

# Therapeutic Efficacy of Aortic Administration of *N*-Acetylcysteine as a Chemoprotectant against Bone Marrow Toxicity after Intracarotid Administration of Alkylators, with or without Glutathione Depletion in a Rat Model<sup>1</sup>

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## ABSTRACT

Modulation of thiol levels may alter both the efficacy and toxicity of chemotherapeutic agents. We investigated cytoenhancement, using L-buthionine-[S,R]-sulfoximine (BSO) to reduce cellular glutathione levels prior to intracarotid alkylator administration. We also evaluated chemoprotection against chemotherapy-induced systemic toxicity when the thiol agents *N*-acetylcysteine (NAC) and sodium thiosulfate were administered into the descending aorta to limit brain delivery. BSO treatment reduced rat brain and intracerebral tumor glutathione levels by 50–65%, equivalent to the reduction in liver and s.c. tumor. BSO treatment significantly enhanced the toxicity of chemotherapy with carboplatin, melphalan, and etoposide phosphate against granulocytes, total white cells, and platelets. Intracarotid administration of NAC resulted in high delivery to the brain, whereas infusion via the descending aorta minimized brain delivery. When NAC, with or without sodium thiosulfate, was administered via aortic infusion prior to chemotherapy, the magnitude of the bone marrow toxicity nadir was minimized, even with BSO-enhanced myelosuppression. Thus, BSO depleted brain and brain tumor glutathione but thereby increased chemotherapy-induced myelosuppression. Surprisingly, although NAC was found to readily cross the blood-brain barrier when given into the carotid artery, aortic infusion of NAC resulted in minimal exposure to the central nervous system (CNS) vasculature because of rapid clearance. As a result, aortic infusion of NAC to perfuse bone marrow and minimize myelosuppression and toxicity to visceral organs could be performed without interfering with the CNS cytotoxicity of intracarotid alkylators, even after BSO depletion of CNS glutathione.

## INTRODUCTION

The effectiveness of chemotherapy against malignant tumors may be increased by dose intensification (1–3). As an alternative to increasing drug dosages, drug cytotoxicity can be enhanced by reducing the intracellular concentration of the endogenous detoxifying tripeptide glutathione by use of BSO<sup>3</sup> to inhibit glutathione synthesis (4–6). BSO treatment increases the effectiveness of chemotherapeutics *in vitro* (6–9), in animal models (10, 11), and in patients (12, 13). However, even without dose intensification, chemotherapy has toxic side effects, such as bone marrow toxicity, and this can be enhanced with BSO.

It may be possible to reduce the bone marrow toxicity of chemotherapeutic agents by using sulfur-containing chemoprotective agents (thio, thiol, and thioether compounds) to mimic one or many of the

detoxifying activities of glutathione (14), including drug conjugation (15) and antioxidant and free radical scavenging activity (16). Clinically relevant compounds that may provide protection against at least some of the systemic toxicities caused by alkylating chemotherapeutics include ethylol (17), STS (2, 18, 19), and NAC (20–22).

Chemoprotectants have had relatively limited clinical use because of concerns of impaired chemotherapeutic efficacy. We hypothesize that reduction of tumoricidal effects may be avoided by separating chemoprotectant and chemotherapy treatments in time or space. For example, studies of STS chemoprotection have used two routes of administration (i.a. *versus* i.v. or i.p.) to minimize interactions of STS with cisplatin in tumor models (23) and patients (2, 24). Preclinical studies (25, 26) and clinical trials (18, 19) have shown that enhanced platinum chemotherapy delivery to the CNS followed by delayed STS chemoprotection, to provide both spatial and temporal separation, results in marked otoprotection without impairing cytotoxicity.

The purpose of the present study was to test whether infusion of thiol chemoprotectants into the descending aorta in the rat could safely provide bone marrow protection while limiting NAC delivery to brain. Additionally, we determined the effects of BSO on glutathione concentrations in the brain and brain tumors and investigated whether glutathione depletion enhanced chemotherapy-induced bone marrow toxicity in the rat. The results demonstrate a potentially exciting new treatment option to maximize dose intensity in brain or head and neck tumors by use of i.a. (carotid or vertebral) chemotherapy given cephalad, while minimizing systemic toxicity with aortic NAC administration given caudally.

## MATERIALS AND METHODS

Animal studies were performed in accordance with guidelines established by the Oregon Health Sciences University Committee on Animal Care and Use.

**Tumor Implantation.** For assessment of the effects of BSO on glutathione levels, intracerebral and s.c. human small cell lung carcinoma cells were implanted in nude rats from our colony (weight, ~220 g; *n* = 5) as reported previously (25, 27).

**Osmotic BBBD.** BBBD was performed in adult female Long Evans rats (weight, 220–240 g; *n* = 6) for assessment of delivery of NAC to the brain. BBBD was performed via the right external carotid artery catheter by infusion of 25% mannitol into the right internal carotid artery as reported previously (25, 27). In animals receiving NAC i.a. without BBBD, 0.9% NaCl was infused instead of mannitol.

