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ORIGINAL ARTICLE

Prevention of cisplatin-induced hearing loss by administration of a thiosulfate-containing gel to the middle ear in a guinea pig model

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Abstract
Purpose Thiosulfate may reduce cisplatin-induced ototoxicity, most likely by relieving oxidative stress and by forming inactive platinum complexes. This study aimed to determine the concentration and protective effect of thiosulfate in the cochlea after application of a thiosulfate-containing high viscosity formulation of sodium hyaluronan (HYA gel) to the middle ear prior to i.v. injection of cisplatin in a guinea pig model.
Methods The release of thiosulfate (0.1 M) from HYA gel (0.5% w/w) was explored in vitro. Thiosulfate in the scala tympani perilymph of the cochlea 1 and 3 h after application of thiosulfate in HYA gel to the middle ear was quantified with HPLC and fluorescence detection. Thiosulfate in blood and CSF was also explored. The potential otoprotective effect was evaluated by hair cell count after treatment with thiosulfate in HYA gel applied to the middle ear 3 h prior to cisplatin injection (8 mg/kg b.w.).
Results HYA did not impede the release of thiosulfate. Middle ear administration of thiosulfate in HYA gel gave high concentrations in the scala tympani perilymph while maintaining low levels in blood, and it protected against cisplatin-induced hair cell loss.
Conclusion HYA gel is an effective vehicle for administration of thiosulfate to the middle ear. Local application of a thiosulfate-containing HYA gel reduces the ototoxicity of cisplatin most likely without compromising its antineoplastic effect. This provides a minimally invasive protective treatment that can easily be repeated if necessary.

Keywords Cisplatin · Thiosulfate · Sodium hyaluronan · Topical administration · Guinea pig · Ototoxicity

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Introduction

Cisplatin is a chemotherapeutic agent that has been widely used for more than three decades against various solid malignant tumors. Its dose-limiting side effects are nephrotoxicity and ototoxicity [1]. While nephrotoxicity can be prevented by increased hydration and forced diuresis, there is still no cure or preventive treatment for ototoxicity. In pediatric cancer patients, loss of hearing is particularly disabling as it may hamper the patient's speech, cognitive, and social development. The first signs of ototoxicity include hearing loss in the high frequency range and

tinnitus [2]. The main targets of the ototoxic effects seem to be the outer hair cells (OHC) in the organ of Corti, the spiral ganglion cells, and the cells of the stria vascularis in the basal part of the cochlea [3].

The main cytotoxic effects of cisplatin are believed to be mediated by the monohydrated cisplatin complex (MHC), which reacts with nuclear DNA, forming platinum–DNA adducts [4]. The cytotoxicity includes the generation of reactive oxygen species (ROS) [5], and therefore, antioxidants have been in focus in the search for protection against cisplatin-induced ototoxicity for many years. Several sulfur-containing compounds are antioxidants with high affinity to platinum species [6]. They may prevent cisplatin- and MHC-induced toxicity in a dual manner, both by decreasing the effect of ROS and by chelating cisplatin and MHC, forming inactive complexes. Among the sulfur-containing antioxidants that have shown otoprotective potential in experimental studies are *N*-acetyl-cysteine [7], sodium thiosulfate [7], and D-methionine [8]. However, when administered systemically, such compounds may reduce the efficacy of cisplatin-based chemotherapy due to drug interactions [9]. Moreover, their uptake from the systemic circulation to the cochlea is limited due to the blood-labyrinth barrier that is physiologically similar to the blood–brain barrier [10]. Intracochlear administration of otoprotective compounds is not a clinical option since the cochlea in the human is located in the base of the skull and such treatment would involve invasive surgery that may cause irreversible injury to aural sensory structures. Because of these limitations, there is an increasing interest in local administration where the protective drug is given to the middle ear cavity from which it can pass to the cochlea, most probably through the round window membrane (RWM) [11]. In a previous study, our research group investigated the kinetics of reactions between cisplatin/MHC and sulfur-containing compounds aimed for local otoprotective administration and found sodium thiosulfate to be a promising candidate due to its fast chelation rate [6]. In the present study, we investigated the otoprotective efficacy of a thiosulfate-containing high viscosity formulation of sodium hyaluronan (HYA gel) administered to the middle ear prior to cisplatin treatment in a guinea pig model. The aims of the study were as follows:

1. To determine the release of thiosulfate from HYA gel in vitro.
2. To quantify thiosulfate in scala tympani perilymph of the cochlea after middle ear administration of a thiosulfate-containing HYA gel in vivo.
3. To investigate the efficacy of middle ear administration of thiosulfate-containing HYA gel against cisplatin-induced hair cell loss in vivo.

