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PREVENTION OF TRAUMA-INDUCED COCHLEAR FIBROSIS USING INTRACOCHLEAR APPLICATION OF ANTI-INFLAMMATORY AND ANTIPROLIFERATIVE DRUGS

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Abstract—Cochlear fibrosis is a common finding following cochlear implantation. Evidence suggests that cochlear fibrosis could be triggered by inflammation and epithelial-to-mesenchymal cell transition (EMT). In this study, we investigate the mechanisms of cochlear fibrosis and the risk/benefit ratio of local administration of the anti-inflammatory drug dexamethasone (DEX) and antimitotic drug aracytine (Ara-C). Cochlear fibrosis was evaluated in cochlear fibrosis models of rat cochlear slices *in vitro* and in KLH-induced immune labyrinthitis and platinum wire cochlear implantation-induced fibrosis *in vivo*. Cochleae were invaded with tissue containing fibroblastic cells expressing α-SMA (alpha smooth muscle actin), which along with collagen I, fibronectin, and laminin in the extracellular matrix, suggests the involvement of a fibrotic process triggered by EMT *in vitro* and *in vivo*. After perilymphatic injection of an adenoviral vector expressing GFP *in vivo,* we demonstrated that the fibroblastic cells derived from the mesothelial cells of the scalae tympani and vestibuli. Activation of inflammatory and EMT pathways was further assessed by ELISA analysis of the expression of IL-1β and TGF-β1. Both markers were elevated *in vitro* and *in vivo*, and DEX and Ara-C were able to reduce IL-1β and TGF-β1 production. After 5 days of culture *in vitro*, quantification of calcein-positive cells revealed that Ara-C was 30-fold more efficient in preventing fibrosis, and provoked less sensory hair cell loss, than DEX. In KLH-induced immune labyrinthitis and platinum wire-implanted models, Ara-C was more efficient in preventing proliferation of fibrosis with less side effects on hair cells and neurons than DEX. In conclusion, DEX and Ara-C both prevent fibrosis in the cochlea. Analysis of the risk/benefit ratio favors the use of Ara-C for preventing cochlear fibrosis.

Key words: cochlear implant, fibrosis, dexamethasone, aracytine.

INTRODUCTION

Cochlear implants are electronic devices which bypass damaged hair cells in the inner ear by directly stimulating the auditory neurons, allowing for a restoration of hearing in people suffering from severe to profound hearing loss who obtain poor benefit from conventional hearing aids. The remarkable speech discrimination of patients who have residual low-frequency hearing has led to the consideration of extending the implantation selection criteria to subjects with useful residual low-frequency hearing. Such patients may benefit from hybrid cochlear implants which combine electrical stimulation for the high-frequency loss and acoustic hearing aid technology for the stimulation of any residual low-frequency hearing.

Efforts to allow hearing preservation rely on the use of soft surgery techniques and the implantation of shorter electrode arrays to minimize the trauma caused during the process of electrode insertion (Gantz and Turner, 2004; Skarzynski et al., 2009). However, the persisting unsolved clinical problem is the progressive loss of a patient's residual hearing that can occur within months following a cochlear implantation. One specific problem is the formation of fibrotic scar tissue around the electrode array (Miyamoto et al., 1997; Alexiades et al., 2001), which involves a tissue reaction consisting of fibrosis and new bone formation. Formation of fibrotic scarring severely damages the cochlear architecture, including the ultrastructure of the organ of Corti, thus compromising the survival of sensory hair cells and neurons, resulting in

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Abbreviations: α-SMA, alpha smooth muscle actin; ABRs, auditory brainstem responses; AP, artificial perilymph; Ara-C, aracytine; DEX, dexamethasone; EMT, epithelial-to-mesenchymal cell transition; IHC, inner hair cells; KLH, keyhole limpet hemocyanin; OHC, outer hair cells; PBS, phosphate-buffered saline.

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a loss of residual hearing (Clark et al., 1995; Fayad et al., 2009). In addition, tissue encapsulation of the electrodes increases their impedance (Clark et al., 1995) and consequently elevates the level of current required to achieve an electrical stimulus threshold, thereby decreasing the patients' cochlear implant battery life. In addition to the increased energy consumption of the implant, impedance changes may alter psychophysical percepts (Kawano et al., 1998; Roland and Wright, 2006). Consequently, prevention of cochlear fibrosis may not only improve auditory rehabilitation by preserving low-frequency residual hearing, but also is important in those patients who have no residual hearing, by improving the efficiency of their cochlear implant by allowing for the use of lower voltage levels in the stimulation of spiral ganglion neurons.