**Aortic Infusion Technique.** The left carotid artery (rather than the right carotid to avoid possible first-pass cranial perfusion) was exposed through a ventral neck incision, and a catheter (PE 50) filled with heparinized saline was tied into the left external carotid. To target the descending aorta, the left internal carotid was temporarily occluded with a Biemer vessel clip (9 mm), and the chemoprotectant was administered retrograde down the carotid to the descending aorta via the catheter. The Biemer clip and catheter were then removed, and the external carotid was ligated. The wound was closed with wound clips, and the animal was allowed to recover.

**Radiolabeled Tracer Studies.** Animals for NAC biodistribution studies were evaluated at low (140 mg/kg) and high (1200 mg/kg) NAC doses with

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<sup>3</sup> The abbreviations used are: BSO, L-buthionine-[S,R]-sulfoximine; STS, sodium thiosulfate; NAC, *N*-acetylcysteine; i.a., intra-arterial; CNS, central nervous system; BBBD, blood-brain barrier disruption.

Table 1 Glutathione measurements in rats treated with BSO

	Glutathione ( $\mu\text{mol/g}$ tissue)			
	Liver	Brain	s.c. tumor	Intracerebral tumor
Normal rats	234 $\pm$ 49 ( <i>n</i> = 6)	95 $\pm$ 10 ( <i>n</i> = 6)	23.3 $\pm$ 5.5 ( <i>n</i> = 2)	27.3 $\pm$ 6.4 ( <i>n</i> = 2)
BSO-treated rats	87 $\pm$ 12 ( <i>n</i> = 5)	44 $\pm$ 13 ( <i>n</i> = 8)	11.4 $\pm$ 2.4 ( <i>n</i> = 3)	9.1 $\pm$ 2.0 ( <i>n</i> = 3)
Decrease (%)	64 <sup>a</sup>	54 <sup>a</sup>	51	67 <sup>a</sup>

<sup>a</sup> *P* < 0.05 by Student's *t* test.

four different delivery routes. Radiolabeled NAC (12.5  $\mu\text{Ci}$  of [<sup>14</sup>C]NAC; American Radiolabeled Chemicals, St. Louis, MO) was mixed with unlabeled NAC and then administered either i.v. or i.a. into the right internal carotid artery, by aortic infusion, or i.a. immediately following BBBD. At 10 min post-NAC administration, a serum sample was collected, and the animal was perfused with normal saline. A postperfusion serum sample was collected to determine clearance of radioactivity from the vascular circulation. Tissue samples from the right and left cerebral hemispheres, the liver, and the kidney were weighed and then solubilized. Quench-corrected <sup>14</sup>C activity in serum and tissue samples was determined.

**Drug Treatment.** BSO (supplied by the National Cancer Institute) was administered i.p. at 10 g/m<sup>2</sup> twice daily for 3 days. A tridrug regimen was administered to all rats in the bone marrow toxicity study. The chemotherapeutic agents carboplatin (200 mg/m<sup>2</sup> Paraplatin; Bristol Meyers-Squibb, New York, NY), melphalan (10 mg/m<sup>2</sup> Alkeran; Glaxo-Wellcome), and etoposide phosphate (100 mg/m<sup>2</sup> Etopophos; Bristol Meyers-Squibb) were each diluted with sterile NaCl to a volume of 1 ml and administered as a bolus injection over 1 min via the catheter, retrograde into the internal carotid artery. STS (Sigma Chemical Co., St. Louis, MO) and NAC (Mucomyst; American Reagent Laboratories, St. Louis, MO) were infused i.v. or by aortic infusion.

**Blood Collection.** Blood samples (0.5 ml) were obtained at baseline (prechemotherapy), at the treatment nadir (day 6), and in the recovery phase (day 9). A pilot study was done on days 9–12 to assess the recovery time course, but all recovery data reported are from day 9. Samples were immediately transferred to EDTA-coated Microtainer tubes, briefly agitated, and stored at 4°C until analysis. The levels of WBCs, granulocytes, and platelets in blood samples were determined blindly by an independent contract laboratory (IDex Veterinary Supply, W. Sacramento, CA).

**Glutathione Measurements.** Glutathione in serum and tissues was assayed using the Bioxytech GSH-400 Assay Kit (Oxis International, Portland, OR). Samples were homogenized in 5% metaphosphoric acid, and centrifuged at 4°C at 3000  $\times$  g for 10 min. The supernatants were assayed directly with a Beckman DU-64 spectrophotometer.

**Statistical Analysis.** Radiolabeled NAC distribution data were determined for the right and left hemispheres of the brain, the liver, and the kidney and are expressed as the mean  $\pm$  SE for percentage of delivered dose per gram of tissue. Tissue glutathione levels are expressed as the mean  $\pm$  SE for mol glutathione/ml of serum or g of tissue. Mortality was determined by time to death or when morbidity necessitated early sacrifice. All surviving animals were sacrificed 9 days after treatment. Kaplan-Meier product limit survival analysis was performed, and significance was assessed with the log-rank test, using JMP software (SAS Institute Inc., Cary, NC).

