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Review

Preservation of Inner Ear Functions: Extending Glucocorticoid Therapy by Tissue-Protective α1-Antitrypsin

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Abstract: Protecting tissues from excessive inflammation by glucocorticoids results in an effective blockade of inflammation; however, it does not instigate processes of inflammatory resolution or tissue repair. Moreover, glucocorticoids have side effects such as a susceptibility to infections. In otolaryngology—specifically, within the inner ear—surgical and non-surgical pathologies include cochlear implantation, stapes surgery, perilymph fistulas and Meniere's disease. For these, steroids are indicated in order to prevent excessive inflammation that might lead to hearing and vestibular failure. Unless tissue homeostasis is restored, the compromised tissue is at risk of a functional loss. α1-Antitrypsin (AAT) is a circulating inflammation-modulating molecule that rises during the molecular signs of a tissue injury; it manipulates inflammation towards an inflammatory resolution and advances tissue repair. Lifelong infusions of AAT are currently indicated for genetic AAT deficiencies and are safe. In the present review, we discuss the advantages and downfalls of glucocorticoid treatments across several surgical inner ear injuries alongside evidence of the beneficial attributes of treatments with AAT. Collectively, the present knowledge places AAT treatments, wither independent or in combination with glucocorticoids, as adding focus on tissue repair in the context of unmet medical needs in otolaryngology.

Keywords: cochlear implantation; hearing preservation; inflammation; perilymph fistula; stapes surgery; tissue repair

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1. Introduction

Hearing loss represents a significant handicap that gravely impacts the quality of life. Normal hearing function depends on the mechanical and physiological integrity of the middle and inner ear structures and their associated nervous system. The middle ear is composed of the tympanic membrane and the ossicles: malleus, incus and stapes. Accordingly, middle ear pathologies primarily involve the mechanical compromise of the eardrum or its associated ossicles due to, for example, an infection, a fluid accumulation or trauma.

The inner ear is enclosed in a bony cavity—the otic capsule—and has two mobile windows, one oval and one round. The portion of the inner ear that processes audio signals is the cochlea whereas the portion that is required to maintain balance involves the vestibular organs of the inner ear. Within the cochlear organ, hydrodynamic and mechanochemical forces are converted into signals that are transferred through the spiral ganglion to the auditory nerve and determine both what we hear and, more ambitiously, where it came from. To achieve such an accurate conversion of soundwave energy into electrochemical signals, the brittle structures and specialized cell types of the inner ear must stand up to a standard, often in the face of a myriad of challenges. For example, the cochlear function requires a potent cochlear duct and the micromechanical and cellular

integrity of the organ of Corti as well as local biochemical and bioelectric homeostasis. Thus, cochlear-related hearing loss might arise due to damage to the structures of the cochlea (e.g., perilymph-filled scala tympani, vestibules and an endolymph-filled cochlear duct) as well as damage to specific cell types (e.g., the inner hair cells and outer hair cells). Cochlear failure ultimately leads to an impaired signal transduction to the auditory nerve and, subsequently, hearing loss.

The causes of inner ear disorders range from age- and genetic-related circumstances to local mechanical trauma to iatrogenic damage caused by ototoxic compounds, including several classes of antibiotics and a few types of chemotherapy. Importantly, surgical procedures might be accompanied by perilymph leakage, as in the case of cochlear transplantations.

1.1. Glucocorticoid Use in Inner Ear Pathologies

Excessive inflammatory processes play a universal role in inner ear diseases, including in several etiologies where a detailed pathogenesis has yet to be determined [1–3]. Corticosteroids, a class of steroid hormones, include glucocorticoids. Practically every cell in a vertebrate animal has a glucocorticoid receptor. A glucocorticoid therapy such as a dexamethasone treatment is widely used both systemically and locally to treat inflammatory flares; inner ear disorders are not exempt. A patient with Meniere's disease or with idiopathic sudden sensorineural hearing loss as well as patients who suffer from vestibular neuronitis, an autoimmune inner ear disease or noise-induced hearing loss will generally receive a steroid therapy. Steroids are also used during inner ear surgery, as in the case of cochlear implantations and stapes surgery. This therapeutic strategy is justified by the effort to retain inner ear functionality in the face of excessive inflammation.

The side effects of a systemic steroid therapy are avoided by the topical administration of steroids. Beyond that, an intratympanic administration results in significantly higher concentrations of steroids in the inner ear (Figure 1) [4,5]. Nonetheless, at the molecular level and regardless of the route, corticosteroids will block the inflammatory processes at the expense of proactive tissue repair.

