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Cochlear Detoxification: Role of Alpha Class Glutathione Transferases in Protection against Oxidative Lipid Damage, Ototoxicity, and Cochlear Aging

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Abstract

Age-related hearing loss (AHL) is the most common form of hearing impairment. AHL is thought to be a multifactorial condition resulting from the interaction of numerous causes including aging, genetics, exposure to noise, and exposure to endogenous and exogenous toxins. Cells possess many detoxification enzymes capable of removing thousands of cytotoxic xenobiotics and endogenous toxins such as 4-hydroxynonenal (4-HNE), one of the most abundant cytotoxic end products of lipid peroxidation. The cellular detoxification system involves three phases of enzymatic detoxification. Of these, the glutathione transferase (GST) detoxification system converts a toxic compound into a less toxic form by conjugating the toxic compound to reduced glutathione by GST enzymes. In this review, we describe the current understanding of the cochlear detoxification system and examine the growing link between GST detoxification, oxidative lipid damage, ototoxicity, and cochlear aging with a particular focus on the alpha-class GSTs (GSTAs). We also describe how exposure to ototoxic drugs, exposure to noise, or aging results in increased 4-HNE levels, how 4-HNE damages various cell components under stress conditions, and how GSTAs detoxify 4-HNE in the auditory system.

Keywords

detoxification; 4-HNE; GST; NRF2; estrogen; cochlea; aging

1. Introduction

Hearing loss is the third most prevalent chronic health condition affecting older adults and age-related hearing loss (AHL) is the most common form of hearing impairment (Gates & Mills, 2005; Ozmeral, Eddins, Frisina, & Eddins, 2016; Yamasoba et al., 2013). AHL is thought to be a multifactorial condition resulting from the interaction of numerous causes

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including aging, exposure to noise and ototoxic chemicals, genetics, epigenetic variables, comorbidities, and lifestyle (Gates & Mills, 2005; Yamasoba et al., 2013). The inner ear contains two sensory systems: the auditory system (cochlea) which detects sound waves (Hudspeth, 1997) and the vestibular system (three semicircular canals, utricle, and saccule) which detects head movements and linear motion or gravity (Angelaki & Cullen, 2008). The major sites of age-related cochlear pathology include inner hair cells (IHC), outer hair cells (OHC), spiral ganglion neurons (SGN), synaptic loss, and stria vascularis (SV) cells (Liberman & Kujawa, 2017; Liu & Yan, 2007; Someya & Prolla, 2010; Yamasoba et al., 2013). The IHCs are the true sound receptors that relay sound wave information to the central auditory system through the SGNs (Hudspeth, 1997). Post-mitotic hair cells and SGNs are particularly susceptible to injury from a combination of noise exposure, ototoxic chemicals, and oxidative damage (Someya & Prolla, 2010; Yamasoba et al., 2013). The blood vessels coursing through the cochlea are also essential for transporting oxygen and nutrients such as calcium, potassium, amino acids, cholesterol, and glucose into the cochlea. Therefore, age-related changes in the structures and functions of those cochlear cells typically disrupt auditory function and result in irreversible and permanent hearing impairment.

Cells possess many detoxification enzymes capable of removing thousands of cytotoxic xenobiotics and endogenous toxins such as 4-hydroxynonenal (4-HNE) (Henderson & Wolf, 2011; Nebert & Dalton, 2006; Simic, Savic-Radojevic, Pljesa-Ercegovac, Matic, & Mimic-Oka, 2009). The cellular detoxification system involves three phases of enzymatic detoxification. Of these, the glutathione transferase (GST) detoxification system converts a toxic compound into a less toxic form by conjugating the toxic compound to reduced glutathione (GSH) by GST enzymes. In this review, we describe the current understanding of the cochlear detoxification system and examine the growing link between GST detoxification, oxidative lipid damage, ototoxicity, and aging with a particular focus on the alpha-class GSTs (GSTAs). We also describe how exposure to ototoxic drugs, exposure to noise, or aging results in increased levels of 4-HNE, one of the most abundant cytotoxic end products of lipid peroxidation (Awasthi et al., 2004; Dalleau, Baradat, Gueraud, & Huc, 2013; Di Domenico, Tramutola, & Butterfield, 2017; Jaganjac et al., 2019; Singhal et al., 2015), how 4-HNE damages various cell components under stress conditions, and how GSTAs detoxify 4-HNE in the auditory system.

2. Role of GST detoxification in protection against aging and hearing loss

2.1. Cellular detoxification system

Cells are continuously exposed to endogenous toxins such as superoxide, hydrogen peroxide (H_2O_2), and 4-HNE and thousands of xenobiotic cytotoxic chemicals such as formaldehyde and pesticide chemicals throughout the course of life. Cells possess many detoxification enzymes capable of removing those toxins. The cellular detoxification system is highly complex, and involves three phases of enzymatic detoxification (Figure 1) (Henderson & Wolf, 2011; Nebert & Dalton, 2006; Simic, Savic-Radojevic, Pljesa-Ercegovac, Matic, & Mimic-Oka, 2009): In phase I (functionalization reactions), the initial bioactivation or the formation of reactive intermediates of thousands of endogenous and exogenous toxins