Means and SEs for individual blood counts as well as changes from each animal's own baseline values were determined with Excel software (Microsoft). Large SEs are inherent in blood count values that can range over an order of magnitude. Although some treated animals showed a decrease in blood counts at the 6-day nadir in comparison with baseline, some animals with low baseline blood counts in the NAC treatment groups actually showed a large increase over baseline. Bone marrow toxicity between treatment groups (means as well as change from baseline) was assessed by Student's *t* test (Microsoft Excel) and by Wilcoxon/Kruskal-Wallis rank-sums nonparametric analysis (JMP software). Unless otherwise stated, the Wilcoxon analysis of changes from baseline is presented, with significance designated as *P* < 0.05.

## RESULTS

**BSO Modification of Glutathione Levels in Rats.** We evaluated the effect of BSO treatment (10 g/m<sup>2</sup> administered i.p. twice daily for 3 days) on glutathione levels in the brain and tumors of normal and intracerebral tumor-bearing rats (Table 1). BSO treatment consistently resulted in at least 50% reduction in tissue and tumor glutathione levels (*P* < 0.05). Brain and intracerebral tumor glutathione levels were reduced equivalently to liver and s.c. tumor.

**Biodistribution of Radiolabeled NAC.** We tested whether the method of administration of NAC would affect its biodistribution. Intra-arterial infusion retrograde via the left external carotid artery, with transient left internal artery occlusion, was evaluated as a mechanism to essentially perfuse via the descending aorta with limited delivery to the brain. When NAC was administered i.v., negligible amounts were found in brain as determined by the percentage of administered dose of [<sup>14</sup>C]NAC/g of tissue (Fig. 1). Intra-arterial delivery in the right internal carotid artery resulted in high levels of radiolabel in the right cerebral hemisphere, and this was not increased by BBBD. However, aortic infusion minimized brain delivery of NAC (Fig. 1). At the low dose of NAC (140 mg/kg), aortic infusion decreased liver delivery and increased kidney delivery, whereas there was no change in liver and kidney delivery at the high dose of NAC (1200 mg/kg; data not shown). The serum concentration of NAC 10 min after administration of 1200 mg/kg was 2.4  $\pm$  0.6 mg/ml (*n* = 6) as determined by the radiolabel remaining in the blood.

**Effect of BSO on Chemotherapy-induced Bone Marrow Toxicity.** Rats were treated with (*n* = 72) or without (*n* = 68) BSO, i.p., 10 g/m<sup>2</sup> twice daily for 3 days. Baseline blood counts obtained after BSO treatment but prior to chemotherapy demonstrated that BSO treatment did not alter baseline levels of granulocytes or platelets. Total white cell counts were significantly enhanced by BSO treatment (5.9  $\pm$  0.2  $\times$  10<sup>3</sup> cells/ $\mu\text{l}$  without BSO versus 7.5  $\pm$  0.3  $\times$  10<sup>3</sup> cells/ $\mu\text{l}$  with BSO; *P* < 0.0001, Student's *t* test); however, both mean values were within the very large normal range.

The bone marrow toxicity of the tridrug chemotherapy regimen (carboplatin, etoposide phosphate, and melphalan) was assessed in animals pretreated with (*n* = 11) or without (*n* = 6) BSO, with no other addition (Fig. 2A). BSO enhanced bone marrow toxicity, as determined by significantly lower nadir counts compared with base-

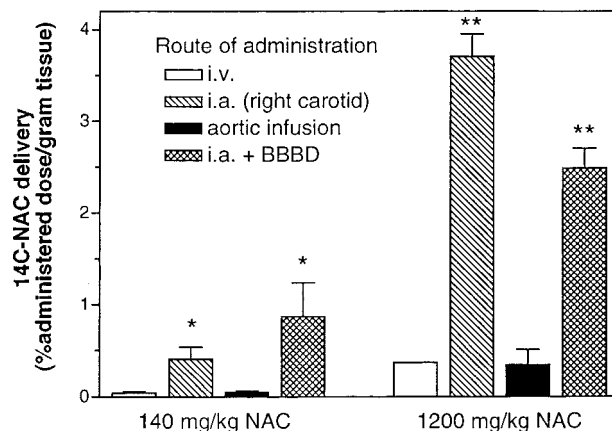


Fig. 1. NAC delivery to rat brain. Radiolabeled NAC in combination with unlabeled low-dose NAC (140 mg/kg) or high-dose NAC (1200 mg/kg) was administered to rats with the following routes of infusion: i.v. ( $\square$ ), i.a. into the right carotid artery ( $\text{▨}$ ), i.a. via the left carotid artery with left internal artery occlusion (aortic infusion;  $\blacksquare$ ), and i.a. (right carotid) with BBBD ( $\text{▩}$ ). Radiolabel in tissue homogenates is expressed as the mean and SE (bars) of the percentage of administered dose of [<sup>14</sup>C]NAC/g tissue (*n* = 3 rats per group). Significant differences from i.v. delivery by Student's *t* test are indicated; \*, *P* < 0.05; \*\*, *P* < 0.001.