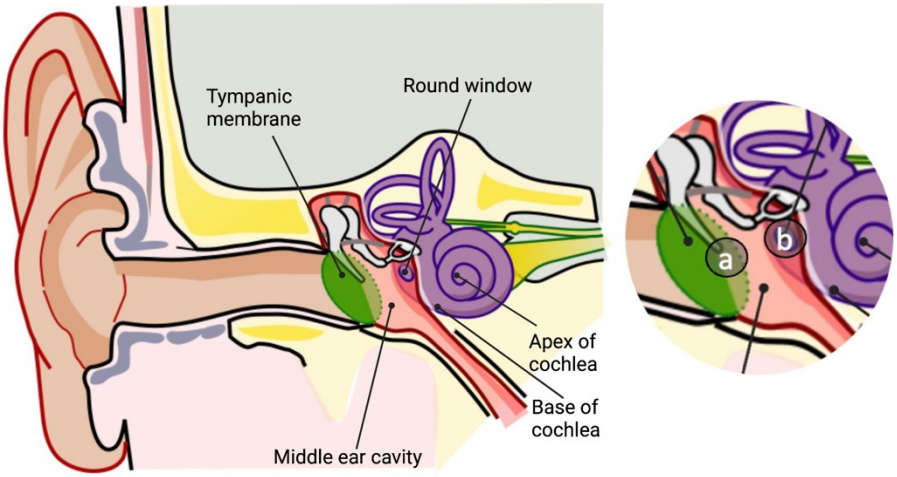

Figure 1. ***Routes of drug administration for inner ear treatments***. Schematic representation of the anatomy of the ear. *Inset*, routes of direct drug injection: (**a**) intratympanic; (**b**) intracochlear through the round window (created with BioRender.com accessed on 9 September 2022).

1.2. Glucocorticoids Are Not Tissue-Protective

Although they are an effective treatment for relatively narrow clinical entities (including acute and chronic inflammations and conditions that require immunosuppression), glucocorticoids are associated with side effects that can involve most major organs and systems. Glucocorticoids increase the risk of an infection due to their immunosuppressive attributes as they reduce neutrophil bactericidal activity, diminish macrophage responses and blunt the expression and spread of inflammatory mediators [6]. Upon binding to

their receptor, glucocorticoids inhibit the translocation of NF-kB family members to the nucleus, thus reducing the induction of the inflammatory expression cassette; that said, this action also paralyses important paths such as angiogenesis, epithelial migration, fibroblast recruitment and restorative macrophage activation at the site of damage as well as fibroblast responses, including the activity of collagen synthesis [7,8]. Under the influence of a systemic glucocorticoid therapy, the hepatic gluconeogenesis rate increases and the glucose uptake by the peripheral tissues decreases, leading to elevated blood sugar levels. In the case of acute glucocorticoid exposure, these events result in impaired wound healing similar to those seen in individuals diagnosed with insulin resistance and poorly controlled diabetes [7]. Other adverse effects become apparent in many and diverse systems, including skeletal, cardiovascular, ophthalmic, skin, adrenal and CNS [6,9]. It is a rather discouraging conclusion that glucocorticoids are effective anti-inflammatory and immunosuppressive drugs, but not tissue-protective agents.

1.3. Alpha1-Antitrypsin Is Tissue-Protective

Alpha1-antitrypsin (AAT) is a circulating tissue-protective glycoprotein that is elevated in response to the molecular signs of tissue damage. Its production is triggered by hypoxia and by inflammatory cytokines. At elevated levels, AAT diverts unwanted inflammation towards an inflammatory resolution, decreases the levels of destructive reactive oxygen species (ROS) and interferes with the apoptosis of several cell types, including neutrophils, which better facilitate a bacterial burden reduction. Together with these more enduring active neutrophils, AAT has been shown to acquire direct antibacterial attributes in the presence of elevated tissue-derived nitric oxide [10]. Unlike in the case of resting cells, in the context of an inflammatory trigger AAT enhances the production of resolution-phase mediators, including IL-10, IL-1 receptor antagonist (IL-1Ra), TGFβ and VEGF, all the while inhibiting the production of IL-1b, IL-6 and TNFα alongside interfering with local cytokine-activating enzymes [11]. This is achieved, in part, by modifying the nuclear translocation profile of selected NF-kB family members in a way that, in response to an inflammatory surge, inflammation-driven resolution genes are preferentially induced. In cell culture experiments, both AAT and dexamethasone were shown to achieve anti-inflammatory outcomes, yet only the AAT treatment increased the production of the anti-inflammatory cytokine, IL-1Ra (Table 1) [12]. Importantly, upon a co-treatment with both AAT and dexamethasone, the production of IL-1Ra was diminished by dexamethasone, presumably as the result of an inability of NF-kB family members to reach the gene promoter of IL-1Ra.

