Dose-dependent Neurotoxicity of High-Dose Busulfan in Children: A Clinical and Pharmacological Study

Gilles Vassal, Alain Deroussent, Olivier Hartmann, Dominique Challine, Ellen Benhamou, Dominique Vallette-Couanet, Laurence Brugières, Chantal Kalifa, Alain Gouyette, and Jean Lemerle

ABSTRACT

Busulfan is known to be neurotoxic in animals and humans, but its acute neurotoxicity remains poorly characterized in children. We report here a retrospective study of 123 children (median age, 6.5 years) receiving high-dose busulfan in combined chemotherapy before bone marrow transplantation for malignant solid tumors, brain tumors excluded. Busulfan was given p.o., every 6 hours for 16 doses over 4 days. Two total doses were consecutively used: 16 mg/kg, then 600 mg/m². The dose calculation on the basis of body surface area results in higher doses in young children than in older patients (16 to 28 mg/kg). Ninety-six patients were not given anticonvulsive prophylaxis; 7 (7.5%) developed seizures during the 4 days of the busulfan course or within 24 h after the last dosing. When the total busulfan dose was taken into account, there was a significant difference in terms of neurotoxicity incidence among patients under 16 mg/kg (1 of 57, 1.7%) and patients under 600 mg/m² (6 of 39, 15.4%) (P < 0.02). Twenty-seven patients were given a 600 mg/m² busulfan total dose with continuous i.v. infusion of clonazepam; none had any neurological symptoms. Busulfan levels were measured by a gas chromatographic-mass spectrometry assay in the plasma and cerebrospinal fluid of 9 children without central nervous system disease under 600 mg/m² busulfan with clonazepam:busulfan cerebrospinal fluid:plasma ratio was 1.39. This was significantly different (P < 0.02) from the cerebrospinal fluid:plasma ratio previously defined in children receiving a 16-mg/kg total dose of busulfan.

This study shows that busulfan neurotoxicity is dose-dependent in children and efficiently prevented by clonazepam. A busulfan dose calculated on the basis of body surface area, resulting in higher doses in young children, was followed by increased neurotoxicity, close to neurotoxicity incidence observed in adults. Since plasma pharmacokinetic studies showed a faster busulfan clearance in children than in adults, this new dose may approximate more closely the adult systemic exposure obtained after the usual 16-mg/kg total dose, with potential inferences in terms of anticancer or myeloablative effects. The busulfan dose in children and infants undergoing bone marrow transplantation should be reconsidered on the basis of pharmacokinetic studies.

INTRODUCTION

Busulfan, a bifunctional alkylating agent, has been the treatment of reference for chronic myeloid leukemia during the past 30 years. Since 1980, high-dose busulfan in combination chemotherapy followed by bone marrow rescue has been used for several malignancies (acute lymphoblastic and myeloblastic leukemias, solid tumors) (1–5) and before allogeneic bone marrow transplantation for nonmalignant diseases (metabolic diseases, immunodeficiencies, thalassemia, osteoporosis) (6–8). These therapies are particularly used in pediatrics, in place of the conventional total body irradiation-cyclophosphamide myeloablative regimen in order to avoid the long-term side effects of radiation therapy.

Since the early preclinical studies, busulfan is known to be neurotoxic in animals (9). Acute neurotoxicity is dose limiting, occurring in mice and rats after a dose greater than 70 mg/kg. Actually, busulfan is a small, lipophilic molecule that does not bind tightly to plasma proteins. Recently, we showed that busulfan crosses easily the blood-brain barrier with a mean CSF:plasma ratio of 100% in children receiving high-dose busulfan (10). Hassan et al. (11) found a mean CSF:plasma ratio of 130% in adults after the same dosage. Several cases of convulsion during high-dose busulfan therapy have been described in adults and the use of anticonvulsive prophylaxis is widespread (12–15). However, this acute toxicity remains poorly characterized and even controversial in children (16).

