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# The effect of hepatic enzyme inducers on busulfan neurotoxicity and myelotoxicity

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**Summary.** Anticonvulsants are commonly used empirically to prevent seizures in patients receiving high-dose busulfan in preparation for bone marrow transplantation. This study evaluates the effects of two anticonvulsants with enzyme-inductive properties, phenytoin and phenobarbital, and an enzyme inducer without anticonvulsant properties, Aroclor 1254, on the myelotoxicity and acute neurotoxicity of busulfan in a murine model. To assess the neuroprotective effects of these agents, we studied the effects of a single dose of 100 mg/kg i.p. busulfan, previously shown in this model to be uniformly lethal due to neurotoxicity. A significantly greater proportion of mice survived when pretreated with phenytoin or phenobarbital as compared with Aroclor 1254 pretreatment or an untreated control group. Busulfan myelotoxicity was studied in another group of mice treated with 135–150 mg/kg given in divided doses over 6 days. The proportion of animals surviving the otherwise myeloablative effects of this regimen were significantly improved by Aroclor 1254, high-dose phenytoin, and phenobarbital pretreatment. We conclude that anticonvulsants offer protection from the acute neurotoxicity of busulfan. However, these enzyme-inducing agents may reduce the myelosuppresive effects as well. These results suggest than an inducible enzyme system such as microsomal or glutathione S-transferases plays an important role in busulfan metabolism, warranting concern over concomitant administration of agents that either induce or inhibit these enzymes.

## Introduction

Busulfan, a bifunctional alkylating agent, is frequently used in high-dose chemotherapy regimens prior to bone marrow transplantation for various malignancies [10, 21, 23]. The elimination of busulfan appears to occur primarily via metabolic conversion, since only about 1%–2% of the dose is eliminated unchanged in the urine [6, 12]. Three metabolites have been identified in the urine of man, and preliminary evidence suggests that glutathione S-transferases are involved in the metabolism [12]. These enzymes have been shown to be inducible by agents such as phenobarbital and polycyclic aromatic hydrocarbons [14, 19]. High-dose fractions of busulfan have been associated with seizures in humans, prompting recommendations for routine prophylaxis with anticonvulsants [9, 16, 17, 25]. Since anticonvulsant therapy may induce microsomal enzymes and glutathione S-transferases, we were concerned that enhanced metabolism and reduced activity of busulfan might be a result of concomitant anticonvulsant therapy. Preliminary data from our laboratory support this hypothesis [8].

This study was conducted in a murine model with two objectives: (1) to compare the effects of enzyme inducers with and without anticonvulsant properties on busulfan neurotoxicity and (2) to determine whether the administration of agents with enzyme-inductive properties reduces the myelosuppressive effect of busulfan.

## Materials and methods

*Animals.* Female B6D2F1 mice (Jackson Memorial Laboratory; Bar Harbor, Me.) aged 7–8 weeks and weighing an average of 20 g were used throughout this study. The animals were allowed free access to food and acidified water throughout the experiment.

*Drugs and treatment.* Busulfan (Burroughs Wellcome; Research Triangle Park, N.C.) was given intraperitoneally in a solution of 10% dimethyl sulfoxide and Hanks' balanced salt solution. Aroclor 1254 (Analabs; Norwalk, Conn.) was given intraperitoneally dissolved in corn oil. Phenytoin (Parke-Davis; Morris Plains, N.J.) and phenobarbital (Roxane Laboratories; Columbus, Ohio) were given by gavage.

To determine the effects of phenytoin, phenobarbital, and Aroclor 1254 on the acute neurotoxicity of busulfan, six groups of animals were studied. All groups received 100 mg/kg busulfan as a single dose. Similar doses have been shown to elicit acute convulsions and death in

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**Table 1.** Effect of pretreatment regimens on the acute neurotoxicity of a single i. p. dose of 100 mg/kg busulfan

| Pretreatment | Number of mice treated | Number (%) of mice surviving 24 h |
|---|---|---|
| No pretreatment | 15 | 0 (0) |
| Aroclor 1254 | 10 | 0 (0) |
| Phenytoin | 20 | 12 (60%) |
| Phenobarbital | 19 | 13 (68%) |

Chi-square $P < 0.001$

another murine model [24]. A control group received no other treatment, and the remaining five groups were pretreated with one of the following: phenytoin (15 or 60 mg/kg), phenobarbital (70 or 140 mg/kg), or Aroclor 1254 (100 mg/kg). Animals were observed for 24 h and the death rates were compared in the different groups.

The effect of these agents on the myelotoxicity of busulfan was also studied. All mice received a myeloablative dose of 135 – 150 mg/kg divided over 6 days. Previously published data demonstrated that similar doses of busulfan resulted in survival of <33% due to myelotoxicity [18]. Four groups of mice were pretreated for 3 days with one of the following: low-dose phenytoin (15 mg/kg per day), high-dose phenytoin (60 mg/kg per day), phenobarbital (35 mg/kg per day), or Aroclor 1254 (100 mg/kg i. p.). These groups were compared with those receiving either busulfan alone or busulfan followed by syngeneic bone marrow rescue comprising $2 \times 10^6$ cells/mouse infused i. v. Pretreatment regimens were continued throughout busulfan therapy. Serial blood counts were performed to assess myelosuppression, and animals were observed for 6 weeks after therapy to assess differences in death rates. The proportion of animals surviving was compared using a chi-square test at a level of significance of 5%. If the overall chi-square statistic was significant, Scheffe's procedure was used to determine which differences existed between groups.