Table 1. ***Comparison between glucocorticoids and AAT***. Mechanism of action, clinical indication, dose and relevance to hearing.

	Glucocorticoids	**α1-Antitrypsin**
Mechanism of action	Comprehensive inhibition of inflammatory-associated molecules inhibiting the inflammatory induction of anti-inflammatory pathways	Immune modulation diverting excessive inflammation towards an anti-inflammatory resolution and tissue repair
Clinical indication	Ear surgery (e.g., cochlear implants, stapes and inner ear fistulas), inner ear disease (e.g., vestibular neuronitis and sudden sensorineural hearing loss)	Genetic AAT deficiency
Dosage	The protocols vary	Lifelong weekly 60–80 mg/kg slow drip i.v. infusions
Potential benefit to hearing system	Inhibition of excessive inflammatory damage and minimization of damage to hair cells	Facilitation of early inner ear tissue repair, inhibition of excessive inflammatory damage and preservation of hair cell viability

In alignment with the protective attributes of AAT, patients with a genetic AAT deficiency exhibit signs of poor tissue repair, most typically in the form of lung alveolar wall degradation; for these individuals, human plasma-derived affinity-purified AAT is administered by weekly intravenous slow drip perfusion sessions. In recent years, interest in the potential benefits of a clinical-grade AAT therapy for enhancing tissue repair outside a genetic AAT deficiency has gained attention [13–16].

In this review, the anatomically minuscule inner ear compartment serves as an archetype for the detrimental intersection between a standard of care glucocorticoid therapy, tissue damage to the point of functional failure and presumed insufficient liver-derived circulating AAT. For scope considerations, focus is placed on iatrogenic surgical damage to the inner ear as the underpinning of inner ear tissue damage.

2. Inner Ear Injury and Glucocorticoid Therapy

Numerous attempts have been made to prevent and to treat inner ear deficits brought upon by surgical damage from both cochlear implantations and stapes surgery. Obviously, the opening of inner ear barriers during these procedures will result in a trauma to inner ear structures and to varying degrees of hearing loss. Although an optimal surgical technique is certainly the mainstay of successful hearing preservation, a pharmacologic protection may help to increase the success rates. Michael et al. reported positive outcomes of systemic and local therapies with antioxidants, including vitamin E, vitamin C, melatonin and lazaroids [17]. Mora et al. reported positive outcomes of an intravenous tissue plasminogen activator therapy [18]; Miller et al. showed that neurotrophins could enhance spiral ganglion cell survival after inner ear hair cell loss [19] and Lalwani et al. described the benefits of the introduction of an adeno-associated virus into the cochlea of a guinea pig [20]. However, as a corticosteroid therapy has been mainly effective in stopping, decreasing or correcting an auditory impairment in numerous other etiologies of hearing loss [21–24], a steroid therapy has also been encouraged in the field of surgical inner ear interventions.

2.1. Cochlear Implantation and Glucocorticoid Therapy

The now commonly practiced cochlear implantation procedure aims to restore the hearing capacity and improve the quality of life. This surgical procedure is fairly routine and is typically performed under general anesthesia. A small retro-auricular incision is made, the implant is then placed under the skin and the electrode is inserted into the cochlea through the round window (Figure 1). Nevertheless, during the procedure, the delicate structures of the cochlea are exposed to a direct trauma [25,26]. Rapid inflammation ensues as the cascading molecular agents of the tissue injury rage onward, including an avalanche of ROS molecules; these events lead to hair cell death and a subsequent loss of the residual biological hearing capacity [27–31]. Later on, fibrosis forms around the implanted electrode, a physiological phenomenon that occurs around practically any foreign object, and jeopardizes the conductive functionality of the device [32]. The procedure of a cochlear implantation is, therefore, still evolving.

