In Vitro and Animal Studies of Sodium Thiosulfate as a Potential Chemoprotectant against Carboplatin-induced Ototoxicity


Abstract

When carboplatin (cis-diammine-1,1-cyclobutane-dicarboxylato-platinum) delivery to brain tumors is optimized with osmotic blood-brain barrier disruption (BBBD), high frequency hearing loss can result. Treatment with sodium thiosulfate (STS) blocked carboplatin cytotoxicity against the LX-1 human small cell lung carcinoma cell line in vitro. STS decreased carboplatin-induced ototoxicity in a guinea pig model, as determined by electrophysiological measurements and analysis of inner ear outer hair cells. Protection was found when STS was administered up to 8 h subsequent to carboplatin but not 24 h after carboplatin. In a rat model of osmotic BBBD, STS was neurotoxic when given immediately after BBBD but not when given 60 min after BBBD, when the barrier is reestablished. Thus, delayed administration of STS may provide a mechanism to reduce the cochlear toxicity caused by BBBD-enhanced carboplatin delivery to the brain.

Introduction

When delivery of carboplatin across the BBB to intracerebral tumors was optimized with osmotic BBBD, this chemotherapeutic agent was effective for the treatment of some human brain tumors (1, 2) but also caused irreversible high-frequency hearing loss (1). Statistical analysis showed that furosemide diuresis, age, and vertebral artery BBBD might be contributing factors to the carboplatin-induced ototoxicity (1, 3). Use of a chemoprotective agent (4), such as STS, which can convert the alkylating drug into a noncytotoxic compound (4–6), may provide a mechanism to rescue from carboplatin toxicity. STS has been shown to be a useful protective agent against another platinum-based chemotherapeutic, cisplatin, but STS rescue of carboplatin toxicity in vivo has not been reported previously. Treatment with STS can prevent cisplatin toxicity in vitro (7), reduces cisplatin nephrotoxicity in animal models (8), and has shown favorable results against human cancers, allowing cisplatin dose escalation while decreasing the nephrotoxicity of the drug (9–11). The BBB allows very low delivery of STS into the brain (4, 12), effectively producing two compartments. The presence of the BBB, therefore, provides a solution to the potential drawback of chemoprotectant-mediated drug inactivation. We hypothesize that carboplatin might be delivered to intracerebral tumors with osmotic BBBD, whereas STS, administered after the BBB permeability has declined to baseline (13), could be used to reduce the ototoxicity of the circulating drug. The current series of studies was undertaken to determine if STS was protective against carboplatin in vitro and in vivo, if it could reduce the ototoxicity caused by carboplatin, and if it could be used in conjunction with osmotic BBBD.

Materials and Methods

Reagents. Carboplatin (Paraplatin) was obtained from Bristol Myers-Squibb (Seattle, WA) and was dissolved in H₂O at 10 mg/ml (26.9 mM). STS pentahydrate was obtained from Mallinkrodt and was dissolved in H₂O at a concentration of 500 mg/ml (2.0 M).

In Vitro Studies. LX-1 small-cell lung carcinoma cells were seeded at 10⁴ cells/well in 96-well tissue culture plates. For the time course study, the cells were treated with carboplatin (100 μg/ml), STS (2 mg/ml), or no treatment. Some cells that had received carboplatin subsequently received STS at 0, 2, 4, 6, 8, or 18 h after carboplatin treatment. Toxicity was assessed 3 days after the addition of experimental agents. The cells remaining attached to the well were lysed in 0.2% SDS, and the absorbance of total nucleic acid at 260 nm was measured. The absorbance increases linearly with the log of cell numbers between 10³ and 10⁶ cells. The methylene blue method for measuring STS was used to reduce the ototoxicity of the circulating drug. The current series of studies was undertaken to determine if STS was protective against carboplatin in vitro and in vivo, if it could reduce the ototoxicity caused by carboplatin, and if it could be used in conjunction with osmotic BBBD.