Since 1983, we have been using high-dose busulfan in combined chemotherapy with bone marrow rescue in children with malignant solid tumors. Until 1987, we administered p.o. the usual 16-mg/kg total dose of busulfan and we did not see any significant neurotoxicity. In 1987, we started calculating the busulfan dose on the basis of body surface area, i.e., 600 mg/m², in order to better approximate the adult total dose. This new dose was followed by increased neurotoxicity that led us to add systematically an anticonvulsive prophylaxis starting in March 1989. Since busulfan distribution to the CSF was defined in our previous study (10), we designed a new pharmacokinetic study in children receiving 600 mg/m² with anticonvulsive therapy in order to understand the pharmacological mechanisms of busulfan neurotoxicity. We report here our experience in terms of neurotoxicity in 123 children, along with a pharmacological study of busulfan in the central nervous system.

MATERIALS AND METHODS

Patients. From March 1983 to April 1990, 123 children were treated with a combined chemotherapy including high-dose busulfan followed by autologous (n = 118) or allogeneic (n = 5) bone marrow rescue, in the Pediatric Department of the Institute Gustave Roussy, Villejuif, France. These patients (median age, 6.5 years; range, 1–17 years) had malignant solid tumors, brain tumors excluded.

Ninety-six patients did not receive anticonvulsive prophylaxis. From March 1983 to July 1987, 57 patients (group 1) were given 16 mg/kg busulfan (total dose). From July 1987 to March 1989, 39 patients (group 2) received 600 mg/m² busulfan.

From March 1989 to April 1990, 27 children (group 3) were treated with a combined regimen including 600 mg/m² of busulfan and an anticonvulsive prophylaxis with clonazepam (0.1 mg/kg/day, continuous i.v. infusion).

Treatment. Busulfan was always associated with one or two other alkylating agents. The different protocols used are described in Table 1. Busulfan (Techni-Pharma Laboratory, Monaco Principality) was given p.o., on an empty stomach, every 6 h for a total of 16 dosings over 4 days. A 600-mg/m² dose was designed to better approximate the adult total dose; for an adult patient weighing 65 kg with a body surface area of 1.73 m², 16 mg/kg is equal to 600 mg/m². The dose calculation based on body surface area results in higher doses, as expressed in mg/
kg, in young patients than in teenagers (Fig. 1). Actually, the total dose varies from 16 to 28 mg/kg in groups 2 and 3.

Chemotherapy was given along with a 3-liter/m² i.v. hydration. Usually, no antiemetic drugs were used during the 4 days of busulfan course.

Neurotoxicity. Seven children (7 of 96, 7.5%) with a median age of 7 years (3.5 to 14 years), developed 8 episodes of acute neurotoxicity during busulfan therapy without prophylaxis of convulsion. They were treated for stage IV neuroblastoma (n = 3) and non-Hodgkin’s malignant lymphoma (n = 4). Two children were in second complete remission after CNS relapse. Their CSF was normal. Six children received a total dose of 600 mg/m² busulfan (i.e., 20.5 to 26.5 mg/kg) and one a total dose of 16 mg/kg. All the details are given in Table 2.

When a seizure occurred, a complete neurological examination was done along with the following: plasma and urinary ionic determination (8 of 8); fundoscopic examination (7 of 8); lumbar puncture (7 of 8); EEC (3 of 8).

Pharmacological Study. CSF and plasma samples were obtained in 9 children from group 1 (i.e., 16 mg/kg), 3.25 to 7 h after the 16th busulfan dosing. None of these patients had seizure. None of them had CNS disease. These data have been already published (10).

<table>
<thead>
<tr>
<th>Table 1 Combined high-dose chemotherapy followed by bone marrow rescue used in 123 children</th>
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<td>Drugs</td>
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</tr>
<tr>
<td>Busulfan</td>
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<tr>
<td>Busulfan-cyclophosphamide</td>
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<td>Busulfan-melphalan</td>
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<td>Busulfan-cyclophosphamide-melphalan</td>
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CSF and plasma samples were obtained in 10 children from the group 3 receiving 600 mg/m² (i.e., 17.1 to 26.5 mg/kg), 4 to 5 h after the 15th busulfan dosing. One of these 10 children had meningeval lymphoma dissemination at the onset of chemotherapy. The other 9 children did not have meningeval disease. No child experienced seizure. Details are given in Table 3.