Factors that benefit hearing preservation during a cochlear implantation have thus far included modified surgical practices (the angle and speed of the electrode insertion), flexible electrode arrays and the use of steroids [33]. The standard of care most widely includes dexamethasone. Due to its potent anti-inflammatory and anti-proliferative properties, dexamethasone decreases post-operative inflammation and diminishes the fibrotic processes. There is, however, no consensus regarding the optimal timing, dosage and route of the steroidal administration as all methods to date have led to relatively satisfactory hearing preservation [33,34]. A glucocorticoid treatment in the context of a cochlear implantation can also be systemically administered. Several studies suggest that systemic glucocorticoids prior to a cochlear implantation improve hearing preservation. According to a prospective non-randomized study, patients treated with prolonged glucocorticoid regimens of combined oral and i.v. routes beginning 3 days before surgery had significantly superior hearing preservation outcomes 6 months post-surgery compared with

the untreated control group. Positive hearing preservation differences were detected not only when compared with the control group, but also when compared with the standard treatment group, which began an i.v. treatment 30 min prior to surgery [35]. When testing short-term single-dose regimens, a double-blind placebo-controlled trial found that patients undergoing a cochlear implantation and treated immediately before surgery with a single high dose of i.v. methylprednisolone had no difference in hearing preservation or electrode impedance compared with the placebo group [36].

To avoid a systemic glucocorticoid exposure, the treatment may be introduced using an intratympanic delivery. Prospective clinical studies have shown that an intratympanic glucocorticoid therapy prior to an electrode insertion whilst repeatedly delivering glucocorticoids throughout the surgical procedure results in better post-operative hearing preservation [37]. This was tested using intratympanic methylprednisone immediately after an intubation [38] and intratympanic dexamethasone 15 min before an electrode insertion [39]. Yet, there are several limitations to these studies; dexamethasone was applied together with hyaluronic acid, which minimizes the surgical trauma and seals the cochlea, thereby increasing the exposure to the drug. Accordingly, a double-blind placebo-controlled randomized trial showed that applying a polymeric sponge soaked with methylprednisolone onto the round window membrane during cochlear implantation surgery resulted in lower post-operative inner ear symptoms [40]. Moreover, the glucocorticoid treatment group also exhibited a lower electrode impedance from the middle portion of the electrode array, possibly pointing towards a minimal accumulation of fibrosis around the electrode array. This also suggests that the drug reached the mid-range frequency zone of the cochlea, raising the possibility that a longer exposure time may allow glucocorticoids to reach more distally inside the cochlea.

Unlike in the case of a systemic or intratympanic glucocorticoid delivery, a direct intracochlear delivery places the drug at the site of damage, avoiding the reliance on drug diffusion. A clinical study by Passche et al. showed that a single intracochlear dose of triamcinolone prior to an electrode insertion lowered the electrode impedance [41]. The difference in the electrode impedance levels between the treated and the control groups was maintained for up to 17 days post-operation, but was then lost. Prenzler et al. speculated that this significant, albeit short-term, effect was due to a low concentration of the drug and conducted a study using a 5-fold greater dose and a custom-designed catheter that reached deeper into the cochlea [42]. The results confirmed that there was a significant difference between the high-dose group and the control group for up to an average of 39.53 days. When the high-dose and low-dose groups were compared, there was no significant difference at any point of the trial. Nevertheless, the significance between the low-dose group and the control group was lost at an earlier stage than with the high-dose group. It is important to note that when subdividing the electrode surfaces into basal, middle and apical contact sites, a significant difference was only observed in the basal and middle regions despite the deep insertion of the drug [42].

Glucocorticoid delivery routes may also be combined. A retrospective cohort study compared an intratympanic treatment during surgery with an intratympanic treatment plus a systemic two-week oral glucocorticoid taper beginning three days prior to surgery [43]. The results suggest that patients who received the combined regimen had a higher degree of hearing preservation. In that study, the three days of preoperative oral glucocorticoids may have resulted in higher perilymph glucocorticoid concentrations, augmenting the effect of the intratympanic glucocorticoid therapy. According to a randomized controlled trial, a combination of transtympanic glucocorticoids 24 h prior to surgery then local glucocorticoids during surgery achieved superior pure-tone average outcomes compared with either monotherapies [44]. It is reasonable to propose that a preoperative glucocorticoid administration, whether systemic or local, may precondition the tissue and, together with a local intratympanic treatment during surgery, lead to improved outcomes. As the great majority of cochlear implants are planned ahead of time, the concept of preconditioning is worth exploring.