Guinea Pig Ototoxicity Studies. Animal studies were performed in accordance with guidelines established by the Oregon Health Sciences University Committee on Animal Care and Use. Topeka strain guinea pigs having an active Preyer pinna reflex were used for the ototoxicity studies (n = 34). The animals were anesthetized using pentobarbital (32 mg/kg i.p.), and a polyethylene catheter (PE 50) was surgically placed into the left external jugular vein. The animals were then treated with carboplatin (24 mg/kg s.c.), followed after 1 h by furosemide (100 mg/kg i.v.); then the i.v. catheter was removed. Subsequently, animals were treated i.p. with either normal saline (n = 8) or STS (1.83 g/kg, n = 26) at 2, 4, 8, or 24 h after administration of carboplatin. The animals were then maintained for 4 weeks to allow the drug effects to stabilize.

After the stabilization period, each animal was anesthetized using allobarital (60 mg/kg) and urethane (240 mg/kg) i.p. An endotracheal tube was inserted, the animal was mechanically ventilated, and body temperature was maintained at 38.5°C. The middle ear was exposed, a small silver ball electrode was placed on the round window membrane, and the compound action potential (N1) threshold was determined at six different frequencies from 2 kHz through 32 kHz. The ability of the cochlea to generate a 10 μV isopotential at the same six test frequencies as well as the maximum output of the AC cochlear potential at 10 kHz was determined.

After all electrophysiological studies were completed, the guinea pigs were killed and cochleas were fixed in Dalton's fixative containing 1% osmium tetroxide. The cochleas were dissected using microdissection tools, and the cochlear hair cells were counted with the surface preparation technique (15).

BBBD and Administration of STS in the Rat. Adult, female Long Evans rats weighing 220–240 g were anesthetized with isoflurane inhalant (5% induction, 2% maintenance). The right external carotid artery was surgically exposed and catheterized, and mannitol was infused for BBBD as described previously (13, 16). Animals were given 11.6 g/m² STS, which corresponds to

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3 The abbreviations used are: carboplatin, cis-diammine-1,1-cyclobutane-dicarboxylato-platinum; BBB, blood-brain barrier; BBBD, blood-brain barrier disruption; STS, sodium thiosulfate.
Thiosulfate Rescue of Carboplatin Ototoxicity

Sound intensity required to generate 10 μV of AC cochlear potential was tested (Fig. 2B). Carboplatin with furosemide reduced the AC cochlear potential maximum output at 10 kHz to less than 20 μV, but in the presence of STS, the output was maintained in the normal range at over 280 μV (Fig. 2B).

The percentage of cochlear outer hair cells that were missing from the organ of Corti of the treated animals is represented in Fig. 3. The

the dosage used to counteract cyanide poisoning in people (17) and, in a 220-g rat, is equivalent to the dosage of STS (1.83 g/kg) given to guinea pigs. The STS was administered as follows (n = 4/group): (a) intracarotid infusion (0.2 ml/min) immediately after mannitol; (b) i.v. infusion immediately after mannitol; (c) i.v. infusion 30 min after mannitol; (d) i.v. infusion 1 h after mannitol; or (e) i.v. infusion without BBBD. Serum sodium and potassium levels were monitored before, immediately after, and 2 h after STS administration. All animals were sacrificed with an overdose of sodium pentobarbital (400 mg/kg intracardiac) 2 h after STS administration.

Results

STS Rescue of Carboplatin Cytotoxicity in Vitro. LX-1 cells were treated with carboplatin at concentrations ranging from 1–200 μg/ml. The LD50 of carboplatin, after 3 days incubation, was approximately 20 μg/ml, while 100 μg/ml routinely resulted in 80–90% cytotoxicity. STS toxicity was also determined in vitro. At concentrations of 10 mg/ml and above, STS was toxic to LX-1 cells, perhaps due to high osmolarity. Lower STS concentrations had no effect on cell growth. When cells were incubated simultaneously with a lethal dose of carboplatin (100 μg/ml) in combination with STS, concentrations of STS above 0.5 mg/ml (a molar ratio of 7.5) completely blocked the cell killing by carboplatin. A time course experiment was performed to determine how late after the addition of carboplatin the STS would retain its protective effect. Treatment with STS anytime from 0–6 h after the addition of carboplatin reduced the toxicity of this drug by >90% (Fig. 1). By 8 h after the addition of carboplatin, STS was only 60% effective in reducing cytotoxicity, and it was ineffective at 18 h (Fig. 1).