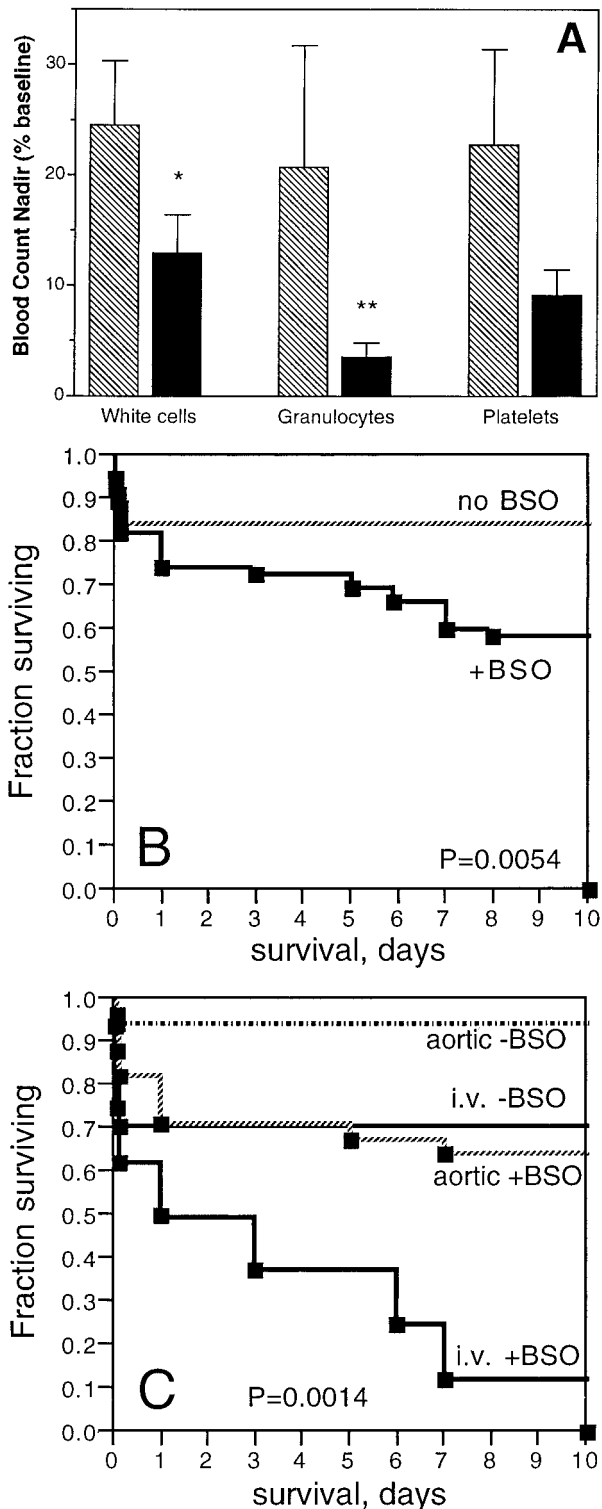


Fig. 2. Toxicity of BSO treatment. A, effect of BSO on chemotherapy-induced myelosuppression. Rats received either no pretreatment (▨;  $n = 6$ ) or treatment with BSO (10 mg/kg i.p. twice daily for 3 days; ■;  $n = 11$ ) prior to treatment with chemotherapy (200 mg/m<sup>2</sup> carboplatin, 100 mg/m<sup>2</sup> etoposide phosphate, 10 mg/m<sup>2</sup> melphalan). Total WBC, granulocyte, and platelet counts were determined prior to chemotherapy, at the blood nadir (6 days), and in the recovery phase (9 days after treatment). Data are expressed as the mean  $\pm$  SE (bars) for nadir as a percentage of each animal's own baseline, with significance determined by Wilcoxon analysis (\*,  $P < 0.03$ ; \*\*,  $P < 0.02$ ). B, effect of BSO on mortality. A Kaplan-Meier product limit analysis was used to evaluate the mortality attributable to treatment with BSO (10 mg/kg i.p. twice daily for 3 days) prior to treatment with chemotherapy (200 mg/m<sup>2</sup> carboplatin, 100 mg/m<sup>2</sup> etoposide phosphate, 10 mg/m<sup>2</sup> melphalan). Animals were grouped with (■;  $n = 42$ ) or without (▨;  $n = 48$ ) BSO, regardless of whether they also received chemoprotectant.  $P = 0.0054$ , Wilcoxon analysis. C, effect of route of NAC administration on mortality. A Kaplan-Meier product limit analysis was used to evaluate the mortality attributable to

line blood counts for both total white cells ( $P < 0.03$ ) and granulocytes ( $P < 0.02$ ). The enhancement of the platelet nadir was not significant. No difference in the blood count recovery (9 days after chemotherapy) was found when BSO-treated and untreated rats were compared.