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primarily occurs in the liver, although it can also take place in kidneys and other tissues. The phase I detoxification enzymes include cytochrome P450 (CYP), hydroxylases, reductases, aldoketoreductases, flavin-containing monooxygenases, lipooxygenases, cyclooxygenases, peroxidases, epoxygenases, and oxidases (Nebert & Dalton, 2006). Of these, CYPs comprise 70–80% of all phase I detoxification enzymes. Humans possess 57 CYP genes in 18 families. The members of the CYP1 to CYP4 families oxygenate thousands of xenobiotics, whereas all members of the CYP5 family primarily metabolize endogenous toxins in a highly substrate-specific manner. Most phase I enzymes are capable of both detoxification and metabolic activation. Phase II detoxification (conjugation reactions) involves conjugation of toxic compounds, potential carcinogens, and pharmacologically active compounds, to the -SH groups of reduced glutathione (GSH) by GST enzymes (Figure 2) (Henderson & Wolf, 2011; McLaren & Moroi, 2003; Simic et al., 2009). Glutathione conjugates are metabolized further by cleavage of the glutamate and glycine residues, followed by acetylation of the resultant free amino group of the cysteinyl residue to produce a very hydrophilic product, mercapturic acid.

The phase II detoxification enzymes include GSTs, uridine diphosphate glucuronosyltransferases, sulphotransferases, acyltransferases, acetyltransferases, methyltransferases, and transaminases. Phase III detoxification involves the elimination of mercapturic acid from the cell through the transmembrane transporters, completing the detoxification cycle (Nebert & Dalton, 2006; Simic et al., 2009). The combined effects of phase I and phase II detoxification enzymes usually result in the detoxification of xenobiotic chemicals. However, CYP-mediated formation of reactive oxygenated intermediates produce some highly electrophilic compounds which can readily bind covalently to DNA or proteins, causing genotoxicity and mutation (Nebert & Dalton, 2006). Hydrophobic xenobiotics can enter the cell passively, while others enter the cell through receptors, membrane-bound transporters or ion channels. Most detoxification enzymes, including GSTs have both cytosolic and membrane-bound forms, while some phase I and phase II detoxification enzymes such as CYPs are membrane-bound in the endoplasmic reticulum, mitochondria, or plasma membrane (Nebert & Dalton, 2006; Simic et al., 2009). Moreover, many phase I and II enzymes, including CYPs, do not show substrate-specific binding or have greater flexibility in substrate binding. Detoxification metabolism of endogenous and xenobiotic chemicals is thought to occur in all eukaryotic cells. Importantly, each detoxification gene shows a high level of time-specific, organ-specific, tissue-specific, cell type-specific, and/or sex-specific expression (Nebert & Dalton, 2006; Simic et al., 2009).

2.2. GST detoxification system

GSTs are a family of phase II detoxification enzymes that catalyze the conjugation of a wide variety of endogenous and xenobiotic toxins, including potential carcinogens, to the -SH groups of GSH, resulting in the formation of a glutathione conjugate, a more water-soluble form compared with the original compound (Figure 2) (Henderson & Wolf, 2011; McLaren & Moroi, 2003; Simic et al., 2009). GSTs are divided into three main families: cytosolic, mitochondrial, and microsomal (membrane-associated eicosanoid/glutathione metabolism (MAPEG) protein superfamily). Of these families, the cytosolic GSTs constitute the largest

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family. Cytosolic GSTs are divided into several classes on the basis of their primary structure (McLaren & Moroi, 2003; Simic et al., 2009).

Over 20 mammalian cytosolic GSTs have been identified to date. The most well characterized cytosolic GST classes have been named alpha (GSTA), mu (GSTM), pi (GSTP), and theta (GSTT) (Table 1). Cytosolic GSTs are dimeric, with subunit molecular weights of approximately 25 kDa. Each subunit contains a catalytically independent active site that consists of a GSH-binding site in the amino-terminal domain, and a site that binds the xenobiotic substrate in the carboxyl-terminal domain (Laborde, 2010). Most cytosolic GST classes show a high degree of polymorphism (Simic et al., 2009). Because of the diversity of potential xenobiotics and stressors, GSTs display extensive functional diversification in gene expression, enzyme activities, and substrate specificities. Most GSTs can bind structurally diverse non-substrate ligands such as steroids, heme, and bilirubin.

2.3. Effects of GSTs on cancer drugs

The action of GSTs can lead to resistance to a range of anticancer drugs in tumor cells. For example, GSTP1 has a role in the regulation of mitogen-activated protein kinases (MAPK), which are involved in the induction of apoptosis (Laborde, 2010; Simic et al., 2009). A variety of stresses can activate c-Jun N-terminal kinase (JNK), a member of the MAPK family, which in turn, phosphorylates c-Jun, a component of the activator protein-1 (AP-1) transcription factor, leading to induction of AP-1-mediated target genes involved in apoptosis (Adler et al., 1999). An earlier study has shown that GSTP1 inhibits JNK through direct protein–protein interaction, forming a c-Jun–JNK complex (Adler et al., 1999). In this non-enzymatic role, GSTP1 sequesters JNK in the complex, inhibiting the phosphorylation of c-Jun by JNK, thereby blocking JNK-mediated apoptosis and leading to resistance to a range of anticancer drugs.

2.4. Roles of GSTs in GH/IGF-1 signaling, aging, and age- and noise-related hearing loss

A growing body of evidence indicates a role for GST detoxification in aging and longevity. Adequate amounts of growth hormone (GH) and insulin-like growth factor-1 (IGF-1), the main mediator of GH actions, are essential for normal growth and development for children (Rogol, 2010; Rogol, Roemmich, & Clark, 2002). Accordingly, the levels of GH and IGF-1 in circulating blood are higher early in life and begin to decline soon after physical and reproductive maturation (Bartke, 2008). In line with these observations, suppression of GH/IGF-1 signaling leads to lifespan extension in worms, fruit flies, and mice (Bartke et al., 1998; Berryman, Christiansen, Johannsson, Thorner, & Kopchick, 2008). Thus, GH/IGF-1 signaling is thought to be an evolutionarily conserved regulator for aging in a variety of species.

