprol (melaminyl-phenyl arsenoxide, supplied as Arsobal®; Fig. 1) was provided in 6-ml ampoules containing 36 mg/ml of drug dissolved in a 3.6% solution of propylene glycol and was purchased from Rhône Poulenc Rorer (Paris, France). The dose and schedule currently employed for the treatment of trypanosomiasis was used [8]. Melarsoprol was administered as a brief intravenous injection daily for 3 days, repeated weekly for 3 consecutive weeks. The initial dose was 1 mg/kg on day 1, 2 mg/kg on day 2, and 3.6 mg/kg on day 3 and on all days thereafter, up to a maximum daily dose of 200 mg. Patients who showed clinical improvement were eligible for one additional 3-week course of therapy, identical in dose and schedule to the induction course, and beginning no less than 2 weeks after the completion of the first treatment.

During the study, patients were monitored with serial determinations of blood counts, serum biochemical analyses, including liver function tests (ALT, AST, LDH, alkaline phosphatase), electrolytes, and creatinine, urinalyses and bone marrow aspirates and/or biopsies. The clinical protocol was reviewed and approved in advance by this center's Institutional Review Board. Signed informed consent was obtained from all patients.

Pharmacokinetic study

All patients underwent serial blood sampling on the first treatment day and on one or more days thereafter. On the first treatment day, 2 ml of whole blood was collected in a heparinized tube before injection, immediately after the injection, and again after 1, 2, 4 and 6 h. On all subsequent treatment days, pre- and posttreatment blood samples were obtained. Whole blood samples were centrifuged and the plasma was separated and stored in opaque containers at −20 °C until assayed. On each treatment day 24-h urine collections were obtained. After the total volume had been measured, a 100-ml aliquot was removed and stored in an opaque container at −20° C until assayed.

Analytical Procedure

Melarsoprol parent and metabolite concentrations in biological fluids were determined using a newly developed HPLC method [9–11]. Briefly, plasma was extracted using a Bond-Elut C2 cartridge column (Varian, Harbor City, Calif.) eluted with the HPLC mobile phase. HPLC analysis was then performed using a Ranin C18 5-mm column (4.6 × 250 mm) with a mobile phase consisting of 35% acetonitrile/65% potassium dihydrophosphate and 1% triethylamine, at a flow rate of 1 ml/min, and monitored at 280 nm. Using this method, the lower limit of detection was 10–50 ng/ml using a 20-ml injection volume. Recovery compared to the unextracted mobile phase was >90%. Inorganic (free) arsenic was measured using a standard induction-coupled plasma-mass spectroscopy method (MedTox Laboratories, St. Paul, Minn.).

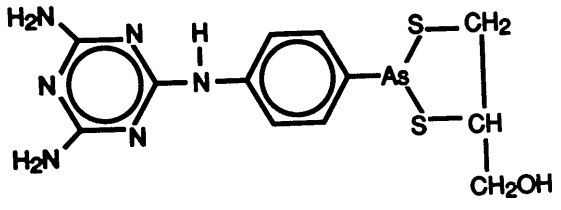

Fig. 1 Chemical structure of melarsoprol (melaminyl-phenyl-arsenoxide)

Results

Patient characteristics

Eight patients were enrolled on this study; they had the clinical characteristics presented in Table 1. Six patients had acute myelocytic leukemia (AML), two of whom had the morphologic phenotype of acute promyelocytic leukemia (APL). Of the latter two patients, one had the classic cytogenetic translocation found in this disease which involves the gene encoding for the retinoic acid receptor-alpha (RARα) on chromosome 17 and the promyelocytic leukemia gene (PML) on chromosome 15 (t[15; 17]), and the other had a cytogenically variant form involving a translocation between chromosomes 11 and 17 (t[11; 17]). This latter form of APL is less likely to respond to retinoic acid therapy and therefore portends a worse prognosis. One patient had chronic myelocytic leukemia (CML), and one patient had chronic lymphocytic leukemia (CLL). All patients had been treated with multiple chemotherapy regimens and were considered to have either resistant (seven patients) or primary refractory (one patient) disease.