Materials and methods

Study design

First, we explored the release of thiosulfate from HYA gel in vitro. Then, we investigated the pharmacokinetics of thiosulfate after middle ear administration of thiosulfate-containing HYA gel in a guinea pig model. After 1 and 3 h, the gel was removed and samples of perilymph were taken from scala tympani for quantification of thiosulfate. The thiosulfate concentration in blood and cerebrospinal fluid (CSF) was also explored. In light of these results, we determined a suitable time for administration of thiosulfate-containing HYA gel to the middle ear prior to cisplatin treatment in a final in vivo experiment. In this part of the study, HYA gel without thiosulfate was administered to the contralateral ear of the guinea pig, serving as control. The animals were decapitated 96 h after cisplatin injection when inner ear changes are known to be seen after cisplatin treatment [12], and the cochleae were dissected for surface preparation to evaluate the potential otoprotection through hair cell count.

Chemicals

Sodium hyaluronan, used for the HYA compositions, and the Carbopol gel (C940) were kind gifts from Advanced Medical Optics (Uppsala, Sweden) and Noveon Inc. (Breeksville, OH, USA), respectively. Sodium thiosulfate pentahydrate was purchased from Sigma Chemical Co. (St. Louis, MO, USA).

Two different gels were prepared for the in vivo studies; a control gel containing 0.5% (w/w) HYA in PBS and an active formulation containing 0.1 M thiosulfate and 0.5% (w/w) HYA in PBS. A stock gel containing 1% (w/w) HYA in PBS was prepared by slowly dispersing the polymer in PBS while stirring vigorously. Parallel to this, a solution of 0.2 M thiosulfate in PBS was made. The stock gel was then autoclaved, before being mixed (50:50) either with the thiosulfate solution or with PBS to form the active gel and the control gel, respectively, at about pH 7.2. The osmolality of the gels was investigated, using a Vapro Osmometer 5520 (Wescor, UT, USA).

The gels for the in vitro studies were prepared in a similar manner. However, to prevent the polymer in the HYA gels from immediately diffusing out of the containers used in the drug release setup, the gels also contained 1% (w/w) Carbopol; this forms a matrix where small drug molecules are able to diffuse freely, but where the diffusion of large HYA polymers is very limited. A reference gel containing 0.1 M thiosulfate in 1% (w/w) Carbopol was also prepared. Methods for preparing Carbopol gels are described elsewhere [13].

Ketamine (Ketalar®, 50 mg/ml, Pfizer AB, Täby, Sweden) and xylazin (Rompun® vet, 20 mg/ml, Bayer Health Care AG, Leverkusen, Germany) were used for general anesthesia and lidocaine (Xylocain®, 20 mg/ml, AstraZeneca, Södertälje, Sweden) for local anesthesia. Atropine (Atropin Mylan, 0.5 mg/ml diluted with saline to 0.05 mg/ml) was from Mylan (Stockholm, Sweden). The animals were hydrated and rehydrated with Ringer-Acetat (Fresenius Kabi AG, Bad Homburg v.d.H., Germany). Sodium heparin (Heparin LEO, 5,000 IU/ml, diluted with water to 500 IU/ml) was provided by LEO Pharma (Malmö, Sweden). Cisplatin was purchased from Meda (Cisplatin Meda 1 mg/ml, Solna, Sweden). Atipamezol (Antisedan® vet., 5 mg/ml) and buprenorfin (Temgesic®, 0.3 mg/ml diluted with saline to 0.03 mg/ml) were from Orion Pharma Animal Health (Sollentuna, Sweden) and Schering-Plough (Stockholm, Sweden), respectively. Monobromobimane (≥95%, Fluka, Sigma–Aldrich, Steinheim, Switzerland), succinic acid, and acetonitrile (≥99.8%, both from Merck, Darmstadt, Germany) were used for HPLC quantification of thiosulfate.

Animals

In the pharmacokinetic study, 16 albino guinea pigs (mean weight 421 g, range 340–497 g) and in the otoprotection study, 12 albino guinea pigs (mean weight 297 g, range 239–340 g) were used. All animals had otoscopically normal tympanic membranes. The hearing thresholds of the animals used in the otoprotection study were verified as being normal before treatment by electrophysiological examination of acoustically evoked auditory brainstem response (ABR). Animals were of both sexes from a local breeder and maintained on a 12:12 h light/dark cycle with unrestricted access to food and water. The care and use of the animals were approved by the local animal care and use committee, Stockholms Norra Djurförsöksetiska Nämnd (N 50/07, N 334/08), according to the Declaration of Helsinki.