Ames dwarf mice lack GH, prolactin, and thyroid-stimulating hormone, exhibit a 50% increase in lifespan compared to their normal littermates and many symptoms of delayed aging (Brown-Borg & Rakoczy, 2005). Interestingly, both young and old Ames dwarf mice (*Prop1*$^{df/df}$) display increased levels of liver glutamate-cysteine ligase (GCL), the rate-limiting enzyme in the glutathione biosynthesis pathway, and increased total GST activity in the kidney. McElwee and co-workers (McElwee et al., 2007) conducted a multi-level cross-

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species comparative analysis to compare gene expression changes accompanying increased longevity in mutant *C. elegans* (*daf-2*), *D. melanogaster* (*chico*$^{1/+}$), Ames dwarf mice (*Prop1*$^{df/df}$), and Little dwarf mice (*Ghrhr*$^{lit/lit}$), well-established models of longevity associated with reduced GH/IGF-1 signaling. Interestingly, a number of gene categories were significantly enriched for genes whose expression changes in long-lived animals of all three species: up-regulated categories include GSTs and several other categories linked to CYP metabolism of endogenic and xenobiotic toxins, suggesting that enhanced GST detoxification is associated with reduced GH/IGF-1 signalling and longevity.

In humans, earlier studies suggested a role for GST detoxification in protection against age-related and noise-induced hearing loss: A significant association between AHL and *GSTT1* and *GSTM1* null polymorphisms was found in a Finnish population (Van Eyken et al., 2007) and a Hispanic population (Bared et al., 2010). A significant association between noise-induced hearing loss and *GSTM1*, *GSTP1*, and *GSTT1* null polymorphisms was also detected in a population of noise-exposed workers in Taiwan (C. Y. Lin et al., 2009). Moreover, a *GSTM1* null polymorphism was associated with poorer high-frequency otoacoustic emissions in individuals who have been working in noise exposure jobs, suggesting the protective role of GSTM1 against noise-induced hearing loss (Rabinowitz et al., 2002). Collectively, a decline in GST detoxification function may increase susceptibility to age- and noise-related hearing loss.

3. Roles of GSTAs in reducing oxidative lipid damage and ototoxicity and slowing cochlear aging

3.1. Role of GSTAs in protection against oxidative lipid damage

Up to today, ~20 mammalian GSTs have been identified (Laborde, 2010; McLaren & Moroi, 2003; Simic et al., 2009). The alpha-class GSTs consist of 5 distinct members, GSTA1, GSTA2, GSTA3, GSTA4, and GSTA5 (Awasthi et al., 2004; Balogh & Atkins, 2011; Henderson & Wolf, 2011; Simic et al., 2009; Singh, Zimniak, & Zimniak, 2010). Of these, GSTA1, GSTA2, GSTA4, and GSTA5 are thought to be the major determinants of the intracellular concentration of 4-HNE, one of the most abundant cytotoxic end products of lipid peroxidation and which contributes to neurodegenerative diseases and age-related diseases (Awasthi et al., 2004; Dalleau, Baradat, Gueraud, & Huc, 2013; Di Domenico, Tramutola, & Butterfield, 2017; Jaganjac et al., 2019; Singhal et al., 2015). GSTA1 and GSTA2 have selenium-independent glutathione peroxidase (GPX) activity and can catalyze GSH-dependent reduction of fatty acid hydroperoxides (FA-OOH) and phospholipid hydroperoxides (PL-OOH) (Figure 3) (Awasthi et al., 2004; Balogh & Atkins, 2011; Singh et al., 2010; Singhal et al., 2015). FA-OOH and or PL-OOH are then reduced to the corresponding alcohols with oxidized glutathione (GSSG) and water as by- products, thereby blocking the formation of 4-HNE. GSTA4 and GSTA5 conjugate 4-HNE to GSH, forming a GSH-4-HNE conjugate (GS-HNE), which is then eliminated through the transmembrane transporters (Figure 3). Unlike the other GSTA isoforms, GSTA3 catalyzes the double bond isomerization of precursors for progesterone and testosterone during the biosynthesis of steroid hormones (Dourado, Fernandes, Mannervik, & Ramos, 2014). Of these GSTA isoforms, conjugation to GSH by GSTA4 is thought to be a major route of 4-

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HNE elimination. Therefore, GSTAs play important roles in reducing oxidative lipid damage.

3.2. The cytotoxic lipid peroxidation product 4-HNE

4-HNE is an α,β- unsaturated hydroxyalkenal mostly formed by the peroxidation of linoleic acid, linolenic acid, and arachidonic acid (Figure 4) (Dalleau et al., 2013; Di Domenico et al., 2017; Jaganjac et al., 2019). 4-HNE readily reacts with various cellular components, such as DNA, proteins and lipids, containing nucleophilic thiol (–SH) or amino (–NH2) groups. The chemical structure of 4-HNE possesses three reactive functions: a C2=C3 double bond, a C1=O carbonyl group and a hydroxyl group on C4. These reactive functions make this electrophilic molecule highly reactive toward nucleophilic thiol and amino groups. 4-HNE can react with proteins, particularly those containing histidine, cysteine and lysine residues (Di Domenico et al., 2017; Jaganjac et al., 2019). This includes plasma membrane transporters, growth factor receptors, neurotransmitters, mitochondrial electron transport chain proteins, chaperones, proteasomal proteins and cytoskeletal proteins. It is estimated that 1–8% of the 4-HNE formed in cells target proteins. 4-HNE can also react with lipids containing amino groups and nucleic acids, mostly with the guanosine moiety of DNA.