Clinical efficacy

Due to the rapid emergence of central nervous system (CNS) toxicity, only four of the eight patients enrolled in the study completed the first planned course of therapy. No patient obtained a partial or complete remission as conventionally defined. The patient with CLL had significant reduction in both peripheral lymphadenopathy and splenomegaly (i.e. spleen decreased from 12 cm to 4 cm below the costal margin) but with no significant change in peripheral blood or bone marrow lymphocyte counts. Despite having a minor response, this patient could not receive further therapy due to neurotoxicity.

Table 1 Clinical characteristics of patients treated with melarsoprol

Number of patients enrolled	
Total	8
Males	6
Females	2
Age (years)	
Median	64.5
Range	26–68
Diagnosis	
Acute myeloid leukemia	6[a]
Chronic myeloid leukemia	1
Chronic lymphocytic leukemia	1

[a] Two of these patients had APL (FAB subtype, M3)

One patient with t(11; 17)-variant APL who had been refractory to chemotherapy and all-*trans* retinoic acid had slight improvements in both his leukocyte and platelet counts with the first course of treatment. He then received a second 3-week course without further improvement and was removed from the study. Three other patients were removed from study during their second week of treatment due to adverse reactions.

Adverse reactions

The most common adverse reactions were local irritation and burning at the infusion site during direct push injection of this highly viscous agent (Table 2). Nausea and vomiting occurred shortly after the drug injection in six patients, one of whom required hospitalization to control persistent nausea.

Neurotoxicity was the limiting adverse effect. Three patients experienced generalized seizures during their second week of therapy, which were treated with parenteral diazepam and phenytoin. The first seizure occurred in an elderly woman with an antecedent seizure history who was found to have a subtherapeutic level of phenytoin. When her phenytoin dosage was increased, she had no further episodes. The second patient developed intention tremors and expressive aphasia 1 day preceding a seizure. Electroencephalogram (EEG) in this individual revealed an abnormal nonspecific spike in the right centroparietal region and intermittent right frontotemporal theta activity. A repeat EEG performed 3 days later was completely normal, and this patient recovered without sequelae. The patient with relapsed t(15; 17) APL developed pulmonary hemorrhage and a generalized seizure in the setting of fever and coagulopathy, from which she ultimately expired. Despite evidence of a clinical response after the first course of therapy, the patient with CLL developed intention tremors and stuttering. In view of the neurotoxicity encountered in other patients, we did not offer this individual further treatment; his neurotoxic symptoms resolved 3 weeks after the last dose.

Table 2 Adverse reactions associated with melarsoprol (NCI grading scale)

Reaction	Grade			
	1	2	3	4
Fatigue	3	1	1	
Nausea	3	2	1	
Vomiting	3	1	1	
Diarrhea	1			
Vertigo	2			
Fever		1		
Neurocortical (seizures)				3
Back pain	3			
Headache	2			
Injection site irritation	7			

Other neurologic reactions included vertigo (two patients) and paresthesia (one patient), both of which resolved after discontinuing therapy. The patient who received two complete treatment cycles experienced the most severe peripheral neuropathy. This individual developed bilateral lower extremity numbness and tingling that extended below the knees, which caused moderate functional impairment. A nerve conduction study revealed nonspecific findings. The patient did not return to this center for follow-up; however, all symptoms were reported to have resolved approximately 5 weeks after the last day dose.