Thiosulfate release from HYA gel in vitro

The release of thiosulfate was studied by a modified USP paddle method. The gels were placed in custom-made cylindrical gel containers, with fixed volumes of 6 cm^3 and fixed areas of 21 cm^2, covered with a coarse plastic net and, at the top, a stainless steel net. The gel containers were lowered in the glass cylinders of a Pharma Test PTW bath, containing 300 ml 0.9% NaCl and with paddle speeds of 30 rpm. The release medium, kept at 37°C, was continuously pumped through a Shimadzu UV-1601 spectrophotometer with which the released amount thiosulfate was quantified at 246 nm. The measurements were performed in triplicates. Plots of the release of thiosulfate were constructed, and the release rate was calculated as diffusion coefficients according to Eq. 1:

$$Q = 2 \times C_0 \times \left(\frac{D \times t}{\pi}\right)^{1/2} \quad (1)$$

where Q is the amount of drug released per unit area, C_0 is the initial drug concentration in the gel, D is the diffusion coefficient, t is the time elapsed since the start of the experiment, and π is the mathematical pi. The equation is valid for the initial 60% of the release, where it allows an approximation of Fickian diffusion during sink conditions [14].

Thiosulfate in scala tympani perilymph after middle ear administration of thiosulfate-containing HYA gel in vivo

The animals were randomized for administration of thiosulfate-containing HYA gel to the left or right middle ear cavity. They were anesthetized with ketamine (40 mg/kg b.w., i.m.) and xylazine (12 mg/kg b.w., i.m.). Additional doses of ketamine and xylazine were administered when needed to maintain an adequate depth of anesthesia. During the experiment, the animals were kept on a Harvard homeothermic surgical table maintaining constant rectal temperature of 38°C. Before surgery, they were hydrated with Ringer-Acetat (5 ml s.c.) and anesthetized with lidocaine. The internal jugular vein was exposed, and a catheter was inserted for blood sampling and replacement of lost fluids. To prevent blood clots, heparin (ca 0.5 ml in total) was administrated in the catheter. A small opening was made in the tympanic membrane to prevent rupture of the RWM during application into the middle ear. Through a fine needle (BD Microlance™ 30G; external diameter 0.3 mm), 0.15 ml of thiosulfate-containing HYA gel was injected into the auditory bulla through the skin of the auricle. The point of injection was determined, and the flow of the gel into the middle ear cavity was observed through an operating microscope. The animals were kept on their stomach with the head in a vertical position between the injection and the aspiration of the gel.

After 1 h in 8 animals (group 1-h) and 3 h in the remaining eight animals (group 3-h), the bulla was opened wide using a dorsolateral approach, the gel was aspirated for thiosulfate quantification, and the middle ear cavity repeatedly rinsed with 0.9% NaCl (1 ml in total). To avoid contamination with thiosulfate, the mucosa of the basal turn of the cochlea was removed before a small hole was drilled through the otic capsule. The tip of a 1-µl syringe (Hamilton Bonaduz AG, Bonaduz, Switzerland) was quickly lowered into the scala tympani with

the aid of a micromanipulator. One microliter of scala tympani perilymph was gently aspirated over approximately 10 s. Blood samples of approximately 0.35 ml were taken in every animal. In group 1-h, blood samples were collected 20 min, and 1 h after gel administration, the third was taken after the final CSF sampling. In group 3-h, blood samples were taken at 20 min, 2 h, 3 h, and after the final CSF sampling. After each blood sampling, the animals were given an equal volume of 0.9% NaCl to rinse the catheter and to replace lost fluids. CSF samples were taken immediately after perilymph sampling by percutaneous puncture of the cisterna magna with a 50-µl syringe (Hamilton Bonaduz AG) using a suboccipital approach described by Reiber and Schunck [15]. Thiosulfate samples were handled, stored, and analyzed as described previously [16]. After the final blood sampling, the animal was decapitated while still under anesthesia.

Efficacy of middle ear administration of thiosulfate-containing HYA gel against cisplatin-induced hair cell loss in vivo

Treatment

The left and right ears of 12 animals were randomized for application of either HYA gel with (treated ear) or without (control ear) thiosulfate before the i.v. cisplatin injection. The animals were anesthetized, kept on a homeothermic pad, and hydrated as described above. The right and left external auditory canals, surrounding skin and neck, were cleaned with iodine and 70% ethanol. The animals were injected intratympanically as described above with a thiosulfate-containing HYA gel in one ear and with a HYA gel without thiosulfate in the contralateral ear. They were then kept on their stomach with the head in a vertical position until the internal jugular vein was exposed and a catheter was inserted for administration of cisplatin (8 mg/kg b.w., infusion time approximately 3 min) 3 h after gel application to the middle ear. The animals were woken up using Atipamezol (0.15 ml i.m.) and returned to the animal department. Temgesic (0.3 ml s.c.) was given every 12 h as postoperative analgesic. The animals were rehydrated daily with Ringer-Acetat (5 ml s.c.). Four days (96 h) after cisplatin injection, they were decapitated under deep anesthesia and the cochleae were immediately dissected for surface preparation.