The 4-HNE concentration in human blood is estimated to be about 0.05–0.15 mM, but under pathological conditions, it can increase to over 100 mM (Dalleau et al., 2013). Thus, higher concentrations of 4-HNE cause the alteration of a wide range of biological activities, including disruption of glutamate transport, impairment of Na^+/K^+ ATPase activity, activation of caspase pathways, disruption of Ca_2^+ homeostasis, the suppression of nuclear factor-kappa B (NF-kB) activity or altered protein homeostasis.

3.3. Role of 4-HNE in age-related neurodegenerative diseases

The primary cause of biological aging is thought to be accumulation of oxidative damage at the molecular level (Balaban, Nemoto, & Finkel, 2005; Beckman & Ames, 1998; Finkel & Holbrook, 2000). In support of this view, reactive oxygen species (ROS) cause the oxidation of polyunsaturated fatty acids, leading to the formation of 4-HNE (Dalleau et al., 2013; Di Domenico et al., 2017; Jaganjac et al., 2019). The central nervous system and the peripheral nervous system are particularly sensitive to ROS damage because of the high levels of polyunsaturated fatty acids (PUFA) in neuronal cell membranes. Elevated levels of 4-HNE have been observed in brain tissues of Alzheimer disease, Parkinson disease, and Huntington disease patients and animal models of these neurodegenerative diseases (Di Domenico et al., 2017). Accumulation of 4-HNE is also linked to age-related diseases associated with increased levels of oxidative stress or redox imbalance, including cancer, atherosclerosis, and liver diseases. In addition, 4-HNE is considered as an apoptosis inducer *in vitro* and *in vivo* (Dalleau et al., 2013). These reports suggest that 4-HNE plays a role in the development of age-related neurodegenerative diseases.

3.4. Effects of Gsta4 deficiency and the resultant increase in 4-HNE levels on cell death and age- and noise-related hearing loss

GSTA4 possesses high catalytic efficiency toward 4-HNE (Balogh & Atkins, 2011) and is thought to be the major determinant of the intracellular concentration of 4-HNE. In support

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of this this view, knockdown of either *gst-5* or *gst-10*, which have high catalytic activity toward 4-HNE, shortens lifespan, while overexpression of *gst-10* or murine *Gsta4* extends lifespan in *C. elegans* (Ayyadevara et al., 2007; Ayyadevara et al., 2005). In mice, short-term CR up-regulated mRNA expression of phase 2 detoxification genes, including *Gsta4*, in the liver (Fu & Klaassen, 2014), while treatment with paraquat significantly shortened the survival of *Gsta4* KO mice compared to wild-type mice (Zimniak et al., 1994). In human erythroleukemia cell lines, overexpression of *GSTA4* decreased the levels of 4-HNE (Cheng et al., 1999) and protected cells against apoptosis (Yang, Sharma, Sharma, Awasthi, & Awasthi, 2003). 4-HNE can also be detoxified by aldose reductase (AR) and aldehyde dehydrogenase (ALDH) (Black et al., 2012; Singhal et al., 2015; Srivastava, Chandra, Bhatnagar, Srivastava, & Ansari, 1995): elderly patents show lower activities of aldehyde- and lipid hydroperoxide-detoxifying enzymes, including ALDH1, ALDH2, ALDH3, GSTA4, and AR, in the thyroid arterial compared to young adult controls (Lapenna et al., 2019). Moreover, our lab has recently shown that elevated levels of 4-HNE were observed in the cochlea of aged mice compared to young controls (Park et al., 2020). This was associated with decreased SGN density, and reduced hair cells and SV thickness in the cochlea of aged mice. Interestingly, prolonged noise exposure also resulted in hair cell loss and increased levels of 4-HNE in the cochlea of rodents (Du et al., 2013; Fetoni et al., 2009; Fetoni et al., 2015; Xiong, He, Lai, & Wang, 2011; Yamashita, Jiang, Schacht, & Miller, 2004). Together, we speculate that GSTA4-mediated detoxification of 4-HNE likely plays a role in protection against cell death, noise exposure, and age-related hearing loss.

3.5. Effects of Gsta4 deficiency and the resultant increase in 4-HNE levels on cisplatin ototoxicity

Cisplatin is one of the most widely used chemotherapeutic agents for the treatment of a broad spectrum of cancers (Ding, Allman, & Salvi, 2012; Roy, Ryals, Van den Bruele, Fitzgerald, & Cunningham, 2013; Rybak & Ramkumar, 2007). However, cisplatin chemotherapy commonly causes permanent hearing loss in 40–80% of patients of all ages. Cisplatin-induced hearing loss is generally dose-dependent, irreversible, and associated with loss of sensory hair cells, SGNs and/or SV cells. Cisplatin is thought to exert its cytotoxic effects through DNA crosslinking and generation of ROS following binding to cytoplasmic proteins, leading to increased oxidative damage and cell death (Ding et al., 2012; Ober & Lippard, 2008; Rybak & Ramkumar, 2007). Previous studies have shown that cisplatin cytotoxicity is associated with increased levels of 4-HNE and malondialdehyde (MDA), major end products of lipid peroxidation, and decreased levels of GSH in the kidney of ICB mice (W. Li et al., 2016) and Wistar albino rats (Noori & Mahboob, 2010). Cisplatin administration also resulted in increased levels of 4-HNE in the cochlea of Wistar rats (Fetoni et al., 2014).