In an attempt to optimize the impact of dexamethasone within the inner ear compartment, *drug-eluting electrode arrays* were developed. In this method, dexamethasone-loaded silicone rings are introduced between the stimulating channels that diffuse dexamethasone directly into the cochlea [32]. The rationale behind these eluting electrode arrays is that they are able to release dexamethasone for an extended duration, possibly addressing a later-appearing fibrosis. Positive outcomes have been demonstrated in guinea pig and non-human primate models. In the guinea pig model, three dexamethasone doses were tested, resulting in significant dose-dependent positive outcomes; at two months post-surgery, the electrode impedance was still superior to the control electrode arrays [45]. The immunostaining of the organ of Corti depicted preserved outer hair cells whereas the control group showed missing patches of both inner and outer hair cells. In a parallel study, the fibrosis around the electrode was diminished alongside an electrode impedance [46]. These observations may offer an important development as they advance into clinical trials such as in the CI-DEX study (NCT04750642).

2.2. Stapedectomy and Glucocorticoid Therapy

A stapedectomy is performed to treat hearing loss caused by otosclerosis. Surgery is usually performed as an outpatient procedure under local or general anesthesia. Using an operating microscope, the surgeon elevates the eardrum and observes the stapes immobility as part of the diagnosis. Using delicate instruments and preferably a laser, the upper part of the stapes is removed. In order to re-establish the chain of soundwave transmissions, a small hole is made in the remaining part of the stapes, the footplate. A prothesis is then inserted into the hole and attached to the second bone in the ossicle chain, the incus. Inevitably, during a stapedectomy, the barrier to the cochlear labyrinth is breached, causing a trauma to the vessels of the perilymphatic space and manifesting as serous labyrinthitis and/or a reparative granuloma [47–49]. The incidence of a partial or complete loss of sensorineural hearing due to this procedure is 0.6% and 3%, respectively [47]. The perioperative administration of corticosteroids is not under a consensus as it has been advocated by a few [50–53] and disputed by others [54].

2.3. Perilymphatic Fistula and Glucocorticoid Therapy

A perilymphatic fistula is an improper connection between the perilymph-filled inner ear and either the cavity of the middle ear, the mastoid bone or the cerebral cavity. It may be trauma-induced or idiopathic and caused cochlear and vestibular symptoms in both cases. There are essentially two types of treatment for a perilymphatic fistula: conservative and surgical. Conservative management is favored based on the etiology and severity of the condition; it tries to avoid any measure that might raise intracranial pressure or inner ear pressure and focuses on intratympanic or systemic steroids in cases of an acute decompensation [55,56].

2.4. Post-Surgical Facial Paralysis and Glucocorticoid Therapy

The facial nerve may become paralyzed following ear surgeries, particularly those performed in the middle ear. Such a direct iatrogenic paralysis of the facial nerve, which can occur during or just after surgery, has been the subject of extensive research. Delayed facial paralysis is defined as occurring at least 48 h after surgery and occurs after 0.2 to 1.9% of middle ear surgeries [57]. The mechanism behind this latent phenomenon can be one of three: facial canal dehiscence (the partial or total separation of previously approximated wound edges due to a failed wound healing process); a neural edema; or herpes virus reactivation. An edema, for example, is implied in 17% of stapes surgery procedures [58]. It leads to a compression of the nerve fibers and a disruption of the blood supply causing facial nerve weakness; the process peaks on the fifth day post-surgery and almost completely recedes within 14 days [57]. By default, a steroid therapy is applied with the aim of, at the very least, reducing the edema.

3. Unmet Medical Needs in the Context of a Glucocorticoid Therapy

Glucocorticoid therapies increase the risk of a local infection. In particular, a cochlear implant device risks being utilized by bacteria as a route of entry. Whilst the anti-proliferative attributes of glucocorticoids may postpone tissue growth and protect from an electrode impedance, they simultaneously extend the time required for cochlear sealing, thus increasing the risk of a bacterial invasion [32].

Additionally, a glucocorticoid therapy disrupts the process of surgical site healing. During a cochlear implantation, the insertion of the electrode causes a trauma to the cochlea, which results in an inflammatory cascade that gravely risks the occurrence of hair cell death and hearing loss. Inflammatory resolution mediators such as blood supply restorative VEGF and architectural reparative TGFβ/collagen are all blocked by glucocorticoids. The concentration of ROS molecules in this typically sterile small space is toxic to hair cells [59]. To tip the scales in favor of a positive outcome, it is necessary to explore therapies that harbor antioxidative properties and, ideally, tissue-protective activities.