Surface preparation

The cochleae were dissected free from the surrounding temporal bone. A small hole was made in the apex, the RWM was perforated, and a fixative [4% paraformaldehyde in PBS (pH 7.4)] was gently flushed from the round window to apex. The cochleae were left in the fixative for 1 h and then transferred to 0.5% paraformaldehyde and stored at 4°C until the organ of Corti was dissected. The basilar membrane with the organ of Corti was incubated with phalloidin-TRITC (1 mg/ml; Sigma–Aldrich) to stain structures containing f-actin. After several rinses in PBS, the cochleae were cut into 3-mm pieces and placed on an 8-well slide. The hair cells were blindly counted with a fluorescence microscope (Zeiss Axio Observer Z1 inverted microscope) using a 40 oil objective. The criterion for hair cell loss was scar formation.

Ototoxic evaluation

To describe the ototoxic effect of cisplatin, cytocochleograms were plotted displaying the percent of hair cell loss as a function of distance from the round window [17]. Difference in hair cell loss between the control and treated ear was calculated and plotted in a cytocochleogram for each animal. The ototoxic effect of cisplatin and potential protective effect of thiosulfate treatment could thereby be evaluated separately for each animal. This internal control is important because animals with only minor ototoxic damage in the control ear would tend to show a smaller otoprotective effect than animals where ototoxic damage in the control ear is more pronounced.

Evaluation of the hair cell loss was focused on the basal turn of the cochlea since this is the main site for ototoxic damage caused by cisplatin [12]. The basal turn of the cochlea was considered to be the first 9 mm from the round window [18].

Statistics

Two-tailed Mann–Whitney test was used to compare two independent groups. To compare more than two groups, Kruskal–Wallis and Friedman test were employed for independent and dependent groups, respectively. Dunn's multiple comparison test was employed to see which groups differ from which other groups.

Approximately 95% confidence interval for median difference in hair cell loss between the control ear and the thiosulfate-treated ear was calculated based on the Wilcoxon matched-pairs signed-ranks test [19]. Differences were accepted as statistically significant when the confidence intervals did not overlap $y = 0$ [20].

Effect of treatment was established by the sign test. P value was calculated using the sign test for two related samples [21].

$P < 0.05$ was considered statistically significant.

Results

Thiosulfate release from HYA gel in vitro

The in vitro release of thiosulfate from two different gels one containing 0.1 M thiosulfate in HYA (0.5% w/w) and Carbopol (1% w/w) and another containing 0.1 M thiosulfate in only Carbopol (1% w/w) (reference gel) is shown in Fig. 1. The mean diffusion coefficients of thiosulfate (±SD) with and without HYA were $9.57\ 10^{-6} \pm 0.21\ 10^{-6}$ and $8.95\ 10^{-6} \pm 0.45\ 10^{-6}$ cm^2/s, respectively. This difference was not statistically significant.

Thiosulfate in scala tympani perilymph after middle ear administration of thiosulfate-containing HYA gel in vivo

An intratympanic bolus injection of thiosulfate in HYA gel resulted after 1 and 3 h in scala tympani perilymph concentrations as shown in Fig. 2.

The thiosulfate concentration in blood remained low during the entire study in both the 1- and 3-h gel groups (Fig. 3). However, similar to the concentrations in scala tympani perilymph, there was a large inter-individual variability. In a previous study on guinea pigs, the thiosulfate concentration in blood was about 0.52 μM after an i.v. bolus injection of saline (1 ml/0.3 kg b.w., $n = 2$; for details see [16], similar to what has been found in normal human serum [22]. The thiosulfate concentrations in blood found in the present study did not differ from that. There was no difference in thiosulfate concentrations at any time point within each group or between the groups. Thus, no sign of higher thiosulfate concentrations in blood after an exposure time of 3 h compared to 1 h could be seen. Neither was there a trend of increasing levels with increasing exposure time within the groups.