The effect of BSO pretreatment on the mortality attributable to chemotherapy was determined by assessing deaths in the bone marrow toxicity study. When we compared animals that received either no chemoprotectant or received NAC with or without STS, BSO significantly increased mortality ( $P = 0.0054$ ; Fig. 2B). In both groups, 15–20% of all animals died within hours of treatment because of procedural complications or drug toxicity. The significant difference in BSO-treated animals came as a second phase of toxicity, 5–7 days after treatment, corresponding to the blood count nadirs.

**Toxicity of NAC.** A dose escalation of NAC was performed in the rat. Initial doses of 140–800 mg/kg NAC (22), administered i.v. immediately after chemotherapy ( $n = 4$ ), were well tolerated but provided no detectable bone marrow protection. Doses of NAC  $>1200$  mg/kg ( $n = 3$ ) were toxic, whereas there was no toxicity at 1200 mg/kg. Therefore, 1200 mg/kg NAC was used for the bone marrow protection studies except when administered in conjunction with STS, where a dose of 1000 mg/kg was used because of volume considerations.

The toxicity of NAC was determined when infused for bone marrow protection. The mortality attributable to NAC was significantly dependent on the route of administration, with i.v. administration significantly more toxic than aortic infusion ( $P = 0.0014$ ; Fig. 2C). Pretreatment with BSO markedly enhanced the toxicity of NAC. Groups given i.v. NAC after BSO treatment were halted early because of excessive mortality, with a stopping rule of 75% mortality within  $n = 4$  animals. In selected animals ( $n = 21$ ) from all groups, blood pressure monitoring indicated that most animals that expired experienced persistent acute hypotension, suggesting cardiac toxicity. Of particular note, however, there was no mortality or any evidence of toxicity in 12 animals, without BSO, receiving 1200 mg/kg NAC by aortic infusion 30 min prior to chemotherapy.

**Effect of NAC on Chemotherapy-induced Bone Marrow Toxicity.** Chemoprotection against chemotherapy-induced bone marrow toxicity was determined in BSO-treated and untreated animals given i.a. carboplatin, etoposide phosphate, and melphalan. NAC with or without STS was administered either 30 min prior to chemotherapy or immediately after chemotherapy and was administered either i.v. or by aortic infusion. Chemoprotection was found with NAC, as shown by increased blood counts (WBCs, granulocytes, and platelets) at the nadir compared with no chemoprotectant (Tables 2 and 3). Chemoprotection was effective regardless of whether animals were pretreated with BSO (Fig. 3), and the magnitude of the chemotherapy-induced bone marrow toxicity nadir was minimized and recovery to normal platelet levels was improved.

In animals that did not undergo BSO treatment, pretreatment with NAC (1200 mg/kg) by aortic infusion, with or without follow-up with STS, was the best treatment strategy (Fig. 3, A and C). The chemotherapy-induced decrease in granulocyte counts was completely blocked ( $P < 0.05$ ; Fig. 3A) and platelet toxicity was reduced (Fig. 3C) by aortic infusion of NAC 30 min prior to chemotherapy. There was a trend toward significant protection of bone marrow by NAC

chemoprotection with NAC. Rats were treated with or without BSO (10 mg/kg i.p. twice daily for 3 days) prior to treatment with chemotherapy (200 mg/m<sup>2</sup> carboplatin, 100 mg/m<sup>2</sup> etoposide phosphate, 10 mg/m<sup>2</sup> melphalan). Chemoprotection consisted of NAC (1200 mg/m<sup>2</sup>) or NAC (1000 mg/m<sup>2</sup>) plus STS (8 g/m<sup>2</sup>) given either i.v. (no BSO,  $n = 17$ ; +BSO,  $n = 8$ ) or by aortic infusion (no BSO,  $n = 19$ ; +BSO,  $n = 28$ ).  $P = 0.0014$  by Wilcoxon analysis.

Table 2 Protection against chemotherapy-induced myelosuppression  
The mean and SE are shown for nadir blood counts as a percentage of baseline.

Treatment	No. of rats	White cell nadir (%)	Granulocyte nadir (%)	Platelet nadir (%)
No protectant	6	24.5 ± 5.8	20.7 ± 11.0	22.7 ± 8.7
NAC i.v. postchemotherapy	6	46.0 ± 6.5 <sup>a</sup>	57.9 ± 8.8 <sup>a</sup>	36.8 ± 10.4
NAC i.v. 30 min prior to chemotherapy	8	51.4 ± 9.4	93.5 ± 28.1	53.3 ± 12.7
NAC aortic infusion postchemotherapy	6	25.9 ± 4.9	36.6 ± 17.0	26.8 ± 9.3
NAC aortic infusion 30 min prior to chemotherapy	6	69.7 ± 19.3 <sup>a</sup>	206 ± 125 <sup>a</sup>	59.3 ± 17.9
NAC aortic infusion 30 min prior + STS postchemotherapy	6	53.8 ± 12.8*	83.4 ± 21.3*	47.0 ± 11.5

<sup>a</sup>  $P < 0.05$  compared with no protectant by Wilcoxon/Kruskal-Wallis rank-sums test.

administered by the i.v. route, but this did not achieve  $P < 0.05$  by Wilcoxon nonparametric analysis.