## Results

Death rates due to acute neurotoxicity are summarized in Table 1; the phenytoin and phenobarbital data include both dose groups. A significantly greater proportion of mice treated with phenytoin or phenobarbital survived as compared with either those treated with Aroclor 1254 or untreated controls ($P < 0.001$), demonstrating the neuroprotective effect of anticonvulsants in this model. Data from the myelotoxicity study are summarized in Table 2. The proportion of mice surviving 6 weeks after treatment was significantly greater in the high-dose phenytoin Aroclor 1254, phenobarbital, and marrow transplant groups as compared with the low-dose phenytoin group and animals given no pretreatment ($P < 0.001$). Based on the serial blood counts, the survival advantage appeared to be related to a shorter and less profound myelosuppression (data not shown).

## Discussion

According to our results, phenytoin and phenobarbital offer protection from busulfan neurotoxicity. However, both of these anticonvulsants induce cytochrome P-450 enzymes, and phenobarbital has been shown to induce hepatic glutathione S-transferases. The cytochrome P-450 monooxygenase system is responsible for the biotransformation of many chemicals in the body. These enzymes are

**Table 2.** Effect of pretreatment regimens on the myelotoxicity of busulfan

| Pretreatment | Number of mice treated | Number (%) of mice surviving 50 days | Median (range) days to death |
|---|---|---|---|
| No pretreatment | 37 | 8 (22%) | 19 (16–27) |
| Marrow transplant | 26 | 24 (92%) | 21 (18–24) |
| Phenytoin low dose (15 mg/kg per day) | 20 | 0 (0) | 24 (20–35) |
| Phenytoin high dose (60 mg/kg per day) | 20 | 17 (85%) | 8 (8–10) |
| Phenobarbital | 35 | 31 (89%) | 24 (24–24) |
| Aroclor 1254 | 20 | 20 (100%) | Not applicable |

Chi-square $P < 0.001$

located primarily in the liver but also in the skin, blood, and lungs. Various isoenzymes of cytochrome P-450 exist and are generally classified into a nonspecific form, inducible by phenobarbital, phenytoin, and various other drugs, and a form preferentially induced by polycyclic aromatic hydrocarbons such as methylcholanthrene [2, 4, 20]. Aroclor 1254 is a mixture of polychlorinated biphenyls that can greatly increase the levels of several of the cytochrome P-450 isoenzymes, including the phenobarbital and methylcholanthrene types [4]. Although busulfan metabolism in humans has not been completely elucidated, it has been shown that very little is excreted unchanged in the urine [6, 12]. Three metabolites, sulpholane, 3-hydroxysulpholane and tetrahydrothiophene 1-oxide, have been identified as urinary metabolites in man [12]. These three metabolites were not cytotoxic in a Chinese hamster V79 cell line, implying that busulfan's activity may be the result of the parent compound [11]. Recent pharmacokinetic data in adults show a fall in the half-life of busulfan as measured from the first to the last dose, possibly due to the induction of metabolism [12]. Therefore, the administration of enzyme-inducing agents could potentially diminish the activity of busulfan if the conversion to these inactive metabolites occurs via an inducible hepatic enzyme system. However, this decrease in half-life has not been observed in children [26].

Our data indicate that pretreatment with phenobarbital, high-dose phenytoin, or Aroclor 1254 increases the proportion of animals surviving marrow-ablative doses of busulfan. The high-dose phenytoin and phenobarbital doses used in this study are equivalent to therapy in humans based on interspecies dose-equivalence conversions. As evidenced by differences in blood cell counts, the reduction in death rate appears to be due to reduced myelotoxicity in these groups as compared with the low-dose phenytoin group and animals given no pretreatment. The reduction in myelotoxicity is likely due to the induction of an enzyme system, since Aroclor 1254, phenytoin, and phenobarbital are all classic inducers of hepatic enzymes. This implies that the metabolism of busulfan is mediated at least in part by cytochrome P-450 isozymes, inducible glutathione S-transferases, or both. It is not known whether

the decrease in the myelosuppressive activity of busulfan translates into a reduction in its antitumor effect, as seen in studies using phenobarbital in conjunction with nitrosoureas in a rat model [15]. Additionally, the pharmacologic and clinical effect in man may be less dramatic than that observed in a murine model. A differential effect between animal models and humans has been observed using phenobarbital and cyclophosphamide. Whereas phenobarbital-pretreated animals showed a decrease in circulating active metabolites and antitumor activity in vivo against murine leukemia, the total quantity of alkylating metabolites was not affected in man [1, 7, 13]. The time course of administration of enzyme-inducing agents also affects the applicability of animal data to man. In animal models, 1–3 days of phenytoin or phenobarbital administration have been shown to induce microsomal enzymes and alter the disposition of other drugs [3, 5, 7, 22]. The time course of induction in man may be longer and depends on the inducing agents and their dosing regimen.

Although it would be optimal to choose an anticonvulsant with fewer enzyme-inductive effects, such as valproic acid, the lack of demonstrated efficacy for preventing busulfan-induced seizures and the lack of parenteral dosing forms prevent its widespread application. Optimal anticonvulsant agents and dosing schedules need to be determined to prevent seizures without greatly affecting busulfan's activity. We recommend: (1) giving the lowest dose that maintains therapeutic serum levels, (2) giving loading doses on the day before busulfan is started, and (3) discontinuing therapy as soon as possible. Further investigation quantifying enzyme activity and the effect of induction on the pharmacokinetics and antitumor profile of busulfan is needed. Since it appears that the metabolism of busulfan is mediated by an inducible hepatic enzyme, concomitant administration of agents with inductive or inhibitory effects on these enzymes should be done with caution.

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