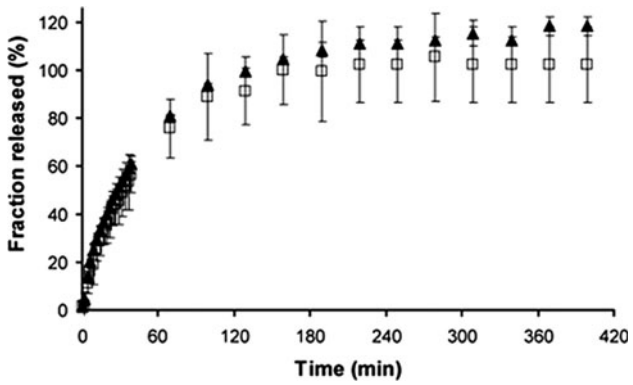

Fig. 1 A comparison of thiosulfate release in vitro from a gel containing 0.1 M thiosulfate in HYA (0.5% w/w) and Carbopol (1% w/w) (*filled triangle*) and from a reference gel containing 0.1 M thiosulfate in Carbopol (1% w/w) (*open square*). Data shown are the mean ± SD of 3 replicates

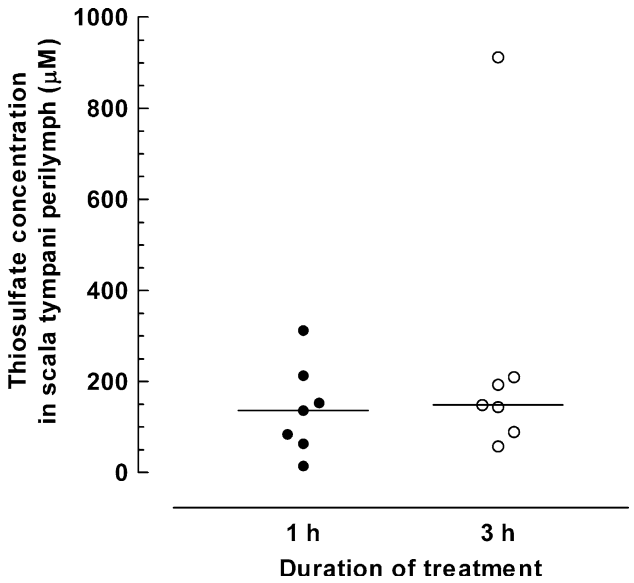

Fig. 2 Thiosulfate concentration in scala tympani perilymph 1 h and 3 h after injection of thiosulfate-containing (0.1 M) HYA gel (0.5% w/w) into the *middle* ear cavity of guinea pigs. *Each symbol* represents one sample, and the *lines* indicate the median of each group ($n = 7$ in each group). There was no statistical difference between the two groups

The thiosulfate concentration was low in CSF samples taken at the end of the experiment. The median concentration was 0.80 μM (range 0.67–0.86 μM) in the 1-h gel group and 0.81 μM (range 0.70–1.0 μM) in the 3-h gel group ($n = 6$ in both groups).

The median thiosulfate concentrations in samples of gel aspirated from the middle ear cavity of guinea pigs in the 1- and 3-h gel groups were 99 mM (range 31–120 mM, $n = 8$) and 90 mM (range 75–110 mM, $n = 6$), respectively. The difference between the groups was not statistically significant.

Efficacy of middle ear administration of thiosulfate-containing HYA gel against cisplatin-induced hair cell loss in vivo

In the in vivo group, cisplatin injection caused OHC loss in the control ear of 11 of the 12 animals. In these 11 control ears given HYA gel without thiosulfate, the hair cell loss was the largest in the first OHC row (OHC1), less in the second (OHC2), and the least in the third (OHC3) OHC row. No inner hair cell loss was seen. Ten of these eleven animals with damaged control ears exhibited only minor OHC loss in the ear treated with thiosulfate-containing HYA gel, thereby confirming a protective effect. In the animal with the smallest otoprotective effect, cisplatin injection induced a minor OHC loss in the control ear and almost no OHC loss in the treated ear, and the protective effect was therefore considered to be relevant. An unusual