In animals pretreated with BSO, good protection for granulocytes was provided by aortic infusion of NAC 30 min prior to chemotherapy, but the best protection and the least mortality was provided by NAC aortic infusion before and STS immediately after chemotherapy (Fig. 3, B and D). The combination chemoprotection (NAC and STS) regimen significantly blocked the toxicity for granulocytes ( $P < 0.01$ ; Fig. 3B) and platelets ( $P < 0.01$ ; Fig. 3D) compared with animals that received no chemoprotection. Chemoprotection also significantly enhanced platelet recovery from chemotherapy. STS alone did not give consistent bone marrow protection (data not shown).

## DISCUSSION

The studies described above demonstrate that BSO administration decreased brain and brain tumor glutathione levels and enhanced chemotherapy-induced bone marrow toxicity as well as mortality in the rat. Infusion of the thiol chemoprotectant NAC into the descending aorta in the rat provides bone marrow protection while limiting delivery of NAC to brain. The results demonstrate a potentially exciting new treatment option to maximize dose enhancement in brain or head and neck tumors while minimizing systemic toxicity.

**NAC Biodistribution.** In the past, NAC has been reported not to cross the BBB after i.v. administration, as determined by low brain concentrations. This was most graphically demonstrated in a rat study of whole body autoradiography showing no brain delivery after i.v. NAC administration (28). By contrast, in this report NAC was found to cross the BBB extremely well when given i.a. into the carotid artery, regardless of whether the BBB was opened with hypertonic mannitol (Fig. 1). NAC is cleared rapidly by uptake into non-CNS tissues (28), resulting in modest exposure of NAC at the CNS vasculature after i.v. or aortic infusion delivery. An additional advantage of the aortic infusion technique reported here is the first-pass delivery of high concentrations of infusate directly to the bone marrow and other organs.

**Enhancement of Chemotherapy Cytotoxicity with BSO.** BSO specifically and potently inhibits  $\gamma$ -glutamylcysteine synthetase, thus blocking glutathione synthesis (4, 5). BSO treatment increases mel-

phalan activity *in vitro* (6, 7, 29) and in xenograft models (10, 11), and this has led to clinical trials of BSO enhancement of melphalan (12, 13). Glutathione depletion with BSO has also been shown to increase the cytotoxicity of cisplatin (6, 8, 29) and carboplatin (6, 9).

Glutathione has multiple detoxifying activities, so depletion of glutathione with BSO can enhance toxicity through a number of mechanisms (14). Glutathione has been implicated in chemical conjugation of platinum agents (15) and in repair of DNA damage after platinum-induced DNA cross-linking (5). Cisplatin-glutathione conjugates are actively pumped from cells, perhaps involving one of the multidrug resistance-associated protein efflux pumps (30). Low glutathione levels also reduce the ability of tissues to scavenge reactive oxygen species and other free radicals. BSO may therefore sensitize cells to oxidative stress, leading to apoptosis (6, 7).

We found that treatment of rats with BSO increased the magnitude of the bone marrow toxicity induced by a clinically relevant alkylator-based combination chemotherapy regimen. Chemotherapy treatment affects all tissues and is especially toxic to rapidly growing normal cells (e.g., bone marrow). In the clinical trials of melphalan potentiation, significantly greater leukopenia and thrombocytopenia occurred in the presence of BSO, compared with melphalan alone (12, 13). This enhanced toxicity against nontumor targets could limit the utility of BSO in the clinic.

**Chemoprotection.** Exogenous thiols can mimic or replace glutathione to reduce chemotherapy toxicity and counteract any additional BSO-enhanced toxicity (6). Two different thiol agents, STS and NAC, were evaluated to assess whether chemotherapy-induced bone marrow toxicity could be rescued. STS is an effective chemoprotectant against alkylator cytotoxicity at the cellular level (6) and is also protective against carboplatin-induced ototoxicity in animal models (25, 26) and in patients (18, 19). However, little bone marrow protection was provided by STS in the current studies. A major mechanism of STS is thought to be direct binding and inactivation of xenobiotics, which occurs at a high molar ratio of STS to drug (14, 15). Therefore, STS may be more effective in preserving bone marrow if administration is delayed until after clearance of a significant portion of the chemotherapy. In support of this hypothesis, recent data suggest that delayed STS may provide platelet protection in brain tumor patients (data not shown).