An earlier study has shown that cisplatin administration resulted in degeneration of SV associated with a decline in the endocochlear potential and degeneration of marginal cells from guinea pigs (Kohn et al., 1988). A subsequent study (Breglio et al., 2017) has shown that while cisplatin was eliminated in most organs over days to weeks, cisplatin remained in the cochlea for months to years in both mice and humans. Importantly, cisplatin levels were higher in the SV region compared to the organ of Corti or SGN region. Our lab has recently

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shown that GSTA4 immunostaining was prominent in the stria vascularis of *Gsta4*$^{+/+}$ mice, suggesting that SV is likely a major site for GSTA4-mediated detoxification of 4-HNE in the cochlea (Park et al., 2019). Cisplatin treatment also stimulated GST activity toward 4-HNE in the inner ear of female *Gsta4*$^{+/+}$ mice. In contrast, *Gsta4* deficiency resulted in increased levels of 4-HNE and more profound loss of SGNs and SV atrophy in the cochlea of cisplatin-treated female mice. Together, these reports suggest that GSTA4 plays an essential role in protection against cisplatin ototoxicity.

4. Potential molecular mechanisms underlying GSTA detoxification of 4-HNE in cochlea

4.1. Role of NRF2 in GSTA detoxification of 4-HNE in cochlea

A growing body of evidence suggests that under physiological conditions, during normal aerobic metabolism, or low levels of ROS, 4-HNE is involved in the intracellular signaling mechanisms for determining whether cells undergo apoptosis, differentiation, or proliferation (Jaganjac et al., 2019; Luczaj, Gegotek, & Skrzydlewska, 2017; Yang et al., 2003). In support of this view, lower intracellular concentrations of 4-HNE (i.e., < 2 μM) appear to be beneficial to cells. The nuclear transcription factor E2-related factor 2 (NRF2) promotes the transcriptional induction of antioxidant genes such as the subunits of GCL, the rate-limiting enzyme in glutathione biosynthesis, and phase II detoxification genes, including *GSTA* and *GSTP* genes (Blackwell, Steinbaugh, Hourihan, Ewald, & Isik, 2015; Itoh et al., 1997; Shen & Kong, 2009). 4-HNE can also act as a direct activator of NRF2 (Boerma et al., 2015). Thus, under physiological conditions, 4-HNE may be involved in modulating NRF2-mediated glutathione synthesis and GSTA detoxification in cochlea. Under pathological conditions or in aged tissues, 4-HNE concentration can increase to over 100 mM, which in turn causes the alteration of a wide range of biological activities (Dalleau et al., 2013). Accordingly, when exposed to noise or ototoxic drug, or under pathological conditions, higher oxidative stress likely leads to accumulation of 4-HNE. This can also trigger the activation of NRF2, which in turn activates GSTA1 and GSTA2, blocking the formation of 4-HNE, and or activates GSTA4 and GSTA5, conjugating 4-HNE to GSH. This results in elimination of 4-HNE and protection of cochlear hair cells, SGNs, and SV cells (Figure 4). In addition, GSH can directly sequester 4-HNE through its cysteine residues by the formation of GS-HNE adducts (Figure 4).

4.2. Effects of estrogen on GSTA detoxification of 4-HNE in cochlea

Evidence indicates that constitutive gene expression of GST isoforms is tissue- and sex-specific (Benbrahim-Tallaa, Tabone, Tosser-Klopp, Hatey, & Benahmed, 2002; Faustino et al., 2012; Gupta, Medh, Leal, & Awasthi, 1990; D. J. Harrison, Kharbanda, Cunningham, McLellan, & Hayes, 1989; Knight, Choudhuri, & Klaassen, 2007; Park et al., 2019). For example, mRNA expression of *Gsta4* was observed to be the highest in the stomach, while lower levels of *Gsta4* mRNA were observed in the liver and kidney of mice (Knight et al., 2007). Female mice also showed higher mRNA expression levels of *Gsta1*, *Gsta2*, *Gsta3*, and *Gsta4* in the kidneys, higher mRNA expression levels of *Gsta4* in the heart (Knight et al., 2007), and higher mRNA expression of *Gsta4* in the inner ears compared to males (Park

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et al., 2019). In contrast, ovariectomy downregulated 25 genes involved in Phase II detoxification, including *Gsta4* in the inner ears. Ovariectomized mice also had significantly lower GSTA4 protein levels compared to ovary-intact female mice, suggesting that ovarian estrogen may modulate gene expression of GST genes.

It is well-documented that women live longer than men in every country in the world (Austad, 2006). A similar pattern of sex differences in longevity is found in many other animals. Numerous studies have also reported gender differences in human auditory perception (Caras, 2013; Chung, Mason, Gannon, & Willson, 1983; Dehan & Jerger, 1990; Jerger & Johnson, 1988; Jonsson, Rosenhall, Gause-Nilsson, & Steen, 1998; F. R. Lin, Niparko, et al., 2011; McFadden & Champlin, 2000). In general, the results of these studies show that the prevalence of hearing loss is lower in women than in men in virtually all age groups. Considerable evidence also suggests that auditory function is diminished following menopause (Hederstierna, Hultcrantz, Collins, & Rosenhall, 2007; Khaliq, Tandon, & Goel, 2003, 2005), whereas estrogen replacement therapy lowers hearing thresholds, shortens auditory brainstem response (ABR) latencies, and increases ABR amplitudes in postmenopausal women (Caruso et al., 2003; Hederstierna et al., 2007; Kilicdag et al., 2004). Numerous studies indicate that estrogen has neuroprotective actions (Bean, Ianov, & Foster, 2014; Bean et al., 2015; Borras et al., 2003; Sherwin, 2009; Torres et al., 2018). For example, liver mitochondria from female rodents produce fewer ROS, had higher activity of GPX, and increased glutathione redox state compared to males, suggesting that female tissues have higher activities of glutathione antioxidant defense enzymes and increased glutathione redox state compared to males (Borras et al., 2003; Torres et al., 2018).