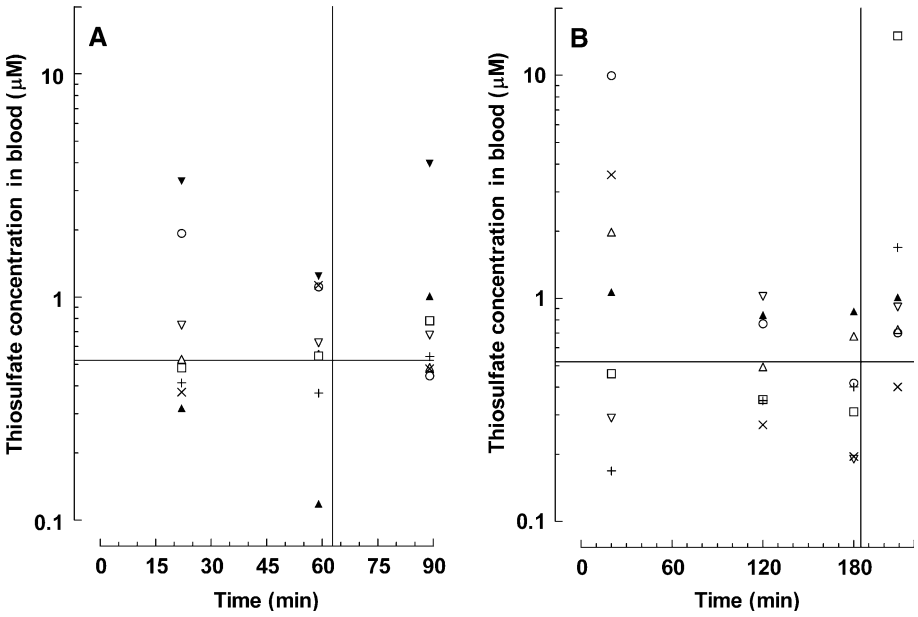

Fig. 3 Thiosulfate concentrations in blood after application of thiosulfate-containing (0.1 M) HYA gel (0.5% w/w) in the *middle* ear cavity for either 1 h (**a**) or 3 h (**b**). *Each symbol* represents one guinea pig. Each animal was sampled 3 times in the 1-h gel group and 4 times in the 3-h gel group. The *solid horizontal lines* at 0.52 μM show the approximate endogenous thiosulfate concentration in guinea pigs, and the *solid vertical lines* indicate the mean end time of thiosulfate treatment

scar formation in the apex of the control ear was seen in the animal that was considered to have no protective effect of treatment. This type of OHC loss is not typical for cisplatin treatment and was therefore not considered during evaluation of treatment.

The median cytocochleograms for control and treated ears are shown in Fig. 4.

Median difference in OHC loss between the control ear and treated ear was statistically significant in the basal turn of the cochlea (Fig. 5). In the apical part of the cochlea, the cisplatin-induced OHC loss was not as pronounced as in the base, and therefore, the difference between the two ears was smaller.

Statistical difference is seen in the basal turn of the cochlea where the Confidence interval does not overlap $y = 0$.

Discussion

In the present study, we used a local administration strategy for otoprotection in a guinea pig model during cisplatin treatment. It was found that administration of a thiosulfate-containing HYA gel to the middle ear cavity 3 h prior to cisplatin injection protected the OHCs. The major benefits of this type of thiosulfate delivery compared to systemic delivery during cisplatin-based therapy are the low risk of interference with cisplatin's antitumor activity and the possibility of obtaining higher cochlear concentrations of the otoprotective drug for a longer period of time. Moreover, the administration could easily be repeated by single injections through the tympanic membrane.

Our study was designed to elucidate different aspects of local application of thiosulfate to the middle ear prior to cisplatin injection. First, we looked at the release of thiosulfate from a HYA gel in vitro and demonstrated that HYA does not impede the release of thiosulfate. Second, we found that the concentration of thiosulfate in the gel was high even 3 h after intratympanic injection in vivo indicating a possible sustained drug uptake from the middle ear cavity to the inner ear compartments. Third, we could show that locally administrated thiosulfate gave a high concentration in scala tympani perilymph in samples taken from the basal part of the cochlea while the levels in blood remained low. Finally, as the main result, we showed that intratympanic administration of thiosulfate-containing

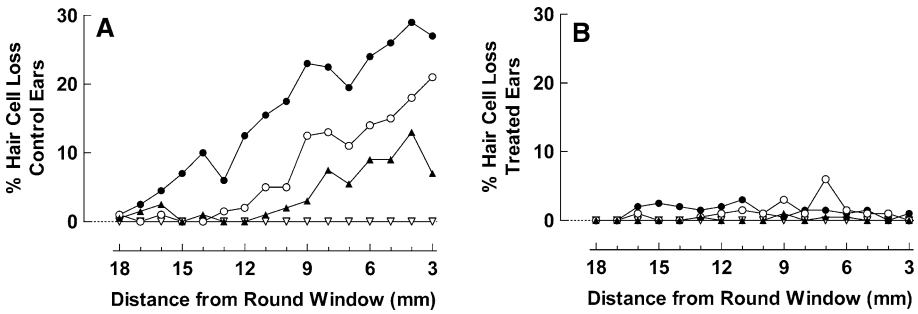

Fig. 4 Cytocochleograms of IHC and OHC loss in control (**a**) and thiosulfate-treated (**b**) ears of cisplatin-treated guinea pigs. IHC (*open triangle*), OHC1 (*filled circle*), OHC2 (*open circle*), and OHC3 (*filled triangle*). *Data* expressed as median values