Pharmacokinetics

Peak plasma concentrations of the parent drug were obtained immediately after injection, which ranged from 1.2 μg/ml on day 1 to 2.4 μg/ml on day 3. Plasma areas under the concentration-times-time curves (AUCs) were proportional to the administered dose, ranging from 0.48 μg · h/ml on day 1 to 1.48 μg · h/ml on day 3 (Fig. 2a). Peak plasma levels were dose-proportional and decayed with a $t_{1/2\beta} \cong 15$ min. Levels of the parent compound were undetectable in plasma at 24 h. There was no detectable level of parent drug in urine at any time. Blood and urine samples were also analyzed for elemental arsenic content. The mean plasma AUC of arsenic on day 1 was 6038 μg · h/ml (range 4497–9095 μg · h/ml), $T_{1/2\alpha}$ was 38 min (range 17–51 min) and $T_{1/2\beta}$ was 19.5 h (range 18.6–47.6 h). There was a gradual increase in trough plasma levels of elemental arsenic over the 3-week treatment course (Fig. 2b).

Discussion

Arsenicals have been used as medicines for thousands of years and are still prominent ingredients of certain folk remedies in Central and Southern Asia [12]. Arsenic was a relatively common anticancer agent in the West up until the 1930 s. For example, Fowler's solution (potassium arsenite) was formerly used to control the leukocytosis associated with chronic myelocytic leukemia [13, 14]. However, with the availability of more conventional therapies (i.e. cytotoxic chemotherapy and radiation therapy), the general use of arsenic was gradually abandoned [15, 16]. In the West, the only remaining therapeutic use of arsenicals has been for the treatment of certain tropical infections.

Melarsoprol was first developed in 1949 as a more effective form of treatment for the late stages of African sleeping sickness [17, 18] that results from CNS invasion by flagellated protozoan parasites (*T. brucei*). Although this agent has excellent antitrypanocidal activity, its use has been limited to advanced stages of the disease because of severe side effects [19]. A "reactive encephalopathy" (Jurisch Herxheimer reaction) occurs in 2% to 10% of patients treated for late trypanosomiasis, a