4. Combining AAT and Glucocorticoid Therapies

Apart from the anti-elastase activity of AAT, it holds attributes that may offer an opportunity to address issues that arise during the glucocorticoid therapy.

4.1. Reduction in the Bacterial Burden

Although it may seem unlikely given the anti-inflammatory nature of AAT, one of its intriguing properties is its capacity to decrease the bacterial burden. A clinical AAT treatment for patients with a genetic AAT deficiency resulted in a significant reduction in bacterial colonization across several clinical studies [60–62]. In one such clinical trial, cystic fibrosis patients were treated with inhaled AAT and, as a result, exhibited a marked reduction in pulmonary *Pseudomonas aeruginosa* numbers [63]; this outcome is consistent with animal studies [64,65]. In a peritoneal sepsis model, mice transgenic to human AAT ($hAAT^{+/+}$) exhibited significantly lower bacterial titers within 1 day after the induction of bacterial sepsis alongside a lower incidence of leukopenia, a lower occurrence of multi-organ dysfunctions and a superior 24 hour survival rate [10]. This outcome was highly unexpected as native AAT does not inhibit bacterial growth in cultures. However, in an environment rich in nitric oxide (NO), AAT is nitrosylated and gains antibacterial properties (Figure 2) [66]. S-NO–hAAT is an inflammatory agent as it transfers the nitrite to the signaling proteins of innate immune cells, thus nitrosylating them and altering the signaling cascade towards macrophage activation; the cells in turn upregulate the production of iNOS and generate more nitric oxide, reaching high bactericidal concentrations. Upon nitrosylation, the enzymatic inhibition profile of AAT changes towards inhibiting cysteine proteases such as caspase-3, thus preventing the apoptosis of neutrophils and prolonging their stay in the infected tissue. The nitrosylation of AAT is reversible, allowing the molecule to continue with local tissue protection after it participates in safely reducing the bacterial burden.

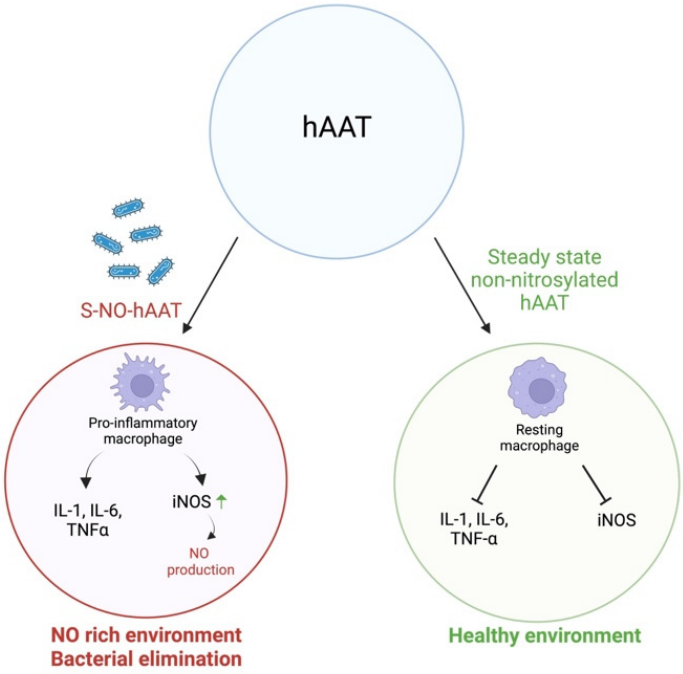

Figure 2. ***Divergence of hAAT functions between a sterile and a non-sterile environment***. Upon exposure to macrophage-derived nitric oxide (NO), hAAT undergoes a covalent change to become S-NO–hAAT (*left*). S-NO–hAAT is inflammatory; activated macrophages increase pro-inflammatory cytokine release and iNOS expression, thereby promoting an antibacterial environment (created with BioRender.com).