There are three major forms of estrogen: estrone (E1), estradiol (E2), and estriol (E3) (Cui, Shen, & Li, 2013): E2 is the most potent and predominant form of estrogen during the premenopausal or reproductive period, while E1 is the dominant form of estrogen after reproductive cessation or menopause. E3 plays larger roles during pregnancy. E1, E2, and E3 are monophenolic compounds, similar to α-tocopherol (vitamin E), which can act as free-radical scavengers (Behl, 2002; Cui et al., 2013; Moosmann & Behl, 1999). The hydroxyl group on ring A of E1, E2, or E3 could donate a hydrogen, thereby detoxifying free radicals such as hydroxyl radical. This direct free-radical scavenging activity of E1, E2, or E3 is independent of the activation of estrogen receptors or of any other estrogen-mediated signaling pathways (Behl, 2002). Estrogen also modulates cell survival by inducing the transcription of neurotrophic factors and their receptors, including nerve growth factor, brain-derived neurotrophic factor (BDNF), neurotrophin 3, and IGF1 (Behl, 2002; Sohrabji, Miranda, & Toran-Allerand, 1995), and BCL2 and BCLX, inhibitors of apoptosis (Pike, 1999; Singer, Rogers, & Dorsa, 1998). Furthermore, estrogen can directly affect neurotransmission by binding to transmembrane ion channels such as GABAA (γ-aminobutyric acid type A) receptor and or NMDA (N-methyl-D-aspartate) receptor, a glutamate and ion channel protein receptor (Behl, 2002; Woolley, 1999). Glutamate is the major excitatory neurotransmitter, whereas GABA is the major inhibitory neurotransmitter in the auditory system (Puel, 1995). Given that females have higher levels of estrogen particularly during the reproduction period, we speculate that estrogen-mediated GSTA detoxification of 4-HNE may play a role in neuroprotection against oxidative lipid damage in cochlea.

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5. Concluding remarks

There is a growing consensus that the primary cause of biological aging is accumulation of oxidative damage at the molecular level (Balaban et al., 2005; Beckman & Ames, 1998; Finkel & Holbrook, 2000; McElwee et al., 2007). We speculate that accumulation of oxidative lipid damage and the resultant increase in 4-HNE levels may play a causal role in age-related diseases associated with increased levels of oxidative damage, including AHL (Balogh & Atkins, 2011; Dalleau et al., 2013; Di Domenico et al., 2017; Jaganjac et al., 2019; Park et al., 2020; Singhal et al., 2015). In addition, the fact that cells possess many detoxification enzymes and antioxidants capable of removing 4-HNE or blocking its formation (i.e., GSTA1, GSTA2, GSTA4, GSTA5, AR, ALDH, glutathione, and carnosine) (Awasthi et al., 2004; Black et al., 2012; Di Domenico et al., 2017; Singh et al., 2010; Singhal et al., 2015; Srivastava et al., 1995) broadly supports the view that elevated levels of 4-HNE play a major role in the pathogenesis of a variety of age-related diseases, including AHL. Of these detoxification enzymes and antioxidants, the GSH/GSSG redox couple is thought to be an intracellular determinant of the antioxidant capacity because the abundance of GSH (10–15 mM) is three to four orders of magnitude higher than the other reductants, including NADPH and NADH (Mari, Morales, Colell, Garcia-Ruiz, & Fernandez-Checa, 2009; Rebrin & Sohal, 2008). Importantly, GSH has the ability to rapidly bind 4-HNE through its cysteine residues and detoxify 4-HNE by the formation of GS-HNE adducts (Figure 4) (Di Domenico et al., 2017). GSH can also detoxify 4-HNE through serving as a co-factor for GSTA1, GSTA2, GSTA4, and GSTA5 (Figure 4) (Awasthi et al., 2004; Balogh & Atkins, 2011; Di Domenico et al., 2017; Singh et al., 2010; Singhal et al., 2015; Yang et al., 2003). Therefore, we speculate that a combination of GSTA-mediated detoxification and glutathione antioxidant defense against 4-HNE plays a major role in protecting cochlear structure and function against oxidative lipid damage, ototoxic drug, noise, or aging.

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Highlights

- A growing body of evidence indicates a role for GST detoxification in aging and longevity.
- GSTA1, GSTA2, GSTA4, and GSTA5 are thought to be the major determinants of the intracellular concentration of 4-HNE.
- Elevated levels of 4-HNE may play a role in the pathogenesis of noise-, drug-, and or age-related hearing loss.
- GSTA-mediated detoxification may play a major role in protecting cochlear structure and function against oxidative lipid damage, ototoxic drug, noise, or aging.

