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Effect of Systemic Lipopolysaccharide-Induced Inflammation on Cytokine Levels in the Murine Cochlea: A Pilot Study

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Abstract

The objective was to detect changes in cytokine expression within cochleae in a murine model of systemic inflammation, with or without aminoglycoside exposure. Four groups of mice received 1 of the following: saline only, systemic bacterial lipopolysaccharides (LPS) for 6 hours to induce endotoxemia and inflammatory responses, systemic gentamicin for 3 hours, or both treatments. After exsanguination, pooled cochleae (4/group) were processed for enzyme-linked immunosorbent assay (ELISA) for 16 cytokines. Gentamicin alone did not change cochlear cytokine levels, while LPS ($\pm$ gentamicin) substantially elevated cochlear expression of several cytokines, particularly interleukin-1 α , interleukin-6, monocyte chemotactic protein-1, macrophage inflammatory protein-1 α , and RANTES. Since cytokines increase blood-brain barrier permeability, we hypothesize that cytokine-enhanced permeability of the blood-labyrinth barrier (BLB) could potentiate aminoglycoside-induced ototoxicity. This pilot study demonstrated the feasibility of detecting cytokine expression in murine cochleae using ELISA and facilitates future studies investigating BLB permeability in animal models of systemic inflammation.

Keywords

aminoglycosides; cochlear cytokines; sepsis; systemic inflammation; ototoxicity

Introduction

Aminoglycosides like gentamicin are life-saving antibiotics, particularly for severe systemic bacterial sepsis. However, aminoglycoside therapy carries the risk of irreversible ototoxicity. Sepsis induces inflammatory responses that include the release of cytokines that can

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Author Contributions

Lourdes Quintanilla-Dieck, conception and design, acquisition of data, analysis and interpretation of data, drafting and revising the article; **Barbara Larrain**, acquisition of data, analysis and interpretation of data; **Dennis Trune**, conception and design, analysis and interpretation of data, drafting and revising the article; **Peter S. Steyger**, conception and design, analysis and interpretation of data, drafting and revising the article.

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modulate the permeability of the blood-brain barrier.¹ These inflammatory-mediated cytokines could also affect the permeability of the blood-labyrinth barrier (BLB) to nutrients and drugs.² The goal of this pilot study was to determine the feasibility of detecting changes in cochlear levels of cytokines in a murine model of sepsis using bacterial lipopolysaccharide (LPS)-induced endotoxemia and subsequent inflammation with multiplex enzyme-linked immunosorbent assays (ELISA).

Methods

Eight C57/B6 mice (28-42 days old) were divided into 4 groups, (a) vehicle controls receiving an intravenous (IV) tail injection of saline at 0 hours, then an intraperitoneal (IP) injection of saline at 3 hours; at the same time points, (b) received saline IV followed by gentamicin (300 mg/kg, a nonlethal dose³) IP; (c) received LPS (1 mg/kg) IV, followed by saline IP; or (d) received both LPS and gentamicin. At 6 hours, mice were anesthetized and transcardially perfused with phosphate-buffered saline (PBS). Excised cochleae from each group were pooled, washed with PBS, immersed in a dithiothreitol and protease inhibitor cocktail, and homogenized using a BioMasher kit (Diagnocine, Hackensack, New Jersey). After centrifugation at 4°C, the 4 supernatant samples were sent to Quansys Biosciences for multiplex cytokine screen analysis (110451MS). Oregon Health & Science University's Institutional Animal Care and Use Committee approved this study.

Results

Substantial elevation in specific cochlear cytokines was observed in mice treated with LPS, with or without gentamicin treatment (**Figure 1**), particularly interleukin-1 α (IL-1 α), interleukin-6 (IL-6), monocyte chemotactic protein-1 (MCP-1), macrophage inflammatory protein-1 α (MIP-1 α), and RANTES (**Table 1, Figure 2**). Gentamicin did not induce substantive changes in cytokine levels. Due to the small sample size, no statistical analyses were performed.