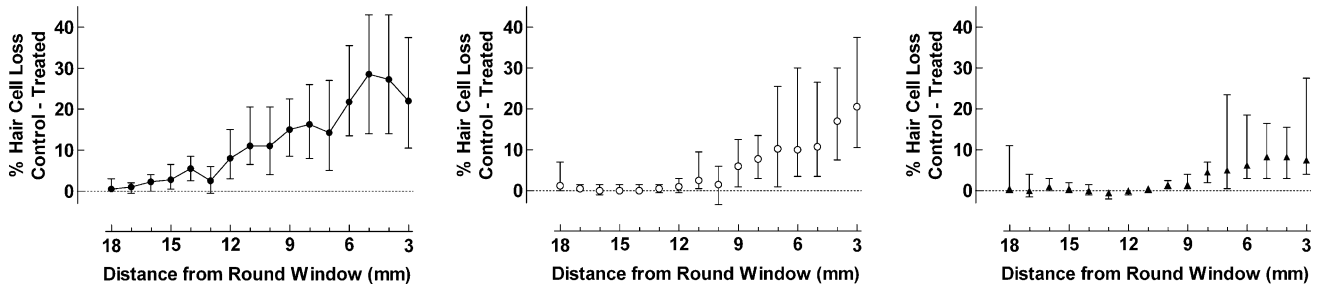

Fig. 5 Cytocochleogram for cisplatin-treated guinea pigs showing difference in OHC loss between control and thiosulfate-treated ears in the three different OHC rows. OHC1 (*filled circle*), OHC2 (*open circle*) and OHC3 (*filled triangle*). Data expressed as median values with approximate 95% CI

HYA gel was efficient against the 8 mg/kg bw of cisplatin. Further study should be performed to confirm the long-term protective effect of thiosulfate-containing HYA gel on higher dose of cisplatin by testing hearing and by cytocochleogram.

Many methods for otoprotection described in experimental studies are limited by use of drugs or surgical interventions that might not easily be transferred to a clinical situation. In this study, we employed a minimally invasive procedure that could in the future be applied in humans. The sulfur-containing antioxidant thiosulfate is a particularly suitable otoprotective candidate for clinical settings, since it is an endogenous ion with low risk of serious side effects. In this study, the blood levels of thiosulfate after local administration did not exceed the endogenous levels from historical controls [6]. Moreover, intracochlear perfusion of thiosulfate in the guinea pig has been shown to prevent inner ear damage without any sign that thiosulfate itself would cause inner ear damage [23]. Another agent that has been proven to partially protect from cisplatin-induced ototoxicity when administrated intratympanically is lactate that may counteract cisplatin toxicity by acting upon cellular metabolism and replacing NADH stores [24].

The most widely used local inner ear therapy to date is transtympanic injection of gentamicin into the middle ear for the treatment of Ménière's disease [25]. In general, the drug vehicle used is a low-viscosity solution, which means that the drug can rapidly be eliminated from the middle ear through the eustachian tube. Methods have been developed to increase the drug's residence time in the middle ear, e.g., by increasing the viscosity of the formulation using fibrin glue [26] and HYA [27]. In humans, resorbable gelatin sponges have been applied to the round window [28], and HYA has been used for intratympanic injections [29] and round window application after exploratory tympanotomi [30]. Animal studies of continuous drug application using partially or fully implantable catheter systems and pumps have also been made [31, 32]. A majority of the above-mentioned methods involve surgical procedures. The less invasive injection of a drug-containing gel into the middle ear cavity is preferable since it will lower the risk of complications. By using a highly viscous gel as vehicle, drug elimination will be retarded and the effect more long-lasting, reducing the need for repeated treatment.

In this study, we chose HYA gel as a volume stabilizer. Since HYA occurs naturally in the extracellular matrix of practically all human tissues and is already in clinical use, general toxicological considerations are redundant [33]. It has also been shown in experimental studies that HYA gel increases the permeability of the RWM [34] without toxic effects on the cochlea [35]. The osmolality of the HYA gels was about 340 mosmol/kg, meaning that the gels were slightly hypertonic. It is commonly known that formulations far from isotonicity (~300 mosmol/kg) may stress the mucosa. Hypertonicity is not as harmful as hypotonicity [36] and a slightly increased tonicity can actually be utilized to improve drug absorption [37]. In the present study, however, the formulation was near-isotonic, and it is unlikely that such small deviations from isotonicity would have had much effect if any.

