Introduction

Otitis externa, also known as swimmer's ear, is a prevalent and often debilitating inflammatory condition of the external ear canal. This inflammatory phenomenon most commonly involves redness, swelling, pain and discharge from the ear [1]. In the malignant form, complications such as skull base infarctions and facial nerve paralysis may occur [2]. Although otitis externa is usually not a life-*threatening* condition, it may cause significant discomfort effecting *quality* of life, as well as posing a considerable burden on healthcare systems worldwide due to its frequent encounter in medical practice.

Up to date, the main strategies of treatment for otitis externa involves topical antibiotics and corticosteroids [3] , aiming at infection control and inflammatory reduction, respectively. Nevertheless, these treatments are accompanied by undesirable effects including microbial resistance in the case of antibiotics, and corticosteroid associated side effects such as fungal infections, local skin reactions and systemic absorption. With these in mind, a call for alternative approaches that are safer, more effective, and sustainable is undoubtedly in place.

In this context, Alpha-1 Antitrypsin (AAT) emerges as a potential novel therapeutic option for otitis externa. AAT, a serine protease inhibitor, is an essential protein synthesized in the liver [4] and is primarly known for its role in protecting lung tissue from proteolytic damage caused by neutrophil elastase. AAT production in hepatocytes and other cell types is triggered by hypoxia and by inflammatory cytokines. Beyond the well-established functions in lung-related disorders, AAT possesses diverse inflammatory-modulating and tissue protective actions, making it an intriguing candidate for managing inflammatory conditions in other organ systems. At elevated levels, AAT *diverts* excess inflammation towards an inflammatory resolution, decreases the levels of destructive reactive oxygen species (ROS) and interferes with apoptosis in several cell types, including neutrophils, which by this facilitate a stronger bacterial burden reduction capacity [5]. Consistent with neutrophil empowerment, AAT has been shown to acquire direct antibacterial attributes in the presence of elevated tissue-derived nitric oxide [6,7]. 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-1β, IL-6 and TNFα alongside interfering with local cytokine-activating tissue-degrading enzymes [8]. By harnessing these capabilities, based on their known mechanisms of action and promising therapeutic benefits, AAT may offer a unique opportunity to address the underlying inflammation and tissue damage associated with otitis externa, leading to improved clinical outcomes and patient well-being.

In this study, we aim to assess the potential therapeutic efficacy of AAT treatment in a murine model of otitis externa. To achieve this, we will investigate various treatment combinations, ranging from AAT monotherapy to co-administration with established standard of care interventions, aiming to compare the effectiveness of AAT against antibiotics and corticosteroids. Furthermore, we will explore the interaction between AAT and conventional treatments when used in combination, considering the necessity of combining AAT with current standard of care in any newly approved treatment protocol.

Results

AAT treatment mirrors the effect of dexamethasone and ciprofloxacin combination

To delineate the tissue's inflammatory profile, we employed real-time PCR analysis, which gauged the expression levels of the IL-1b gene, indicative of an ongoing active inflammatory process. As anticipated, untreated positive control ears exhibited notably elevated IL-1b expression (15.45-fold increase from sham levels). Evaluating ears subjected to the dexamethasone-ciprofloxacin regimen, a conventional treatment approach, yielded predictable outcomes characterized by subdued IL-1b expression (mean of 3.14-fold increase from sham levels). Intriguingly, an examination of locally administered clinical grade AAT treatment on the ears, as well as the ears of the homozygote mice group, revealed IL-1b expression levels closely resembling those observed in dexamethasone-ciprofloxacin treated ears (averaging 5.15 and 6.15-fold increases from sham levels, respectively). Lastly, an exploration of IL-1b expression in ears subjected to a combined AAT and ciprofloxacin intervention exhibited higher expression compared to the other treatment groups (mean of 12.16-fold increase from sham levels).

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