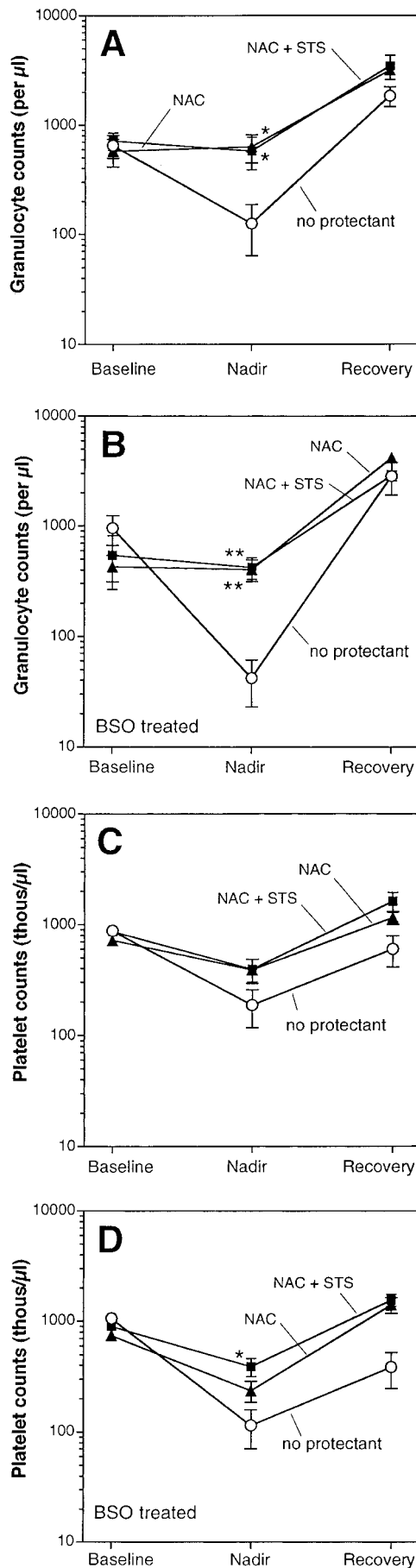
NAC is a cysteine analogue with strong antioxidant activity (20, 22, 31). The protection provided by NAC against kidney toxicity induced by radiographic contrast agents is proposed to be the result of antioxidant activity (21). NAC also induces *de novo* synthesis of glutathione over a period of hours to days (28, 32), which may contribute to long-term protection. In our previous studies of *in vitro* chemoprotection, NAC was the most effective of the thiol agents tested (6). NAC can restore peripheral WBC function in cancer patients (33), and it has therefore been suggested that NAC, or increased glutathione levels, may stimulate blood cell production. When tested as a chemoprotective agent in cancer chemotherapy, NAC reduced ifosfamide nephrotoxicity, but cyclophosphamide-induced leukopenia was not affected (34). NAC also failed to protect against hematopoietic toxicity produced by cisplatin (35) or dimethylbenzanthracene (36). We

Table 3 Protection against BSO-enhanced chemotherapy-induced myelosuppression

The mean and SE are shown for nadir blood counts as a percentage of baseline.

Treatment	No. of rats	White cell nadir (%)	Granulocyte nadir (%)	Platelet nadir (%)
No protectant	11	12.9 ± 3.5	3.5 ± 1.3	9.1 ± 2.3
NAC aortic infusion postchemotherapy	7	13.9 ± 4.2	19.8 ± 14.7	11.7 ± 3.5
NAC aortic infusion 30 min prior to chemotherapy	9	20.1 ± 2.7	115.4 ± 68.8 <sup>a</sup>	23.1 ± 6.2
NAC aortic infusion 30 min prior + STS postchemotherapy	7	30.5 ± 6.5 <sup>a</sup>	121.0 ± 40.2 <sup>a</sup>	39.0 ± 7.4 <sup>a</sup>

<sup>a</sup>  $P < 0.05$  compared with no protectant by Wilcoxon/Kruskal-Wallis rank-sums test.



believe that the difference between our study and previous attempts at bone marrow protection have to do with high-dose delivery via the aortic infusion route.

With regard to safety of chemoprotectants, the route of administration (i.a. versus i.v.) and timing are key issues. When given after osmotic BBB opening, STS administration must be delayed at least 60 min, when the BBB is reestablished (19) or seizures result (25). As with high-dose NAC (1200 mg/kg), high-dose mannitol at doses adequate to open the BBB has to be given i.a. and not i.v. to avoid cardiac toxicity (37). Clearly, i.v. NAC at high doses, particularly after pretreatment with BSO, is more toxic than i.a. NAC, probably because of cardiac toxicity attributable to higher blood levels going to the heart after i.v. infusion than i.a. infusion. In the absence of BSO, even high-dose aortic infusion NAC was without either toxicity or mortality.

**Possible Interactions with Antitumor Efficacy.** The possibility of reduced anticancer efficacy as a result of chemoprotective agents is a major concern limiting their use (14). To minimize interactions between chemoprotectants and chemotherapy, the agents should be separated either in time or in space. Two-route administration has been used in studies of STS given with cisplatin to provide local chemoprotection while sparing local antitumor activity (2, 23, 24). Two-route therapy can consist of, e.g., i.p. cisplatin and i.v. STS for ovarian carcinoma (24) or i.a. cisplatin with i.v. STS for head and neck cancer (2). Iwamoto *et al.* (23) found that addition of STS in two-route chemotherapy actually gave superior antitumor effects against mouse tumors. Our previous preclinical studies showed no significant loss of carboplatin antitumor efficacy when STS was administered 8 h after chemotherapy (26), a time point at which STS was still protective against carboplatin-induced ototoxicity (25).