STS Rescue of Carboplatin-induced Otoxicity in the Guinea Pig. In the guinea pig, administration of carboplatin in combination with furosemide reproducibly causes high frequency hearing loss, as determined by electrophysiological measures and loss of outer hair cells.4 Experiments were performed to determine if STS could reduce the carboplatin-induced auditory damage in vivo. The sound intensity required to generate a compound action potential threshold (N1) is shown in Fig. 2A. A single dose of carboplatin, in conjunction with furosemide, was sufficient to result in severe cochlear damage in this guinea pig model. When STS was administered i.p. 2 h after carboplatin, the N1 thresholds were maintained within the normal range of historic controls (15) at test frequencies ranging from 2 to 32 kHz (Fig. 2A). STS also blocked carboplatin-induced ototoxicity when the


Fig. 1. Time course for rescue of carboplatin toxicity in vitro. LX-1 cells were treated with carboplatin (100 μg/ml), followed by STS (2 mg/ml) at the indicated times. After 3 days, total nucleic acid was analyzed as a measure of cell number. The bar graph indicates cells that received no addition, STS alone, or carboplatin alone during the treatment period. Each value indicates the mean for n = 4 independent wells; bars, SD.

Fig. 2. Rescue of carboplatin-induced electrophysiological auditory damage with STS in the guinea pig. A, compound action potential thresholds. B, maximum AC cochlear potential output at 10 kHz. All guinea pigs (n = 12) were treated with carboplatin, followed in 1 h with furosemide. One h after furosemide, six animals were treated with STS and six with saline. Each value indicates the mean for n = 6 animals; bars, SD.

Fig. 3. Protection of cochlear hair cells by STS in the guinea pig. The percentage of outer hair cells missing from the inner ears was determined for guinea pigs treated with carboplatin followed in 1 h with furosemide (n = 12). One h after furosemide, animals were treated with STS or saline (n = 6 per group). Each value is the mean; bars, SD.
hair cell counts are consistent with the electrophysiological data showing the protective effect of STS. For example, the maximum output of the AC cochlear potential at 10 kHz was about 300 μV for the animals treated with STS, and they lost only about 5% of the outer hair cells. The saline-treated animals, on the other hand, were only able to generate 20 μV of AC cochlear potential and had lost over 60% of their outer hair cells.

A time course experiment was performed to determine the window of opportunity for STS rescue of carboplatin-induced hearing loss. The maximum AC cochlear potential at 10 kHz was maintained at a mild, focal seizure that occurred 15 mm after recovery from anesthesia immediately after BBBD demonstrated less neurotoxicity. One animal had after BBBD. Those animals receiving STS as an iv. infusion immediately after recovery from anesthesia. In two animals, the seizures were controlled with diazepam (7.5 mg/kg i.p.), but in the third animal, the seizures were not controlled and the animal subsequently died 1 h after BBBD. Those animals receiving STS as an i.v. infusion immediately after BBBD demonstrated less neurotoxicity. One animal had a mild, focal seizure that occurred 15 min after recovery from anesthesia and did not require diazepam for control. No signs of neurotoxicity were detected when STS was given as a slow i.v. infusion at 30 or 60 min after BBBD, when the barrier is reestablished (13, 16). Serum sodium and potassium levels did not change as a result of STS infusion.

Discussion

STS Rescue of Carboplatin Toxicity. The results described herein indicate that STS dramatically reduced the level of auditory damage due to administration of carboplatin and furosemide, without BBBD, in the guinea pig. The guinea pig was used as a model of the ototoxicity observed in human brain tumor patients given carboplatin in combination with vertebral artery BBBD. However, the guinea pig model of carboplatin-induced ototoxicity does not completely reflect the clinical situation: (a) high levels of furosemide are required to induce ototoxicity in guinea pigs. Although use of this diuretic perioperatively was associated with increased hearing loss in humans undergoing BBBD, withholding furosemide did not prevent hearing loss (1); and (b) delivery of carboplatin by BBBD is not a necessary component of guinea pig ototoxicity, while vertebral artery BBBD was linked to human ototoxicity (1). Experiments are under way to test STS rescue in a canine model, which may mimic more closely the clinical situation.