4.2. Protection from Free Radicals and Reactive Oxygen Species

AAT holds several mechanisms for inhibiting excessive free radical activities and can harness reactive oxygen species to improve the tissue resolution. Free radicals are primarily formed by transferring an electron from NADPH to oxygen by the enzymatic action of neutrophilic NADPH oxidase. The enzyme is blocked by AAT at several levels downstream and upstream of the free radical formation. Firstly, AAT modulates oxygen consumption by neutrophils, which is an essential component in the creation of free radicals. AAT prevents the phosphorylation of ERK1/2 and inhibits the membrane translocation of the P67phox-P47phox complex, which is crucial for the activation of NADPH oxidase. In a genetic AAT deficiency, patients produce greater amounts of the superoxide radical, O_2^- [67] and exhibit an increase in the abundance of p67phox-p47phox complexes on neutrophils. When these patients received an AAT augmentation therapy, they produced less O_2^- [68].

An intriguing ability of AAT is the utilization of reactive oxygen species for a more efficient resolution process. Early on in tissue injury, neutrophils are the first cells to extravasate and reach the site of damage. As they advance towards the center of the damage they degranulate, releasing tissue-degrading enzymes and, unexpectedly, granule-packaged AAT [69]. Neutrophils readily generate reactive oxygen species facing the direction of their migration; these oxidize AAT and block its ability to neutralize them. By this, neutrophils carve their way through the tissue for the sake of a swift phase of decontamination; throughout this process, AAT diffuses away from their path and regains tissue-protective attributes as it is chemically reduced by the surrounding interstitial fluid. As the surrounding tissue is spared from the destructive path of neutrophils, a greater immune response is achieved as unstunned resident tissue cells are permitted to initiate their complex roles in proactive tissue repair.

4.3. Accelerated Inflammatory Resolution and Improved Tissue Repair

During an inflammation, circulating AAT levels rise. In skin wound models, mice treated with local AAT had a significantly faster wound closure rate compared with the

control mice (4 days versus 9 days, respectively) [70]. Similarly, in a tympanic membrane perforation model, mice treated with local AAT exhibited a more rapid healing process than the control mice and the tympanic tissue presented with a pro-resolution gene profile (*unpublished data*). In a unilateral perilymph fistula model, hAAT-transgenic mice exhibited a significantly more rapid normalization of the vestibular function compared with the control mice (48 h compared with 2 weeks, respectively) [69]. Locally applicated AAT was superior to a steroid treatment in that model (*unpublished data*). Several of these outcomes are suggested to relate to improved revascularization under AAT-rich conditions [71, 72] not excluding the possibility of enhanced neutrophil-mediated extracellular matrix remodeling [70] and the preferential differentiation of local macrophages towards a tissue-restorative phenotype [12].

5. Discussion

Glucocorticoid treatments for inner ear pathologies is a challenging field, especially regarding post-surgical hearing preservation. Presently, there is a lack of uniform clinical guidelines instructing on the timing of treatment, the route of delivery or the dosing. This may be the result of a futile assortment of clinical evidence that focuses on treatment efficacy whilst employing a large variety of medications, dosages and regimens along with different methodological protocols. At the very least, the results do depict limited long-term hearing preservation by glucocorticoid therapies. This highlights the claim by which glucocorticoids are indeed anti-inflammatory, but they are not tissue-protective or tissue-restorative. Enter AAT, a molecule that is physiologically programmed to promote tissue repair and immune tolerance throughout life. It rises in the circulation during an infection as well as during the third trimester of a healthy pregnancy and with an advanced age; it is lost during protein-losing enteropathies in a manner that correlates with the gastrointestinal disease severity [73,74] and has recently been shown to protect patients with steroid-refractory gut GVHD [75,76]. In perilymph samples from patients undergoing cochlear implantations, the levels of endogenous perilymph AAT were inversely associated with cochlear hearing loss [77]; Causse et al. described a relationship between AAT and the enzymatic concept of otospongiosis in cases of cochlear otosclerosis [78]. AAT is not anti-inflammatory per se; it is pro-resolution. Whether an endogenous AAT insufficiency plays a role in a suboptimal recovery from inner ear disruptions is an important topic of ongoing and future exploration.

Is there a direct relationship between AAT and the physiology of the glucocorticoid pathway? AAT was found to prevent the enzymatic degradation of corticosteroid-binding globulin in the blood [79] and to bind to the cytoplasmic glucocorticoid receptor (GR) [80]; conversely, the activation of the GR by glucocorticoids increased AAT production [81]. This receptor is, fundamentally, the channel by which endogenous cortisol generates its responses; it is tempting to suggest, therefore, that stressful conditions in the whole animal may apply this molecular bridge between cortisol delivery to the sites of inflammation and the production of tissue-protective AAT. These data invite further investigations into the pathways that may connect the favorable biological activities of both AAT and glucocorticoids in the context of a whole organism.