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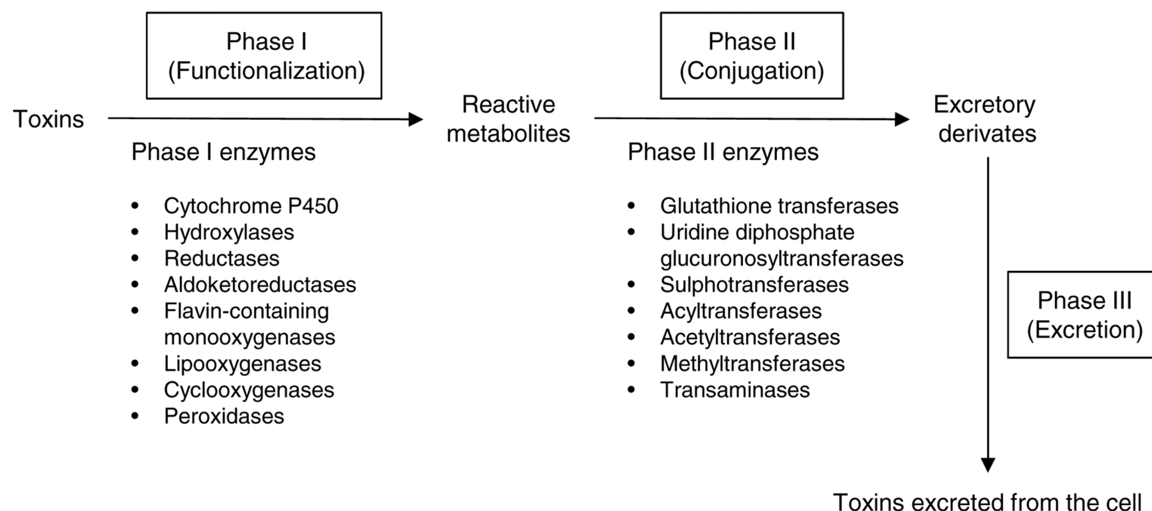

Fig 1. Cellular detoxification system.

The cellular detoxification system involves three phases of detoxification. Phase I is the initial bioactivation or the formation of reactive intermediates of thousands of endogenous and exogenous toxins, and primarily occurs in the liver. The phase I detoxification enzymes include cytochrome P450, hydroxylases, reductases, aldoketoreductases, flavin-containing monooxygenases, lipooxygenases, cyclooxygenases, and peroxidases. Phase II detoxification involves conjugation of toxic compounds to the -SH groups of reduced glutathione (GSH) by GST enzymes. The phase II detoxification enzymes include glutathione transferases, uridine diphosphate glucuronosyltransferases, sulphotransferases, acyltransferases, acetyltransferases, methyltransferases, and transaminases. Phase III detoxification involves the elimination of mercapturic acid from the cell through the transmembrane transporters, completing the detoxification cycle.

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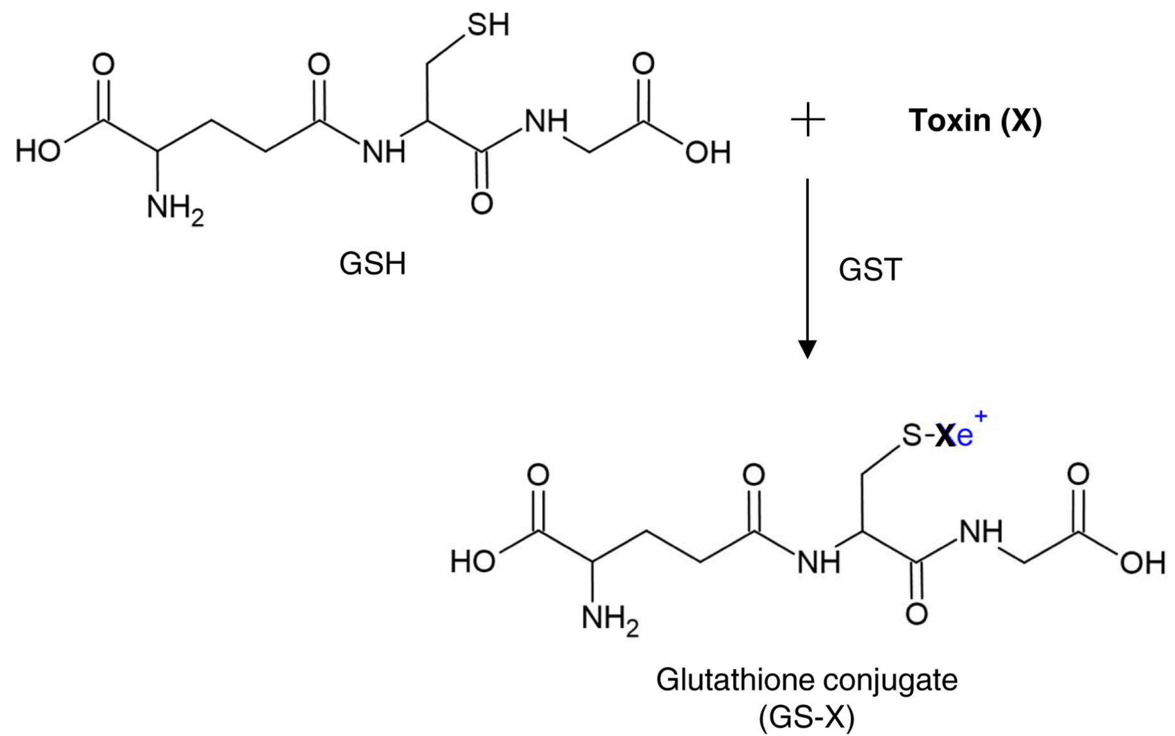

Fig 2. GST detoxification.