Plasma samples were separated by centrifugation at 4°C within 1.5 h and then frozen and stored at −20°C. CSF samples were withdrawn by lumbar puncture within 10 min before or after plasma collection, immediately frozen, and stored at −20°C.

Drug Assay. Busulfan levels in biological fluids were determined by a gas chromatography–mass spectrometry assay using a deuterated busulfan analogue [2,2,3,3-2H]-1,4-butanediol dimethanesulfonate or busulfan-d₄, as the internal standard (17). Briefly, busulfan was extracted into ethyl acetate from biological samples spiked with 100 μl busulfan-d₄ (internal standard) solution in acetone (2.5 μg/ml). Busulfan and busulfan-d₄ were then converted to 1,4-diododobutane and 1,4-diododobutane-d₄, respectively, in 4 M aqueous sodium iodide solution, under stirring at 70°C for 30 min. The organic phase was washed in distilled water and evaporated to dryness. After reconstitution in 20 μl acetone, 0.2 μl was injected into the gas chromatography–mass spectrometry system. Mass spectrometry detection was done with single ion monitoring focused on ions m/z = 182.9671 and ion m/z = 186.9222 for 1,4-diododobutane and 1,4-diododobutane-d₄, respectively. The detection limit of busulfan in biological fluids is 0.5 ng/ml. The relative standard deviation of the method is 5%.

Statistical Analysis. The χ² test was used to compare the neurotoxicity incidence in the 3 groups. The nonparametric Wilcoxon test was used to compare CSF:plasma ratios in groups 1 and 3 and the regression analysis of individual CSF:plasma ratios against the dose of busulfan was tested with a Student t test.

RESULTS

Clinical Study. Eight episodes of acute neurotoxicity were observed in 7 children during the 4 days of busulfan course (n = 5) or within 24 h after the last dosing (18, 19, and 24 h) (n = 3). During busulfan treatment, the onset of seizure was 2 to 4 h after dosing. No convulsion occurred before the 7th dosing.

Six of 39 (15.4%) children receiving 600 mg/m² busulfan had generalized seizure, lasting less than 5 min in 5 patients and 30 min with cyanosis in 1 patient (patient 7). There were neither ionic nor CSF disturbances in any patient. One EEG showed nonspecific alterations. All patients recovered quickly without sequelae. One child (patient 4) developed two seizures, after the 7th and 15th dosings.

Only 1 child (patient 1) of 57 (1.7%) receiving 16 mg/kg developed generalized seizure. Because of an early vomiting after the 8th dosing, this 7.5-year-old girl received a second full dose within 1 h. Then, she had headache and 2 h later developed focal seizure with arms clonus, head and eye deviation to the right, and loss of consciousness for 30 min that needed both diazepam and phenobarbital. She had alteration of consciousness and blindness for 24 h. There were no ionic abnormalities, although she had weight gain. The CSF was normal; no edema was found by fundoscopic examination. The EEG showed focal left parietooccipital δ slow waves. She recovered within 2 days without any sequelae.

None of the other 89 children receiving high-dose busulfan without anticonvulsant prophylaxis developed neurological symptoms. No patient receiving 600 mg/m² along with clonazepam continuous i.v. infusion had neurological symptoms. On the other hand, we never observed any acute neurotoxicity in any child without brain tumor during a high-dose chemotherapy course that does not include busulfan.

Finally, 6 of 39 children (15.4%) in group 2 (600 mg/m²) and 1 of 57 children (1.7%) in group 1 (16 mg/kg) had seizure.
This difference was significant ($P < 0.02$, $\chi^2$ Yates). On the other hand, no child of 27% (0%) in group 3 (600 mg/m$^2$ with clonazepam) developed neurotoxicity. The difference between groups 2 and 3 is clinically compelling but does not reach a statistical significance ($P = 0.08$, $\chi^2$ Yates). More patients would be required to prove statistically the clinical benefit of clonazepam.