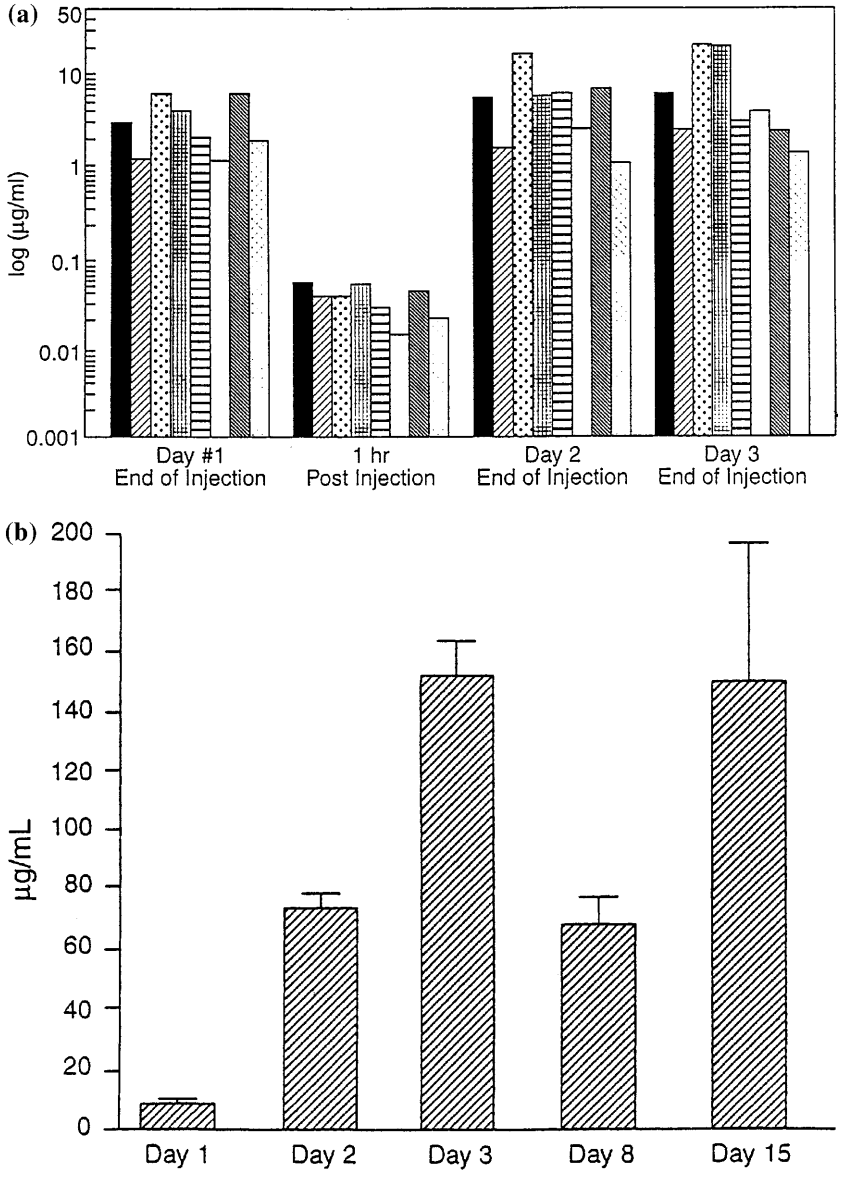

Fig. 2 **a** Peak plasma concentrations of melarsoprol obtained immediately after the end of injection on days 1, 2, and 3 of the first week of therapy, and the first day of treatment on weeks 2 (day 8) and 3 (day 15) of the first cycle. Each *bar* represents a single patient. **b** Trough plasma levels of melarsoprol obtained during the first cycle of treatment

syndrome that is characterized by increased mental excitement, twitching and choreoathetosis, followed by confusion, hyperkinesis, seizures, and occasionally death [19–21]. This reaction has been suspected to result from immunologic effects induced by dying parasites and/or brain damage rather than a direct drug effect [22], in part because its incidence varies and appears to be independent of the administered dose [23]. The severity of the reaction is ameliorated by pretreatment with corticosteroids [20].

In our study using the dose and schedule designed for trypanosomiasis treatment, we observed significant neurotoxicity, including seizures in three of the eight patients treated. We were not able to obtain cerebrospinal fluid (CSF) to assess drug levels in any of these patients. However, in a detailed pharmacokinetic study CSF levels were found to vary significantly between patients, with a range of 21 to 38 ng/ml at 24 h and 0 to 11 ng/ml at 120 h after the last injection [19]. These concentrations were several logs lower than serum concentrations measured at the same times. Nonetheless, in view of our experience, melarsoprol must be presumed to exert a direct toxic effect on the CNS, possibly because of its highly lipophilic chemical structure.

In comparative studies with arsenic trioxide, melarsoprol has been shown to display broad antileukemic effects against both acute and chronic lymphoid and myeloid cell lines [7]. In a detailed study with CLL B-cell lines, melarsoprol caused a dose- and time-dependent inhibition of cell growth and survival, induction of apoptosis, and a decrease or loss of bcl-2 expression [6]. By contrast, arsenic trioxide was less potent. Interestingly, the single patient with CLL exhibited the only significant antitumor response in this study.

The CNS toxicity observed in this study limits the clinical utility of melarsoprol at this dose and schedule. For now, melarsoprol cannot replace arsenic trioxide as the arsenical of choice for APL [24]. Clinical verifica-

tion of the high level of antileukemic activity observed in vitro will require development of a safer dosing regimen.