**Potential for Enhancement and Chemoprotection in Brain Tumor Therapy.** We propose that chemotherapy enhancement and chemoprotection may have a unique role in CNS malignancies as well as head and neck tumor therapy. The BBB effectively generates two compartments, blocking access to the brain of most charged, hydrophilic chemoprotectants and chemotherapy agents. Although NAC did cross the BBB if administered into the carotid artery (Fig. 1), administration by aortic infusion increased delivery to organs below the clavicle, including the bone marrow and visceral organs, but by virtue of high first-pass clearance, CNS delivery was limited (Fig. 4). Thereby, aortic infusion of NAC could be performed prior to chemotherapy for head and neck or brain tumors. Alkylating chemotherapies, such as carboplatin or melphalan, might be delivered into either the vertebral or internal carotid arteries to intracerebral tumors by use of an osmotic BBB opening to enhance CNS delivery and increase dose intensity as has been done in primary CNS lymphoma (1) and other tumors (3, 19). If both safe and efficacious, pretreatment with the enhancer BSO could then be assessed.

We previously assessed this paradigm in the clinical setting, evaluating chemoprotection against carboplatin-induced hearing loss. After osmotic BBB opening to increase CNS delivery, delayed STS in

Fig. 3. Chemoprotection for chemotherapy-induced myelosuppression. Rats received tridrug chemotherapy (200 mg/m<sup>2</sup> carboplatin, 100 mg/m<sup>2</sup> etoposide phosphate, 10 mg/m<sup>2</sup> melphalan), with (▲ and ■) or without (○) chemoprotection. Blood counts were determined prior to chemotherapy, at the blood nadir (6 days), and in the recovery phase (9 days after treatment). Chemoprotection was with NAC (1200 mg/kg) administered via aortic infusion 30 min prior to chemotherapy (▲) or NAC prior to chemotherapy and STS (8 g/m<sup>2</sup>) immediately after chemotherapy (■). In B and D, animals received BSO (10 mg/kg i.p. daily for 3 days) prior to treatment with chemotherapy. A, granulocyte counts without BSO. B, granulocyte counts with BSO [mean ± SE (bars); n = 6 per group]. C, platelet count without BSO. D, platelet count with BSO. Significant differences from the no-protectant groups were determined by Wilcoxon/Kruskal-Wallis rank-sums tests (\*, P < 0.05; \*\*, P < 0.01). thous, thousand.

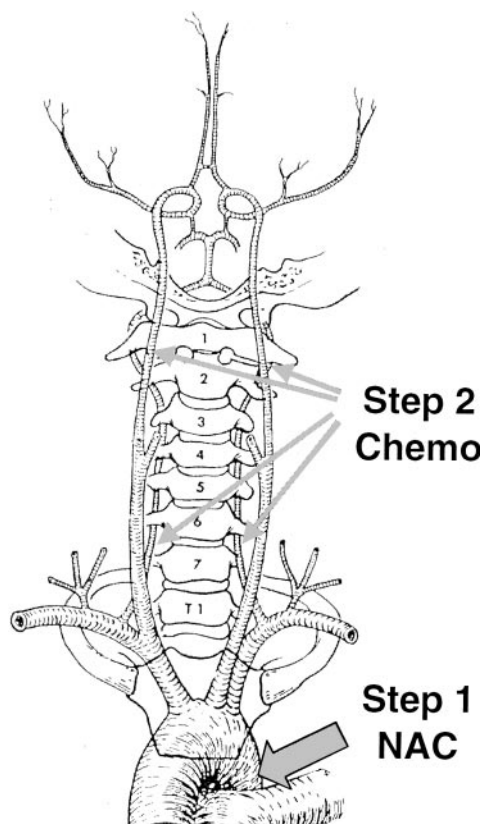


Fig. 4. The "aortic infusion" method. Chemoprotectant, *i.e.*, NAC, is administered into the descending aorta 30 min prior to chemotherapy. Chemotherapy is then administered into the left or right internal carotid artery or the left or right vertebral artery, in conjunction with BBBD. CNS alkylator delivery thus is increased, but because of first-pass clearance, NAC does not abrogate alkylator efficacy. This method thus may increase dose intensity to tumors above the clavicle while permitting tissue targeting of chemoprotective agents to reduce systemic toxicity below the clavicle, without either interference with efficacy against intracerebral tumor or significant toxicity.

patients greatly decreased ototoxicity (18, 19). Indeed, chemotherapy for brain tumor therapy can be safely separated from chemoprotectants not only because of the BBB, as with STS, but also because of different routes of administration, *i.e.*, *i.a.* alkylators cephalad *versus* aortic infusion of NAC caudally. Clinical testing of NAC chemoprotection is warranted, particularly in CNS and/or head and neck tumors with *i.a.*-based chemotherapy.

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