**Pharmacological Study.** The CSF:plasma ratio was determined at the end of busulfan therapy in 19 children. The CSF:plasma busulfan ratio 4 to 5 h after the 15th dosing was 1.39 ± 0.28 (SD) in 9 children without CNS disease receiving a total dose of 16 mg/kg with anticonvulsive prophylaxis, while CSF busulfan level ranges from 268 to 1616 ng/ml. In the previous group of 9 children receiving a total dose of 16 mg/kg, the CSF:plasma ratio was 1.02 ± 0.26, 3.25 to 7 h after the 16th dosing, with a 215- to 881-ng/ml range (Table 3). The difference between the two groups was significant ($P < 0.02$, Wilcoxon test). Moreover, when the individual CSF:plasma ratio was plotted against the dose (mg/kg), a regression line significantly different from zero was found ($r = 0.4942$, $P < 0.05$, Student $t$ test).

A CSF busulfan level of 1603 ng/ml with a CSF:plasma ratio of 2.47 was found in a 6.5-year-old boy with non-Hodgkin's lymphoma meningeal involvement at the time of busulfan therapy.

**DISCUSSION**

Since the introduction of high-dose busulfan in conditioning regimens for bone marrow transplantation, several cases of convulsions have been reported, especially in adults. Usually, they are described as generalized tonic-clonic seizures, occurring on therapy (3, 4, 14, 15). Martell et al. (12) described a case of myoclonic epilepsy and Marcus and Goldman (13) reported losses of consciousness with blurred vision and intermittent muscle twitching in a 31-year-old man. The incidence is estimated at 10% by Santos (18) in 106 adults treated for acute leukemia. Such a neurological toxicity is not described in children receiving high-dose busulfan for malignant or non-malignant diseases (7, 8, 16, 19).

In our retrospective study of 96 children receiving high-dose busulfan without anticonvulsive prophylaxis, the overall neurotoxicity incidence is 7.5%. The clinical features were those found in adults, i.e., generalized tonic-clonic seizures mainly short without neurological sequelae. However, when considering the total dose administered, there was a significant difference between children receiving 16 mg/kg and children receiving 600 mg/m$^2$ in terms of probability of developing an acute neurotoxicity (1.7% versus 15.4%). Only one child in group 1 (16 mg/kg busulfan total dose) had seizure, but she received two full doses at a 1-h interval. This suggests a dose-dependent neurotoxicity, since the dose calculation on the basis of body surface area results in higher doses in patients less than 65 kg than the calculation based on body weight.

Actually, neurotoxicity in children receiving 600 mg/m$^2$ is close to the usual incidence defined in adults. This suggests that such a dosage gives in children a closer approximation of the adult systemic and brain exposures to busulfan than the usual 16-mg/kg dose. Two recent studies defined the busulfan...
plasma pharmacokinetics in adults receiving high-dose therapy (1 mg/kg every 6 h for 16 doses) (11, 20). Their results are given in Table 4 along with the pharmacokinetic parameters determined in children receiving the same dose (10, 21). It shows that, after the first 1 mg/kg busulfan dose, a lower systemic exposure is obtained in children than in adults because a 2 to 4 times faster clearance of the drug. Grochow et al. (20) already showed that the occurrence of venoocclusive disease is correlated with busulfan systemic exposure in adults receiving a high-dose regimen. Low systemic exposures, due to a fast busulfan clearance, may account for the lack of neurotoxicity in children receiving 16 mg/kg.

When seizure occurs during busulfan course, it always appears between 2 and 4 h after the dosing. Recently, in a pharmacological study of high-dose busulfan in children, we found a mean time for maximal plasma concentration of 2.6 h (range, 1.53 to 4.25 h) (10). Moreover, no convulsion occurred before the 7th dosing. In the literature, busulfan neurotoxicity occurs in adults after the second day of therapy, more frequently on the 3rd or 4th day (3, 12–14). On the other hand, Hassan et al. (22) showed no accumulation of busulfan in the CSF in a patient with an Ommaya shunt receiving 1 mg/kg every 6 h for 16 doses. These data show that busulfan neurotoxicity is always delayed after the first dosing and may occur after a certain prolonged brain exposure to busulfan and/or its metabolites.