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References

1. Fenaux P, Le Dely MC, Castaigne S, et al (1993) Effect of all-*trans* retinoic acid in newly diagnosed acute promyelocytic leukemia: results of a multicenter randomized trial. Blood 82: 3241
2. Frankel SR, Eardley A, Miller WH Jr., et al (1994) Treatment of acute promyelocytic leukemia with all-*trans* retinoic acid: results of the New York study. Ann Intern Med 120: 278
3. Soignet S, Fleischauer A, Polyak T, et al (1997) All-*trans* retinoic acid significantly increases 5-year survival in patients with acute promyelocytic leukemia: long-term follow-up of the New York Study. Cancer Chemother Pharmacol 40 [Suppl]: S25
4. Chen GQ, Zhu J, Shi XG, et al (1996) In vitro studies on cellular and molecular mechanisms of arsenic trioxide (As_2O_3) in the treatment of acute promyelocytic leukemia: As_2O_3 induces NB4 cell apoptosis with downregulation of Bcl-2 expression and modulation of PML-RAR alpha/PML proteins. Blood 88: 1052
5. Shen Z-X, Chen G-Q, Ni J-H, et al (1997) Use of arsenic trioxide (As_2O_3) in the treatment of acute promyelocytic leukemia (APL): II clinical efficacy and pharmacokinetics in relapsed patients. Blood 89: 3354
6. Konig A, Wrazel L, Warrell RP Jr., et al (1997) Comparative activity of melarsoprol and arsenic trioxide in chronic B-cell leukemia lines. Blood 90: 562
7. Rivi R, Calleja E, Konig A, et al (1996) Organic arsenical melarsoprol shows growth suppressive activity via programmed cell death on APL and other myeloid and lymphoid leukemia derived cell lines. Blood 88 [Suppl]: 68a
8. Pépin J, Milord F, Khonde AN, et al (1995) Risk factors for encephalopathy and mortality during melarsoprol treatment of *Trypanosoma brucei gambiense* sleeping sickness. *Trans* R Soc Trop Med Hyg 89: 92
9. Berger BJ, Fairlamb AH (1994) High-performance liquid chromatographic method for the separation and quantitative estimation of anti-parasitic melaminophenyl arsenical compounds. *Trans* R Soc Trop Med Hyg 88: 357
10. Koons RD, Peters CA (1994) Axial distribution of arsenic in individual human hairs by solid sampling graphite furnace AAS. J Anal Toxicol 18: 36
11. Nixon DE, Moyer TP (1992) Arsenic analysis II: rapid separation and quantification of inorganic arsenic plus metabolites and arsenobetaine from urine. Clin Chem 38: 2479
12. Klaassen CD (1995) Heavy metals and heavy metal antagonists. In Gilman AG, Goodman LS, Rall TW, Murad F (eds) Goodman's and Gilman's the pharmacological basis of therapeutics, 7th edn. MacMillan, New York, p 1605
13. Stephens DJ, Lawrence JS (1936) The therapeutic effects of solution of potassium arsenite in chronic myelogenous leukemia. Ann Intern Med 9: 1488
14. Forkner CE (1931) Arsenic as a therapeutic agent in chronic myelogenous leukemia. JAMA 97: 3
15. Jackson R, Grainge JW (1975) Arsenic and cancer. Can Med Assoc J 113: 396
16. Cuzick J, Evans S, Gillman M, et al (1982) Medicinal arsenic and internal malignancies. Br J Cancer 45: 904
17. Friedheim EAH (1949) The active form of tryparsamide TPB in the treatment of human trypanosomiasis. Am J Trop Med 29: 173
18. Williamson J, Lourie EM (1948) "Melarsen" and "melarsen oxide". Nature 161: 103
19. Burri C, Baltz T, Giroud C, et al (1993) Pharmacokinetic properties of the trypanocidal drug melarsoprol. Chemotherapy 39: 225
20. Pépin J, Milord F, Guerin C, et al (1989) Trial of prednisolone for prevention of melarsoprol-induced encephalopathy in gambiense sleeping sickness. Lancet i: 1246
21. Reference deleted
22. Pepsin J, Milord F (1991) African trypanosomiasis and drug-induced encephalopathy; risk factors and pathogenesis. Trans R Soc Trop Med Hyg 85: 222
23. Haller L, Adams H, Merouze F, et al (1986) Clinical and pathological aspects of human African trypanosomiasis (*T.B. gambiense*) with particular reference to reactive arsenical encephalopathy. Am J Trop Med Hyg 35: 94
24. Soignet SL, Maslak P, Wang Z-G et al (1998) Complete remission after treatment of acute promyelocytic leukemia with arsenic trioxide. N Engl J Med 339: 1341