Discussion

These results show substantial changes in cochlear levels of selected cytokines in mice 6 hours after administration of LPS at 1 mg/kg, a dose that increased cochlear uptake of aminoglycosides without modulating drug plasma levels (unpublished data). This dose can also decrease expression of several renal transporters/channels, but does not induce arterial hypotension or renal ischemia.⁴ Gentamicin, at a nonlethal 300 mg/kg dose,³ did not affect cochlear cytokine levels within 3 hours.

Cytokines are intricately involved in the pathophysiology of any inflammatory response, and bacterial sepsis is no exception. Cytokines like IL-1 β , IL-6, and TNF- α levels are all substantially increased in plasma at various time points during sepsis in humans and after LPS-induced inflammation as a model for sepsis in mice. In our study, 5 cytokines, IL-1 α , IL-6, MCP-1, MIP-1 α , and RANTES, were all substantially elevated in cochlear tissues 6 hours after systemic LPS injection. Although this study looked at *one time point*, 6 hours after LPS injection, it is likely that some or all cytokines will peak at other time points. A comparison between plasma and cochlear levels of cytokines over time is needed.

In this study, the most upregulated cytokines are primarily involved in cell-mediated immunity and cellular transmigration across endothelial barriers, particularly IL-1, IL-6, and MIP-1 α . Cytokines that promote transepithelial migration may also alter the permeability of the BLB. Further research into the effect of these cytokines on the cochlear BLB will

provide new insight into changes in BLB physiology, molecular trafficking, and the stria microenvironment during systemic inflammation and sepsis.

We propose that systemic inflammation, and sepsis, induce changes in cochlear cytokine levels that alter BLB permeability and the entry of ions, nutrients, and ototoxins into inner ear tissues. These pilot data demonstrate the feasibility in detecting substantial upregulation of specific cytokines in murine cochleae during LPS-induced endotoxemia and inflammation. The data suggest that the enhanced cochlear uptake of aminoglycosides during LPS-induced endotoxemia and inflammation might be modulated by inflammatory-mediated expression of cytokines that can regulate the permeability of the BBB,¹ and likely also the BLB. This assumption needs to be tested in subsequent experiments.

There may be 2 cytokine-related phenomena occurring to regulate the permeability of the BLB: cytokine production within the cochlea and/or cytokine entry across the BLB from serum into the cochlea. Further studies, with larger sample size for statistical power, are needed to distinguish between the 2 phenomena. We can speculate on the potential effect of cytokines on the BLB. An inflammatory response could cause breakdown of tight junctions between stria endothelial cells, leading to paracellular influx of plasma into the stria vascularis, including drugs and proteins into the cochlea. Alternatively, cytokines may affect BLB physiology and transcellular trafficking across endothelial cells to account for the observed increase in cochlear uptake of aminoglycosides across the BLB. We hypothesize that inflammatory-mediated upregulation of cytokines, in serum or in the cochlea, increases cochlear BLB permeability to aminoglycosides and subsequent ototoxicity. Ongoing experiments will test this hypothesis.

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Figure 1.
Cochlear cytokine protein levels for all 4 treatment groups in log scale for each cytokine tested.

Most significant results from protein expression analysis

Figure 2.

Bar graph for selected cytokines with substantial elevations in cochlear protein levels in log scale.

Table 1

Cytokine levels for each treatment group, for the same cytokines as Figure 2.

	IL-1 α (pg/mL)	IL-1 β (pg/mL)	IL-6 (pg/mL)	MCP-1 (pg/mL)	MIP-1 α (pg/mL)	RANTES (pg/mL)
Controls	6.73	19.64	3.35	2.00	5.58	12.20
Gentamicin only	7.11	34.01	3.55	6.57	9.19	17.64
LPS only	351.59	54.48	1826.78	450.27	303.58	623.52
LPS and gentamicin	278.37	37.49	1632.38	375.55	237.40	491.58

Abbreviations: IL-1 α , interleukin-1 α ; IL-1 β , interleukin-1 β ; IL-6, interleukin-6; MCP-1, monocyte chemotactic protein-1; MIP-1 α , macrophage inflammatory protein-1 α ; LPS, lipopolysaccharide.