Otolaryngology–Head and Neck Surgery (2005) 133, 206-210

Penicillin Reduces Eustachian Tube Gland Tissue Changes in Acute Otitis Media

Henrik Andersen, MD, Jens Thomsen, MD, DMSC, and
Per Cayé-Thomasen, MD, DMSC, Copenhagen, Denmark

OBJECTIVE: The volume of the mucous paratubal glands and the number of the mucus-producing goblet cells in the middle ear and Eustachian tube (ET) are increased after experimental acute otitis media (AOM). The present investigation examines a potential effect of penicillin on the changes in goblet cell density and gland structures of the ET during and after AOM.

STUDY DESIGN: Middle ear inoculation of *Streptococcus pneumoniae* in 50 rats. Two days later, 25 rats were given penicillin V as one daily dose for 5 days. Twenty-five rats received no treatment. Five animals from each group were sacrificed on days 4, 8, 16, 90, and 180. The ET was dissected and decalcified, followed by paraffin embedding, serial transverse sectioning, and PAS/alcian blue staining. The goblet cell density and the paratubal gland composition and volume were determined in every 20th section, using a light microscope.

RESULTS: Penicillin reduced the increase of goblet cell density from day 8 and through 6 months, whereas the increase of the paratubal mucous gland volume was unaffected by treatment.

CONCLUSION: We conclude that penicillin reduces the increase of ET goblet cell density during and after acute otitis media, whereas the paratubal gland volume remains unaffected. An increased mucosal secretory capacity and indicated excessive secretion of mucus may contribute to the deteriorated ET function found after AOM and thus predispose, sustain, or aggravate middle ear disease. This may be prevented by penicillin treatment.

Antibiotic treatment of acute otitis media (AOM) remains internationally controversial, due in part to the scarcity of clinical trials without important methodological flaws.[1] Although modest, a clinical effect of antibiotic treatment has been reported by comprehensive literature reviews.[2,3] However, clinical trials have not been able to document an effect in regard to prevention of a subsequent development of secretory otitis media, which as a histopathological hallmark is characterized by an increased secretory capacity of the middle ear mucosa, ie, increased goblet cell density and gland formation.[4]

Most experimental studies on clinical features and histopathological changes in AOM have favored antibiotic administration,[5-8] while some have been unable to document a positive effect.[9,10] The increase of middle ear secretory capacity following AOM is clearly reduced by treatment,[7] implicating prevention of a subsequent development of secretory otitis media. However, an effect on the increase of Eustachian tube goblet cell density[11] and paratubal mucous gland volume[12] is unaccounted for and seems an essential issue, considering the pivotal role of Eustachian tube patency and function in middle ear disease.

This study report on the effect of antibiotic treatment on the increase of Eustachian tube goblet cell density and paratubal mucous gland volume encountered in AOM, employing a well-established rat model.

MATERIAL AND METHODS

The Rat Model

A well-established model of acute suppurative otitis media was employed,[13] using 50 male Sprague-Dawley rats, weighing 200 to 250 g. They were kept under standard laboratory conditions and given food (pellets) and water ad libitum. During anesthesia, induced by a short-acting barbiturate (methohexital; Brietal, Lilly, Sweden) administered through a tail vein, a ventral midline incision was made in the neck. The right tympanic bulla was exposed and the

From the Department of Oto-rhino-laryngology, Head and Neck Surgery, Gentofte University Hospital of Copenhagen.

Reprint requests: Per Cayé-Thomasen, MD, DMSc, Department of Oto-rhino-laryngology, Head and Neck Surgery, Gentofte University Hospital of Copenhagen, DK-2900 Hellerup, Denmark.

E-mail address: peca@gentoftehosp.kbhamt.dk.

