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Pharmacokinetics of sodium thiosulfate in Guinea pig perilymph following middle ear application

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ABSTRACT

Hypothesis: To determine the pharmacokinetics of sodium thiosulfate in the inner ear perilymph following middle ear application in Guinea pigs.
Background: Cisplatin chemotherapy is often associated with a dose-dependent high frequency sensorineural hearing loss. Sodium thiosulfate has been shown to reduce cisplatin-induced ototoxicity when given intravenously, but this may limit the tumoricidal effects of the chemotherapy. Recent animal studies looking at middle ear application of sodium thiosulfate have shown prevention of outer hair cell and hearing loss, but the perilymph pharmacokinetics have not yet been established.
Methods: Twenty Guinea pig ears were split into two groups and administered sodium thiosulfate to the middle ear at either a concentration of 250 mg/mL or 50 mg/mL for 30 min. Perilymph samples were then obtained serially through the round window over 6 h. Sodium thiosulfate concentrations were obtained using high-pressure liquid chromatography.
Results: The 250 mg/mL group had a maximum perilymph concentration of 7.27 mg/mL (±0.83) that decreased to 0.94 mg/mL (±0.03) over 6 h. The 50 mg/mL group had an initial concentration of 1.63 mg/mL (±0.17) and was undetectable after 1 h. The half-life of sodium thiosulfate within perilymph was 0.74 h.
Conclusions: and Relevance: The results of this study show that sodium thiosulfate is capable of diffusing through round window and into the inner ear perilymph. Peak levels decline over several hours after exposure. This has a potential application as a localized therapy in the prevention of cisplatin induced ototoxicity.

1. Introduction

Cisplatin is a commonly used chemotherapy agent in the treatment of a number of malignant tumors, both in children and adults. This type of chemotherapy is often associated with a dose-dependent bilateral high frequency sensorineural hearing loss. The incidence of cisplatin-induced hearing loss is reported to range from 22 to 77% (Schaefer et al., 1985; Bokemeyer et al., 1998; De jongh et al., 2003; Coradini et al., 2007; Knight et al., 2005; Kushner et al., 2006). The range reflects differences in cisplatin dose, as well as in measures of hearing outcomes. Additional risk factors for cisplatin-induced ototoxicity include children under five years in age, prior irradiation to the head and neck greater than 40 Gy, and prior hearing loss (Montaguti et al., 2002; Li et al., 2004; Hitchcock et al., 2009). Vestibular function can also be damaged by platinum-based toxicity (Moroso and Blair, 1983). The exact cellular mechanisms by which cisplatin causes cochlear hair cell loss is unclear. There is believed to be some degree of resultant oxidative stress with subsequent activation of apoptotic pathway in outer hair cells and the stria vascularis (Rybak, 2007). Other possible mechanisms include the activation of voltage-dependent big conductance potassium channels in type 1 spiral ligament fibrocytes of the lateral wall of the cochlea leading to disruption of the electrochemical gradient and the activation of apoptotic pathways (Liang et al., 2005). Finally, other postulated mechanisms include disruption to nuclear excision repair leading to aggregates of cisplatin-induced DNA adducts and inflammation from the induction of pro-inflammatory cytokines in the cochlea (Guthrie et al., 2008; So et al., 2008).

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Sodium thiosulfate (STS) has shown potential as an otoprotective agent against platinum-based chemotherapy (Leitao and Blakley, 2003; Muldoon et al., 2000; Kaltenbach et al., 1997; Doolittle et al., 2001; Neuwelt et al., 1998; Zuur et al., 2007). STS is currently approved by the United States Food and Drug Administration (USFDA) as an antidote for cyanide and nitroprusside toxicity, and it is also used as an off-label treatment for cisplatin-related nephrotoxicity and calciphylaxis (Hall et al., 2007; Bourgeois and De Haes, 2016; Ossorio-Garcia et al., 2016; Yu et al., 2015; Gandara et al., 1990). Unlike several other potential otoprotectants, STS has been shown to act as both an antioxidant as well as a chelating agent (Gandara et al., 1990; Yerram et al., 2007; Marckmann et al., 2008). The chelating properties of the sulfur-thiol functional group are believed to be responsible for the otoprotective effects of STS by binding to and inactivating the platinum (Dedon and Borch, 1987). The thiol compound may also act to scavenge reactive oxygen species produced by the platinum, thus preventing the initiation of the apoptotic pathway (Dedon and Borch, 1987).

STS administered intravenously has been shown to prevent cisplatin-induced ototoxicity in animal models and *in vivo* (Leitao and Blakley, 2003; Muldoon et al., 2000; Kaltenbach et al., 1997; Doolittle et al., 2001; Neuwelt et al., 1998; Zuur et al., 2007). However, a major concern to this mode of delivery is that it may potentially reduce the tumoricidal activity of the platinum. Sodium thiosulfate is believed to bind to cisplatin, forming a complex that is then excreted by the kidneys. Such chelation could negatively impact the desired activity of the platinum. There are conflicting reports of reduced tumoricidal properties of STS in vitro (Harned et al., 2008; Yee et al., 2008). A recent *in vivo* study by Freyer et al. showed that infusion of intravenous 6% STS solution after cisplatin therapy was not found to have a statistically significant otoprotective effect in children with pediatric cancer. Additionally, patients with disseminated disease treated with STS were shown to have significantly lower 3-year overall survival (45%) compared to the control group (84%) (Freyer et al., 2017).

