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Otitis Media: Molecular Impact of Inflammation in the Middle and Inner Ear: Cytokines, Steroids, and Ion Homeostasis

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Abstract

An overview of recent topics under investigation in our laboratory is presented. Topics include the cytokine and ion homeostasis gene response to steroids in the middle ear in a murine model of otitis media and gene chip analysis of inner ear genes affected by trans-tympanic and systemic steroid administration to the mouse.

Keywords

otitis media; mouse model; inflammation; ion homeostasis

Introduction

Several questions have been investigated in our lab over the last few years using a mouse model to study both acute otitis media (Balb/c) and chronic otitis media (C3H/HeJ) effects on the middle and inner ear. We know that inflammation in the middle ear affects the inner ear as well (1,2) and can affect hearing. Steroids are often given either systemically, trans-tympanically or topically for several clinical scenarios. At the gene level, how do steroids work in the middle ear (ME) and inner ear (IE)? Is their impact on genes purely anti-inflammatory? At the gene level, what happens when we give trans-tympanic (TT) and systemic steroids after establishing an inflammatory response in the mouse ME? We know from previous work that TT steroids decrease ME fluid and inflammation *histologically* in mice (3).

How is fluid regulated in the ME? Traditionally, Eustachian tube dysfunction was thought to be the primary issue in persistent middle ear fluid. What genes are in action in these processes? Could ion homeostasis genes play a role in fluid regulation not only in the inner ear, but also in the middle ear in the setting of inflammation?

Methods

Inflammation is studied in the mouse middle ear by inducing an inflammatory response in the Balb/c mouse with TT injection of heat-killed bacteria as previously published (3). The middle ear tissues are then dissected and RNA extracted for qRT-PCR. We use custom gene array plates to quantitatively measure with RT-PCR the following cytokine genes: MIP-2 (Cxcl2), MIP-1a (CCL3), KC (Cxc11) = chemokines; IL-1A, IL-1B, IL-6 (pro-inflammatory interleukins); IL-10 (anti-inflammatory interleukin); TNF (tumor necrosis factor). We also

measure the following ion homeostasis genes: isoforms of Na⁺,K⁺-ATPase ; K⁺, Na⁺, Cl⁻ transport channels; gap junction connexins; water transporting aquaporins; tight junction claudins. We selected these ion homeostasis genes for their known importance in the function of the inner ear and potential role in control of middle ear fluids during inflammation.

Affymetrix® gene chip technology was also used to study gene response to steroids in the inner ear. Mice were studied after receiving prednisolone, dexamethasone or aldosterone trans-tympanically to the middle ear and after receiving one of the three steroids systemically. Analysis was performed to see how many genes were upregulated, downregulated in inner ear tissues and a comparison was made between systemic and trans-tympanic delivery.

Results

Previous work has shown that surprisingly, IH genes in the ME are downregulated in the setting of otitis (acute and chronic) (4). This effect on ion homeostasis genes early in inflammation may partially contribute to otitis media with effusion and other long standing middle ear fluid disorders. Unlike the middle ear response, ion homeostasis genes in the inner ear are upregulated in otitis (C3H) (unpublished data).

Steroid Impact on Ion homeostasis genes in the Middle Ear in Murine Otitis Media

Trans-tympanic steroids given after bacteria to the mouse ME, cause downregulation of all pro-inflammatory cytokines and upregulation of IL-10. This effect was seen both with prednisolone and aldosterone (unpublished data). Aldosterone, and to a lesser degree prednisolone, cause upregulation of ion homeostasis genes in the middle ear. Histology confirmed that aldosterone and prednisolone both caused a decrease in ME fluid and cellular infiltrate (statistically significant).

Gene Chip Analysis Inner Ear Genes

There are about 22,000 genes in the mouse genome. About 17,000 of these are found in the mouse inner ear. Far more genes were up- or down-regulated with trans-tympanic steroid injection to the mouse middle ear than with systemic dosing. Not only were more genes affected by steroids given trans-tympanically, but different genes were affected when given systemically compared to trans-tympanically.

Discussion

Steroids appear to work both as anti-inflammatory mediators and by fluid regulation via the mineralocorticoid properties of the mixed steroids (Prednisone has both gluco-corticoid and mineralo-corticoid properties) (3, 5). In the case of aldosterone, perhaps faster fluid clearance washes out the inflammatory exudate, thereby decreasing the overall inflammatory response.

Conclusion

We can no longer ascribe the effect of steroids to a pure anti-inflammatory action. Our work also confirms that TT delivery of steroids activates more genes than systemic, confirming the clinical evidence that local delivery of steroids is more effective. In an effort to protect hearing, novel interventions for ME and IE disorders can hopefully be investigated as we sort out which genes regulate the response to inflammation and the gene response to steroids in both the glucocorticoid and mineralocorticoid classes. The potential use of topical aldosterone merits further investigation.

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