GSTs are a family of phase II detoxification enzymes that catalyze the conjugation of a wide variety of endogenous and xenobiotic toxins (X), to the -SH group of reduced glutathione (GSH), resulting in the formation of glutathione conjugate (GS-X). Glutathione conjugates are metabolized further by cleavage of the glutamate and glycine residues, followed by acetylation of the resultant free amino group of the cysteinyl residue to produce a very hydrophilic product, mercapturic acid, which is then eliminated from the cell through the transmembrane transporters.

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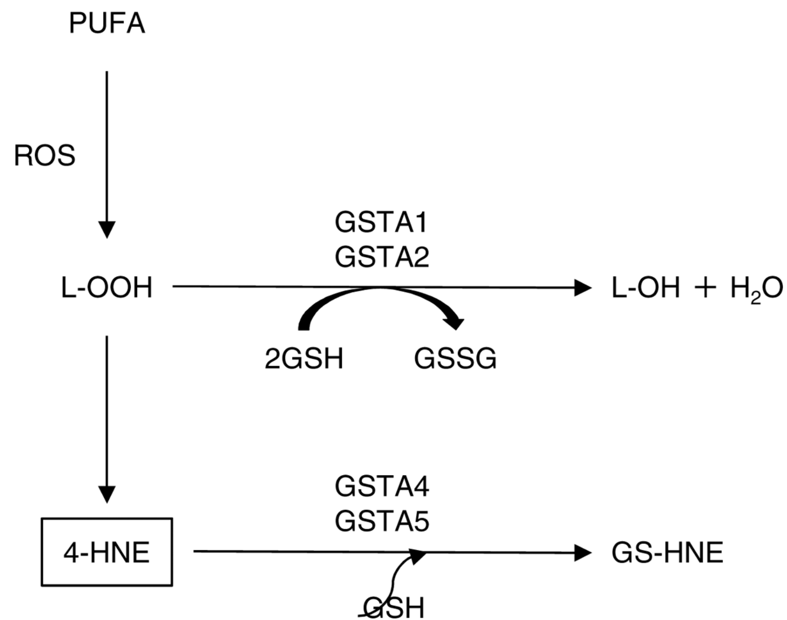

Fig 3. Detoxification of 4-HNE by GSTA isoforms.

GSTA1, GSTA2, GSTA4, and GSTA5 are thought to be the major determinants of the intracellular concentration of 4-HNE. GSTA1 and GSTA2 can catalyze GSH-dependent reduction of lipid hydroperoxide (L-OOH). L-OOH is then reduced to the corresponding alcohol (L-OH) with oxidized glutathione (GSSG) and water as by-products, thereby blocking the formation of 4-HNE. GSTA4 and GSTA5 conjugate 4-HNE to GSH, forming a GSH-4-HNE conjugate (GS-HNE), which is then eliminated from the cell through the transmembrane transporters. PUFA: polyunsaturated fatty acid (i.e., linoleic acid, linolenic acid, and arachidonic acid).

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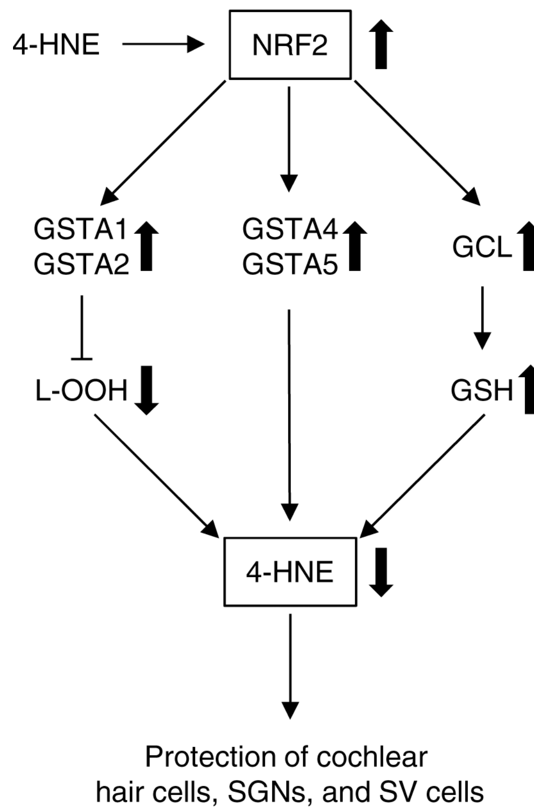

Fig 4. Role of NRF2 in GSTA detoxification of 4-HNE in cochlea.

NRF2 promotes the transcriptional induction of antioxidant genes such as the subunits of glutamate-cysteine ligase (GCL), the rate-limiting enzyme in glutathione biosynthesis, and phase II detoxification genes, including GSTA genes, including *GSTA1*, *GSTA2*, *GSTA4*, and *GSTA5*. 4-HNE can also act as a direct activator of NRF2. When exposed to noise or ototoxic drug or in aged cochlear tissues, higher oxidative stress likely leads to accumulation of 4-HNE. This can trigger the activation of NRF2, which in turn activates GSTA1 and GSTA2, blocking the formation of 4-HNE, and or activates GSTA4 and GSTA5, conjugating 4-HNE to GSH. This results in elimination of 4-HNE and protecting cochlear hair cells, SGNs, and SV cells. In addition, GSH can directly sequester 4-HNE through its cysteine residues by the formation of GS-HNE adducts.

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Table 1.

Classes of the most well characterized cytosolic GSTs.

Class	Symbol	Subunit
Alpha	GSTA	1, 2, 3, 4, 5
Mu	GSTM	1, 2, 3, 4, 5
Pi	GSTP	1
Theta	GSTT	1,2