Because the proliferative fibrotic process is assumed to result from inflammation, both animal and human studies have investigated the role of corticosteroid therapy in prevention of cochlear fibrosis occurring after cochlear implantation. In animals, the protective effects of local or systemic administration of corticosteroids have been widely studied (Dinh et al., 2008; James et al., 2008; Chang et al., 2009; Braun et al., 2011; Connolly et al., 2011; Lee et al., 2013; O'Leary et al., 2013; Stathopoulos et al., 2014; Wrzeszcz et al., 2015). While most of these studies demonstrate a protective effect on acute hearing loss induced by cochlear implantation, long-term effects on hearing loss and prevention of fibrosis are more disparate. In humans, application of corticosteroids directly into the scala tympani of the cochlea during surgery may prevent fibrosis formation as attested by electrode impedance measurements (De Ceulaer et al., 2003; Paasche et al., 2009; Enticott et al., 2011). Corticosteroid treatments were less efficient when restricted to a systemic route of application (Jia et al., 2011). However, whatever the route of administration, the corticoid effects were short-lasting, leading to a non-significant difference in impedances by 6 months (Paasche et al., 2009).

While an abundant literature deals with inflammation, no previous study has explored the targeting of proliferative pathways and epithelial-to-mesenchymal transition (EMT) that are involved in cochlear fibrosis. The EMT process has already been widely reported to occur in lung, kidney, and liver fibrosis (Fernandez and Eickelberg, 2012; Yanagita, 2012; Yoshida et al., 2012). Moreover, use of the antimitotic agent aracytine (aka Ara-C, or cytarabine) has been proposed to prevent cardiac stent initiated fibrosis in humans (Voisard et al., 1993). Compared with other antimitotic agents such as cisplatin, the systemic administration of Ara-C does not cause hearing loss. Finally, Ara-C has been used successfully in organ of Corti explants to prevent fibrosis without any sensory cell loss over a wide range of concentrations (Lefebvre et al., 1993).

In the present study, mechanisms underlying the development of cochlear fibrosis were investigated *in vitro* using rat cochlear slices model of fibrosis and *in vivo* in KLH-induced immune labyrinthitis and platinum wire cochlear implantation, where we gathered data on the expression of inflammation and EMT markers. The anti-inflammatory drug dexamethasone (DEX) and the antimitotic drug Ara-C were then studied in relation to their ability to prevent or lessen the level of cochlear fibrosis when locally applied both *in vitro* and *in vivo*.

EXPERIMENTAL PROCEDURES

Animal care and handling followed the animal welfare guidelines of the 'Institut National de la Santé et de la Recherche Médicale' (INSERM), under the approval of the French 'Ministère de l'Agriculture et de la Forêt'. Adult Wistar rats were anesthetized with an intraperitoneal injection of 2% Rompun (2 mg/kg, Bayer, Leverkusen, Germany) and Zoletil 100 (30 mg/kg Virbac, Carros, France).

ELISA analysis

Levels of TNF-α, IL-1β, and TGF-β1 released in cochleae were measured using ELISA kits (R&D Systems, Minneapolis, MN, USA). For *in vitro* studies, rat cochlear slices (see below) were cultured in 6-well plates in an incubator (37 °C, 5% CO_2) for 5 days. The supernatants were collected and stored immediately at −80 °C before processing. For *in vivo* studies, KLH immunized animals or platinum wire animals (see below) were sacrificed 7 days after the implantation of the osmotic pump, the perilymph was aspirated from the cochlea and was stored immediately at −80 °C before processing.

Samples were tested for TNF-α, IL-1β, and TGF-β1, using a standard ELISA protocol. All experiments were performed in triplicate and reported in pg/ml. For each condition, cytokine secretion was calculated as percentage ± SEM of cytokines detected by comparison with the level in the controls where no drug was used.

Drug preparation

Dexamethasone base (DEX, D4902 Sigma Aldrich, Saint Quentin Fallavier, France) or cytarabine (Ara-C, Sigma Aldrich, Saint Quentin Fallavier, France) were freshly prepared before each experiment. They were diluted in artificial perilymph (AP) or culture media. During each step of preparation, we limited the exposure of drugs to light to avoid any degradation of active components. The composition of artificial perilymph (AP) was: 137 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 1 mM $NaHCO_3$, 11 mM glucose, pH 7.4, osmolarity 304 ± 4.5 mosm/l.

In vitro experiments

Assessment of cochlear fibrosis in cultured cochlear slices. When cochlear slices are cultured over 5 days, cochlear fibrosis develops spontaneously within the scalae. Therefore, we used this model to investigate the proliferative mechanisms of cochlear fibrosis *in vitro.*

Cochlear slice preparation. After an intra-peritoneal overdose injection of sodium pentobarbital, neonatal Wistar rats (P_1-P_2) were decapitated. The half-head was