In vitro, practically, all thiosulfate had disappeared from the gel within 2 h, whereas in vivo there was still a high concentration of thiosulfate when the gel was aspirated from the middle ear 3 h after intratympanic injection. A possible explanation could be that the middle ear in this context can be regarded as a closed compartment, in contrast to the open conditions in the basins of the in vitro study. It is important to establish that thiosulfate can diffuse freely in and out of the gel and that the gel does not retain the drug. Under in vivo conditions, the surrounding mucosa in the middle ear will rapidly be saturated with thiosulfate, and therefore, the gel will continue to have a high concentration, allowing passage over the RWM for a longer period of time. This might be of particular importance for protection against cisplatin-induced ototoxicity, as cochlear injury develops gradually over several days after treatment [12]. However, our research group has shown that ototoxicity caused by platinum-containing antineoplastic drugs is dependent upon the drugs'

pharmacokinetics in the inner ear, particularly on the area under the concentration–time curve [38]. Therefore, the presence of thiosulfate in the cochlea might be most critical when the cisplatin concentration in scala tympani perilymph is the highest, which is during the first hours after a single i.v. injection in the guinea pig [39]. If this is the case, the middle ear administration strategy employed in the present study offers higher thiosulfate concentration in scala tympani perilymph compared to systemic administration, which was investigated previously [16], despite the fact that the mean molar dose of intratympanically administrated thiosulfate was only 7% of the i.v. dose. The perilymphatic thiosulfate level decreased rapidly after systemic administration, in contrast to after direct application of gel in the middle ear. This might play an important role for the otoprotective efficacy. However, it should be pointed out that intratympanic administration of a HYA gel will cause a transient conductive hearing loss. Unpublished results from our group show that the HYA gel remains in the middle ear for about 3 weeks (in manuscript). This prolonged elimination of the gel makes it hard to asses cisplatin-induced hearing loss by ABR.

The time required for degradation of the gel depends on the viscosity and the concentration of HYA. It has been shown that most of a 1.9% HYA gel injected to the middle ear of the guinea pig is eliminated after 28 days [40]. In this study, we used a 0.5% HYA gel that might remain in the middle ear cavity for a shorter period of time.

The most important outcome of this study was the amelioration of OHC damage in animals given an intratympanic injection of thiosulfate-containing HYA gel to the middle ear 3 h prior to i.v. injection of cisplatin (8 mg/kg). Several studies have focused on the reduction in cisplatin ototoxicity after systemic administration of thiosulfate [41, 42]. However, since thiosulfate as well as other sulfur-containing compounds can form inactive complexes with cisplatin and MHC, there is a considerable risk of decreased antitumor effect when the drugs are administered systemically and concomitantly. This has led to an increasing interest in the effect of timing of thiosulfate administration [43, 44]. However, treatment with thiosulfate before cisplatin is risky since thiosulfate could accumulate in tumor tissues and thereby reduce the anticancer effect. Indeed, a reduced antitumoral effect has been observed even when thiosulfate was administered 72 h after cisplatin [44].

The results from the present study may be hard to extrapolate to human beings. Even though the guinea pig is the most frequently used animal for inner ear research, it has been questioned whether it is a useful animal model for intratympanic drug administration. In the guinea pig, the otic capsule is much thinner than in the human cochlea, especially at the apex. Therefore, drug distribution to the guinea pig cochlea will most likely differ from that of the human cochlea. For example, it has been shown that gentamicin and trimethylphenylammonium given in a solution injected to the middle ear can enter the perilymph both through the RWM and the otic capsule in the guinea pig [45].

Experimental studies with the ultimate goal of establishing methods for prevention of cisplatin-induced ototoxicity have great clinical importance. Cisplatin's ototoxicity still limits its use in a number of cancer patients, especially in those undergoing palliative chemotherapy. If this side effect could be prevented, cisplatin treatment would be better tolerated and higher doses could be given when necessary. Considering that the incidence of cisplatin-induced hearing loss in pediatric cancer patients might be as high as 50% [46], prevention of this ototoxicity would lead to a better personal development, education, and social integration for a large number of children treated with the drug.

Conclusion

HYA gel was found to be an effective vehicle for administration of thiosulfate to the middle ear. This otoprotective substance diffused from the gel to the inner ear giving a high concentration of thiosulfate in the perilymph of the scala tympani. Thiosulfate-containing HYA gel administered to the middle ear 3 h prior to i.v. cisplatin treatment yielded significant protection of OHC in the cochlea.

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