The molar ratio of STS to cisplatin is a primary determinant of the extent and rate of neutralization of cisplatin, with as much as a 400-fold excess of STS necessary for rapid inactivation (6, 7). Previous studies found that the concentration of STS achieved in the plasma was inadequate for rapid clearance of the drug. In fact, Goel et al. (9) found that STS reduced plasma cisplatin levels by only 25% (9). Serum STS levels in the rats and guinea pigs were higher than the values reported in humans (9, 14), which may further decrease circulating carboplatin. STS is rapidly concentrated in the kidneys, with urine levels reaching 2000 mg/dl in humans (12, 14) and over 3800 mg/dl immediately after the STS infusion in our rat studies. In contrast to the serum, the urine concentrations are sufficient to rapidly inactivate cisplatin (6), which explains why STS protects against cisplatin-induced nephrotoxicity but not myelosuppression.

STS inactivates carboplatin in an analogous manner to the inactivation of cisplatin in that the thiol binds the electrophilic platinum, forming a complex that can be excreted rapidly (4, 6). Similar to cisplatin, inactivation of carboplatin was also found only at high molar ratios in previous reports (5, 6), although our in vitro tests showed that even a 7.5-fold molar excess of STS was sufficient to protect LX-1 cells from cytotoxicity. The mechanism of STS rescue of carboplatin-induced ototoxicity is unclear. However, extrapolating the cisplatin data to carboplatin, it is reasonable to assume that carboplatin toxicity will be reduced only in tissues that concentrate STS. We hypothesize that the cochlea may act similarly to the kidney to concentrate STS in perilymph or endolymph. High concentrations in these fluid compartments, as in the kidney, may act to inactivate carboplatin at the site of cochlear damage. Measurements of STS concentration in the perilymph in the guinea pig model are being initiated.

Our results differ from the published studies of STS rescue of cisplatin nephrotoxicity, particularly in regards to the timing of STS administration. Studies using two-route chemotherapy with the combination of high dose i.p. cisplatin and i.v. STS have demonstrated that chemoprotection necessitated the addition of STS within 5 min of the cisplatin (6, 8, 11). We found that STS treatment could be delayed considerably after carboplatin administration; STS was withheld for 6 h in vitro, and for 2, 4 or 8 h, but not 24 h, for the guinea pig study of ototoxicity, without toxic effects. This timing difference may be the key to reducing the undesirable side effects of carboplatin while maintaining a high therapeutic effect.

Potential Use of STS in the Clinical Setting. We hypothesize that STS may be useful to decrease some of the undesirable side effects of carboplatin used in BBBD chemotherapy of brain tumors. We, therefore, tested whether STS could be safely given in conjunction with osmotic BBBD. STS did cause seizures if given intraarterially immediately after BBBD. This may be the result of either the high osmolarity of the STS or a direct effect of the reactive thiol by virtue of its ability to pass freely through the osmotically modified BBB. Seizures were not observed at 30 min or 1 h after BBBD. By 30 min after
BBBD, the transient osmotic opening of the BBB has largely returned to baseline, as determined by the permeability to Evans blue albumin, although permeability to smaller molecular weight agents may persist for up to 2 h (13). Thus, delayed administration of STS was safe, which correlates with previous studies that demonstrate that STS is nontoxic and can safely be given i.v. for treatment of treatment of ototoxicity (17).

A possible drawback to the use of a chemoprotectant is the potential to deactivate the desired antitumor effects of carboplatin, as well as reducing the undesired toxicity. Two-route chemotherapy, using the combination of i.p. administration of high dose cisplatin with i.v. administration of STS, has been used in human studies to provide local chemoprotection while sparing antitumor activity (4, 10, 11). The BBB may serve to produce two compartments by limiting STS combination with continued efficacy.

References
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