Local application of STS to the round window may represent an alternative mode of delivery that may allow for higher intracochlear concentrations while minimizing interference with the tumoricidal effects of cisplatin. There have been a few recent studies showing intratympanic STS as an effective means to prevent cisplatin-induced ototoxicity in animals (Wang et al., 2003; Berglin et al., 2011). However, the pharmacokinetics of STS via round window diffusion are not yet well understood. The purpose of this study is to establish pharmacokinetic parameters of STS in perilymph after intratympanic administration.

2. Materials and methods

2.1. Animal preparation and procedures

Ten retired breeder Hartley albino Guinea pigs of both sexes (Charles River, Kißlegg, Germany) were used. Twenty ears were randomly separated into 2 groups based on STS (Hope Pharmaceuticals, Scottsdale, AZ) concentration: 50 mg/mL (5% solution) or 250 mg/mL (25% solution) (Fig. 1). These concentrations of STS were selected based on previous studies determining their safety. A 25% solution of STS has been used ototopically to prevent myringosclerosis in rat models and a 6% solution has also been used safely when administered ototopically in mice models (Park et al., 2010; Stocks et al., 2004). Each Guinea pig was placed under general anesthesia using inhaled 1–5% isoflurane for induction, then intubated endotracheally and placed on 2–4% isoflurane for maintenance anesthesia. The body temperature was maintained throughout the procedure with a temperature-controlled heating blanket. The head and neck was then shaved and 1% lidocaine with 1:100,000 epinephrine was injected in the postauricular soft tissue. A postauricular incision was then made extending to the neck and soft tissue was elevated off of bone. With the aid of an operating microscope, a cutting bur was then used on a high-speed drill to enter the bulla. The vertical portion of the facial nerve was drilled away in order to obtain adequate visualization of the round window. This procedure was then repeated on the contralateral ear.

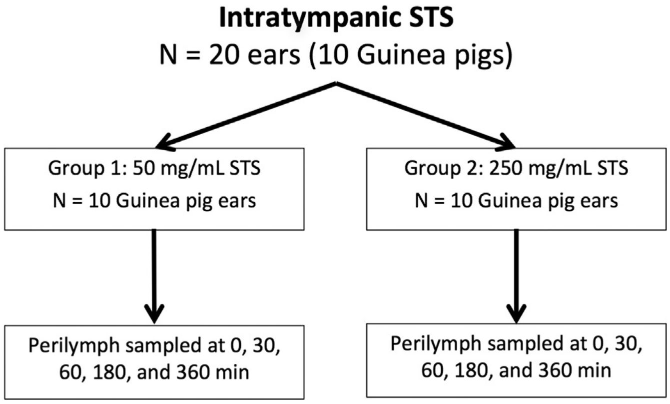

Fig. 1. Flowchart of STS Randomization and perilymph sampling.

The protocol for STS middle ear application and perilymph sampling was adapted from similar studies looking at the pharmacokinetics of intratympanic steroids (Chandrasekhar et al., 2000; Plontke et al., 2008; Parnes et al., 1999; Wang et al., 2011; Hahn et al., 2012; Liu et al., 2006). With the head in a neutral position, STS was placed in the middle ear space for 30 min. An operating microscope was used to confirm the round window was covered with STS. After 30 min, we confirmed that the round window was still covered with STS ensuring none was lost via the Eustachian tube. The bulla was then suctioned and irrigated with normal saline. A 26-gauge needle attached to a 10 μL Hamilton syringe was then inserted into the round window, and 2 μL of perilymph was then aspirated from the scala tympani. Perilymph samples were obtained serially at 0, 30, 60, 180, and 360 min after the bulla was cleaned. In order to prevent perilymph lost between samples, the Guinea pig heads were kept in a neutral position for all samples. Additionally, no gush of perilymph was noted upon perforation of the round window. Samples were stored at −20° C and later analyzed by high pressure liquid chromatography (HPLC). Once all samples were obtained, intraperitoneal pentobarbital was injected for euthanasia. This study was performed in accordance with the Public Health Service Policy on Humane Care and Use of Laboratory Animals, the NIH Guide for the Care and Use of Laboratory Animals, and the Animal Welfare Act. The Institutional Animal Care and Use Committee of SUNY Upstate Medical University approved the animal use protocol.

2.2. High pressure liquid chromatography (HPLC)

STS concentrations were quantitatively analyzed by HPLC using an LC-20AT with a SPD-M20A UV–Vis detector (Shimadzu, Kyoto, Japan) and a LiChrospher RP-select B LiChroCART 250-4 anion exchange column (EMD Millipore Corp, Darmstadt, Germany). All samples were prepared using a method similar to that described by Togawa et al. (1992). Two μL samples were diluted in 8 μL of sterile water. Ten μL of 5 mM monobromobimane in acetonitrile was then added and the mixture was allowed to sit for 60 min in the dark at room temperature. Ten μL of 0.05 M KCl-HCl buffer was then added. Ten μL of this mixture was then submitted to the HPLC system using