Bactericidal/Permeability-Increasing Protein Prevents Mucosal Damage in an Experimental Rat Model of Chronic Otitis Media with Effusion

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Received 7 September 1999/Returned for modification 15 November 1999/Accepted 27 January 2000

In this study, the efficacy of bactericidal/permeability-increasing protein (BPI) was assessed in a rat model of chronic otitis media with effusion. BPI injection prevented disturbance of the mucociliary clearance system of the middle ear. Hence, it is postulated that BPI can be a new therapy for chronic otitis media with effusion.

Chronic otitis media with effusion (OME) is a frequent disease during childhood, and its complications and sequelae often persist into the adult years (7). Two important factors in the development of OME are obstruction or dysfunction of the eustachian tube and bacterial infection. In chronic OME, the majority of cases are caused by gram-negative bacteria (GNB), whereas in acute OME gram-positive bacteria are also frequently isolated (1). Lipopolysaccharide (LPS) is a component of GNB. LPS alone has been shown to induce mucosal inflammation with accumulation of effusion in the middle ears of chinchillas (2) and guinea pigs (14). Furthermore, LPS has been detected in human middle ear effusions (3), and the level of it was found to be significantly higher in children with chronic OME than in children with acute OME (13). Finally, LPS is also thought to be cytotoxic to ciliated epithelial cells (10).

We recently developed an animal model of chronic OME using a combination of eustachian tube obstruction (ETO) and LPS injection (12). This procedure induces an increase in secretory cells of the epithelium and degeneration of cilia, which results in a disturbance of the mucociliary clearance system (MCS) of the middle ear. Comparable mucosal changes have been observed in humans with chronic OME (9, 15). The MCS is considered to be an important defense system of the middle ear cavity, and disturbance of this system is suspected to be an important factor in the development of chronic OME.

Bactericidal/permeability-increasing protein (BPI), a 55-kDa cationic protein present in the granules of polymorphonuclear neutrophils (PMNs), is an antimicrobial protein that has been implicated in the host defensive response to GNB infection (5). Furthermore, in humans, rBPI21 appears to be safe and non-immunogenic and is in phase II/III clinical trials with apparent therapeutic benefit (4, 6, 8). In the present study, we aimed to assess the in vivo capacity of rBPI21 to prevent mucosal damage in chronic OME.

**Induction of chronic OME.** During anesthesia with nitrous oxide, the eustachian tube was reached by a ventral approach, medially to the posterior belly of the digastric muscle, and obstructed by plugging a small piece of Gelfoam (Upjohn Co.) into the tube. Moreover, a drop of tissue glue (Historesin; Braun, Melsungen, Germany) was used to keep the Gelfoam in the tube. In addition, 50 μl of LPS solution (2 μg/ml) in phosphate-buffered saline (PBS) prepared from Salmonella typhimurium (L-6511; Sigma, Zwijndrecht, The Netherlands) was injected through the tympanic membrane. As a control, the other ear was injected with 50 μl of PBS.

After 1, 2, 4, and 12 weeks, the animals were killed with CO2 gas and subsequently decapitated. The middle ear was dissected from the skull, denuded of adhering tissues, and further processed for light microscopy (LM) and scanning electron microscopy (SEM). For LM the specimens were fixed, decalcified, subsequently dehydrated in a graded series of ethanol, and embedded in glycol methacrylate (JB4; Brunschwig Chemie, Amsterdam, The Netherlands). Sections were stained with toluidine blue for histological studies and with alcian blue–periodic acid–Schiff for glycogen histochemistry. Specimens for SEM analysis were fixed, dehydrated in graded series of ethanol, and critical-point dried with liquid CO2. The distribution of the epithelial cells was studied with a Philips 525M scanning electron microscope after specimens were mounted and coated with gold in a Balzers MED010 Sputtercoater. The absolute numbers of ciliated and secretory cells were counted in duplicate in each ear in two standardized areas of the same size in the tympanic orifice. Statistical comparisons were made by the Tukey highest significant difference (HSD) test, with P < 0.05 considered significant, using the Statistical Package for the Social Sciences.

**Histopathological findings.** By LM and SEM, control middle ears remained apparently normal during the whole period. The hypopyonpanum of the middle ear consisted of thin, one-layered squamous epithelium, containing very few ciliated cells (Fig. 1A). In the tympanic orifice of the eustachian tube, a more pseudostratified, cuboidal, or cylindrical epithelium was observed, which contained an abundant number of ciliated cells and few secretory cells (Fig. 2A). This part represents the mucociliary clearance system of the middle ear.

The combination of ETO and LPS injection induced thickening of the middle ear mucosa due to vasodilatation, edema, and infiltration by PMNs, macrophages, and lymphocytes in the hypopyonpanum (Fig. 1B). Compared with control ears after PBS injection, significantly fewer ciliated cells were ob-
served in the tympanic orifice of ETO plus LPS-treated ears (Fig. 3), and severely swollen squamous epithelium was observed by SEM after 3 months (Fig. 2B). Furthermore, a significant increase in secretory cells was observed (Fig. 4). It is clear from these histopathological findings that the MCS was disturbed. Due to hyperproliferation of secretory cells, increased mucus production induced mucoid middle ear effusion. Dysfunction or degeneration of ciliated epithelial cells was responsible for accumulation of surplus fluid in the tympanic cavity. Therefore, clearance of the middle ear was seriously impaired.

**Treatment with BPI.** Two days after induction of chronic OME, 50 µl of 2-mg/ml rBPI21, a recombinant 21-kDa amino-terminal fragment of BPI (Xoma LLC., Berkeley, Calif.), was injected into the middle ear cavity. To ensure complete neutralization of LPS, an amount of rBPI21 equal to 1,000 times the LPS concentration was used (11). It was decided to inject the BPI after 2 days to investigate the effect of BPI during an acute inflammation reaction for prevention of chronic OME. Injection of BPI prevented the thickening of the middle ear mucosa in the hypotympanum seen after ETO in combination with LPS injection. No infiltration of inflamma-

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**FIG. 1.** Light micrographs of the hypotympanum at 12 weeks after PBS injection (A), ETO and LPS injection (B), and ETO plus LPS with rBPI21 injection after 2 days (C). OME induction caused thickening of the mucosa (between arrows) and infiltration of inflammatory cells (arrowheads). This was not observed after rBPI21 injection. Magnification, ×200; bar, 10 µm.

**FIG. 2.** SEM of the tympanic orifice at 12 weeks after (A) PBS injection, (B) ETO and LPS injection, and (C) ETO plus LPS with rBPI21 injection after 2 days. Abundant goblet cells (arrows) and ciliated cells are present. OME induction caused degeneration of cilia and swollen epithelial cells (asterisks). This was not observed after rBPI21 injection, and the cilia had a normal appearance. Magnification, ×1,250; bar, 10 µm.
rBPI21 after OME induction significantly inhibited the increase in the numbers of ciliated cells in the subepithelial layer was observed (Fig. 1C). In the tympanic orifice, an abundance of ciliated cells were present and no significant increase in secretory cells was seen (Fig. 3 and 4). BPI prevented the induction of mucosal changes partly by a grant from the Heinsius Houbolt Foundation.

REFERENCES

Editor: J. D. Clements