Three children had seizure 18 to 24 h after the last dosing. Grigg et al. (15) reported 3 cases of convolution in adults occurring 6 to 24 h after the last busulfan dosing. In the case reported by Hassan et al. (22), the busulfan half-lives in plasma and CSF during the first 12 h after the last dose were 2.6 and 2.8 h, respectively. However, 24 h after the last dosing, busulfan levels in CSF and plasma were 70 and 12 ng/ml, respectively, suggesting a slow equilibrium between brain tissue and the CSF compartment. Recently, Hassan et al. (23) also defined the distribution of busulfan and its metabolites to the rat brain. A mean brain:plasma concentration ratio of 0.75 was shown. After i.p. injection of busulfan or [14C]busulfan, intact busulfan and radioactivity half-lives in brain were 3 and 8 h, respectively. This demonstrates a slower elimination for metabolites than for parent drug. The busulfan metabolic pathway includes a reaction with reduced glutathione catalyzed by glutathione transferases (24). The major metabolites, i.e., 3-hydroxysulfolane, sulfolane, and tetrahydrothiophene 1-oxide, were found in the rat brain. Moreover, these metabolites have been identified recently in the urine of adult patients receiving high-dose busulfan (11). This raises the question of the role of busulfan metabolites in the neurotoxicity. Seizures occurring after the last busulfan dosing could be due to prolonged brain exposure to busulfan and/or its metabolites.

Our pharmacological study confirms the high distribution of busulfan to CSF, unlike other lipophilic alkylating agents such as melphalan and chlorambucil. There was a significant difference in CSF:plasma ratios between 16 mg/kg and 600 mg/m², suggesting a dose-dependent distribution of busulfan into the CSF. However, the regression analysis, with a correlation coefficient of 0.49, means that the dose explains only 25% of the variation in CSF:plasma ratio. On the other hand, the difference observed might represent the difference in sampling times between the two groups (3.25 to 7 h and 4 to 5 h in the 16-mg/kg and 600-mg/m² groups, respectively) with CSF level lower than plasma level before the T_{max} (plasma) and higher than plasma level after the T_{max} (plasma). However, Hassan et al. (22) showed a very rapid distribution of busulfan to the CSF in a patient with an Ommaya shunt, suggesting the lack of delay in the drug penetration into the central nervous system. Finally, these high ratios raise the question of a slower busulfan clearance from the brain tissue and the CSF than from the plasma in some patients who might be at risks of seizure. For obvious ethical reasons, complete pharmacokinetics of busulfan in the CSF could not be performed to address these issues.

In a child with meningeal lymphoma involvement, a CSF:plasma ratio of 2.47 was found 4 h after the 15th busulfan dosing. This child received clonazepam and did not develop neurotoxicity. The increase of drug distribution to the CSF in patients with meningeal disease is well documented, especially for methotrexate (25), but ratios are not as high as with busulfan. Altered blood-CSF and blood-brain barriers may trigger busulfan accumulation in brain tissue and/or decreased clearance from the CSF.

No seizure occurred when busulfan was given with clonazepam. Phenytoin is usually given to adult patients receiving high-dose busulfan, but some cases of neurotoxicity are described with such a prophylaxis (3, 4, 15). Unlike other antiepileptic drugs, benzodiazepines are less suspected to interfere with metabolic pathways than phenytoin and phenobarbital. They may be a better prophylactic agent of convolution due to a drug with a large hepatic clearance.

Finally, busulfan acute neurotoxicity is dose dependent in children and may be due to particular pharmacokinetics and/or metabolism. It occurs very rarely with a conventional total dose of 16 mg/kg that does not need anticonvulsive prophylaxis in children. Since busulfan-induced neurotoxicity is always transient without any sequelae, we are currently using a total dose of 600 mg/m² with anticonvulsive prophylaxis. Since then, no convolution occurred. Potentially, this dosage may have a higher antitumor or myeloablative effect than the usual 16-mg/kg total dose, considering the high systemic clearance in children.

We are currently investigating busulfan pharmacokinetics and pharmacodynamics in very young children undergoing bone marrow transplantation for nonmalignant disease and in children receiving 600 mg/m² busulfan to define the optimal dosage in conditioning regimens for bone marrow transplantation. Experimental studies are required to define the mechanisms of neurological toxicity. This study suggests that busulfan could be a drug of interest for the treatment of CNS malignancies with high-dose regimens. We are currently conducting a phase II study of high-dose busulfan and thiopeta with autologous bone marrow transplantation in children with recurrent brain tumors (26).

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