0194-5998/$30.00
doi:10.1016/j.otohns.2005.02.020

middle ear inoculated through the bony bulla wall with 0.05 mL of a suspension of log-phase type 3 *Streptococcus pneumoniae*, sensitive to penicillin V, prepared as previously described.[13] The surgical procedure was performed under aseptic conditions. The core of the pneumococcal suspension was determined as 5 × 106 colony-forming units (CFU)/mL. The number of CFU was chosen according to prior postinoculation observations of otomicroscopical resemblance to findings in acute otitis media in children.[13] Two days after inoculation, 25 rats were given penicillin V 100 mg/kg body weight orally, as one daily dose for 5 days. To ensure established infection all rats were examined by otomicroscopy on day 4. Previous studies had proven 100% positive cultures of effusion samples on day 4.[13] The left tympanic bulla of rats from the untreated group was sham-inoculated suspension medium only, serving as control. On days 4, 8, 16, 90, and 180, five rats from the treated group and five rats from the untreated group were randomly selected, anesthetized, and sacrificed by perfusion fixation with formol-alcohol through the left cardiac ventricle. The perfusion lasted 10 minutes, after which the rats were decapitated and the head transferred into a vial for postfixation in the same fixative for at least 1 week. All rats were examined by otomicroscopy on day 4 to ensure established infection and rats sacrificed at 3 and 6 months were examined otomicroscopically every 14 days to rule out spontaneous infection.

Preparation for Microscopy

The middle ear bulla and Eustachian tube were removed from the cranium, followed by opening of the middle ear and micro-dissection of middle ear mucosa, which was used for other studies. The remaining Eustachian tube and bony middle ear bulla were decalcified by submersion in 18.41% formic acid for 1 week, followed by separation of the Eustachian tube from the middle ear by a scalpel transcision preserving the tympanic orificium in the Eustachian tube specimen, which was embedded in paraffin. Serial 5- to 10-micrometer sectioning was performed perpendicular to the longitudinal axis of the Eustachian tube.

Microscopical Methods

Morphologic characteristics, as well as the type and stainability of the tubal glands, were determined by light microscopy of every 20th section, stained with periodic acid-Schiff (PAS)/alcian blue. The density of tubal mucous and serous gland acini were determined by counting all such structures in every stained section, using magnification ×200. The overall and sub-type gland volume were determined by counting the number of grid cross-points positioned in a gland acinus, using an ocular with a grid and magnification ×100, and with a fixed position of the microscopic field. Distance between grid cross-points was 100 micrometers, when using magnification ×100. The goblet cell density was determined by counting all goblet cells in the Eustachian tube epithelium of every stained section. Using an ocular with a grid, the length of the epithelial surface was measured and the number of goblet cells per millimeter epithelium subsequently determined. The individual and inter-individual median gland volume, number of gland acini, and number of goblet cells per millimeter epithelium in each of 5 anatomical localizations were determined. The rat Eustachian tube has a length of approximately 4 mm, yielding 25 to 30 stained and investigated sections from each tube preparation. The stained sections were divided into 5 groups depending on anatomical localization: the tympanic orifice, the tympanic third, the mid-, and the pharyngeal third of the Eustachian tube, as well as the pharyngeal orifice (Figure 1). The median numbers from each anatomical localization constituted the basis of statistical analysis of differences between the untreated and treated group. For graphic illustration, the overall mean gland volume and goblet cell density was determined on all days of sacrifice.

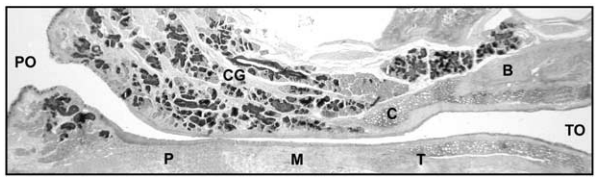

Figure 1 Longitudinal section through the roof (dorsal part) of the rat Eustachian tube, providing an overview of the squamous epithelium lining and the tympanic (TO) and pharyngeal (PO) orifice, as well as the tympanic (T), mid-portion (M), and pharyngeal third (P) of the tube. These 5 localities were the basis of the morphometric quantitation of gland acini, gland composition, and volume, as well as epithelial goblet cell density in normal conditions and during and following untreated and penicillin-treated acute otitis media. B is part of the temporal bone, C is part of the cartilagenous tube frame, and CG is the caudal paratubal gland. PAS/alcian blue, original magnification ×25.

Statistical Methods

The Mann-Whitney test was used as statistical method, with $P < 0.05$ as critical level of significance. Counting was performed "blinded" in regard to treatment or no treatment. Recounting was performed in 30 randomly selected sections, to determine intra-observer counting variability.

Ethics

All animal handling was done as gently as possible and during full anesthesia, to avoid unnecessary stress and pain during procedures. All procedures with living animals were performed in the laboratory of the Department of Oto-rhino-laryngology, at the University Hospital of Lund/Malmö, Sweden, and were approved by the local committee for ethics on animal experiments (Malmö/Lunds djurforsöksetiska nämnd, approval M30-95 and M29-98).