Does a decision have to be made between AAT and glucocorticoids, or can the two be used in synergy? Very few studies address an AAT-augmented steroid therapy. In an in vitro study, LPS-stimulated macrophages were exposed to a concentration gradient of either AAT, dexamethasone, or both. The IL-6 and IL-1Ra levels were determined in supernatants; the ratio these largely signified the inflammatory versus anti-inflammatory outcomes. Consistent with the literature, LPS-stimulated AAT-treated cells exhibited low IL-6 levels and high IL-1Ra levels and LPS-stimulated dexamethasone-treated cells exhibited both low IL-6 and low IL-1Ra levels [82]. According to the outcomes, the highest IL-1Ra/IL-6 ratio was achieved by a combination of low concentrations of dexamethasone and high, yet clinically relevant, concentrations of AAT [83]. In contrast, lower concentrations of AAT were blocked by the addition of dexamethasone. This suggests that a

low-dose glucocorticoid treatment still allows for an inflammatory modulation by AAT whilst carrying the desired immunosuppressive effects of glucocorticoids. These unique results support the idea that the tissue repair and inflammatory resolution may be therapeutically enhanced by combining glucocorticoid and AAT therapies at ideal dose ratios or as interleaving treatment protocols to achieve optimized outcomes in which inflammatory branches are executed for the sake of decontamination and tissue repair whilst the desired immunosuppressive effects of glucocorticoids persist. It is also reasonable to propose that such a combination may allow for a reduction in glucocorticoid doses, thereby minimizing the adverse effects of a glucocorticoid therapy.

The role of AAT in the taming of excess free radicals brings to mind antioxidant approaches for the conditions of tissue damage. Many destructive inflammatory processes are mediated by free radicals, including trauma-related processes in the inner ear and exposure to ototoxic drugs that subsequently lead to hair cell death. With this notion in mind, a trial conducted in Hannover Medical School, Germany, tested the effect of a treatment with an antioxidant dietary supplement combination (including vitamins A, C, E and magnesium) on the preservation of residual hearing [84]. The treatment was provided to patients for a duration of 106 days, starting 2 days prior to surgery. Although a statistical significance was not observed, the results showed a positive trend as the treated patients exhibited a 4.15 dB lower threshold than the placebo group at 500 Hz three months after the first fitting. One year after surgery, this disparity increased to 6.45 dB between the groups. It is suggested that a possible mechanism of action of AAT regarding hair cell protection could be in modulating excessive oxidative stress. This action may benefit inner ear disorders, including as an adjunct therapeutic alongside glucocorticoids.

Cochlear implants, stapes surgery and idiopathic perilymph fistulas are all examples of *sterile inner ear injuries.* As such, a local inflammation arises independent of pathogens as the cell injury and mechanical stress that occur within the cochlear microstructure readily spread the inflammation. This is not unlike a sterile inflammation in, for example, cardiac ischemias and cerebral strokes as well as kidney ischemia-reperfusion injuries; the injured cells are extremely inflammatory. In these circumstances, AAT has been shown to be of benefit at multiple levels, including in regard to neutrophilic infiltration, TNFα production and systemic CRP levels [15,85–87]. AAT has been tested for middle ear treatments in animals and did not exhibit ototoxicity [88,89]; in humans, the effect of AAT was tested on middle ear effusions and was found to interfere with destructive enzymes [90]. Considering the pattern of the intimate protection of injured tissues by AAT, in the context of inner ear disorders, it would be tempting to explore the possibility of applying AAT in the electrode-eluting arrays that presently infuse dexamethasone directly into the cochlear compartment or for improving the outcomes of the novel technology of vestibular implants [91].

In conclusion, the steroid inhibition of the early inflammatory surge is, in many ways, detrimental because it prevents the unfolding of the repair processes that are essential for the actual resolution of wounded tissues. There is strong experimental and clinical support for the capacity of an AAT therapy to advance the tissue healing processes in a safe and physiologically appropriate setting either alone or possibly in a calculated conjunction with glucocorticoids. The idea of treating a tissue repair rather than treating an inflammation opens opportunities for many more unmet clinical indications outside the scope of the current review. Further research is required to better understand the delicate synergy between glucocorticoids and AAT and to identify optimal timings and dosages across the various pathologies.

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