A pilot study to assess the feasibility of pseudomonas aeruginosa and streptococcus pneumoniae infection in the cat.


Department of Otolaryngology, University of Melbourne, The Royal Victorian Eye and Ear Hospital, 32 Gisborne St, East Melbourne 3002, Australia.

ABSTRACT

An experimental model for the induction of otitis media in cats is described using pseudomonas aeruginosa and streptococcus pneumoniae. Until now the cat has been regarded as being resistant to streptococcus pneumoniae infections, whereas pseudomonas aeruginosa is known to cause a most virulent otitis media in this animal. A successful inoculation using streptococcus pneumoniae, however, can be achieved by direct inoculation of a highly concentrated suspension of microorganisms in the bulla, retention of the organisms by Gelfoam*, and enhancement of virulence by intraperitoneal inoculation in mice. The model promises to be an important contribution in studying the effects of pneumococcal otitis media in Cochlear Implants.

INTRODUCTION

The University of Melbourne Cochlear Implant Program is now more than 16 years old, and much of the current success is due to the extensive animal experimentations carried out by this Department over this period of time. The experimental animal being used is the cat. In view of the desire to extend the Cochlear Implant Program to children, it became especially important to be able to demonstrate that the round window seal achieved following cochlear implantation was resistant to spread of infection from the middle ear. This question is particularly vital because of the susceptibility of children to develop recurrent otitis media. Extension of infection to the inner ear can cause loss of ganglion cells and nerve fibres and thus lead to impairment in function of the Cochlear Implant.

A number of models have been described for the experimental induction of otitis media using different organisms, different experimental animals and a variety of methods of inoculation. These models have included transtympanic inoculation (Giebink et al., 1980; Hodges et al., 1984), obstruction of the Eustachian tube (Kowata et al., 1980), and nasal inoculation combined with brief application of negative pressure (Giebink et al., 1980).

In this Department, direct inoculation of the cat bulla has been carried out using group A beta haemolytic streptococci alone (Franz et al., 1985), and in combination with staphylococcus aureus (Clark et al., 1984). However, only a low grade otitis media was achieved and the inoculated organisms could not always be cultured from the bulla at the time of sacrifice a few days later.

In an attempt to improve the intensity of the inflammatory reaction, to create a worst case situation, and to have an animal experiment that is closely related to the situation in the human, a more useful model was sought. The organisms chosen for inoculation were pseudomonas aeruginosa and streptococcus pneumoniae group 2. Pseudomonas aeruginosa was selected as it is regarded as the most virulent cause of otitis media in the cat and often proceeds to otitis interna (Sullivan, 1984). This organism was expected to successfully create an otitis media in the cat. Streptococcus pneumoniae accounts for over one third of all cases of acute bacterial otitis media (Rohn et al., 1972; Klein, 1960).

The virulence of pseudomonas aeruginosa is due to the elaboration of a number of exotoxins. In contrast streptococcus pneumoniae does not elaborate any toxins but possesses a type specific polysaccharide capsule which neutralizes the antibody before it can bind to the microorganism.

Cats are regarded as being resistant to pneumococcal infection (Davis et al., 1980), and experimentally induced otitis media has not been previously described in cats, although it has been described in a number of other animals (Hodges et al., 1984). Based on our past experience with streptococci and staphylococci (Franz et al., 1985; Cranswick, 1985; Clark et al., 1984) we felt encouraged to create a new model in our experimental animal using pseu-
Pseudomonas aeruginosa and streptococcus pneumoniae for inoculation.

METHOD

Two healthy adult cats were used in this study. Surgery was performed under general anesthesia and strict aseptic conditions. One animal was bilaterally inoculated with streptococcus pneumoniae group 2 and one with pseudomonas aeruginosa. The inoculation technique was a simple procedure. A suspension of $10^6$ microorganisms was directly introduced into the surgically opened bulla which had been packed with Gelfoam to retain the bacteria and prevent expulsion through the Eustachian tube. Antibiotics were withheld after the inoculation. In order to ensure a successful infection with pneumococci, the virulence of this organism was enhanced by intraperitoneal inoculation in mice. The mice were sacrificed two days later and the spleens were cultured yielding the more virulent pneumococci.

RESULTS

Pseudomonas aeruginosa and streptococcus pneumoniae group 2 were cultured ten days after inoculation. Histologically streptococcus pneumoniae infection caused a chronic proliferative inflammation of the bulla mucosa and the round window membrane (Fig. 1). Inflammation with pneumococcus was characterized by an exudate and a cellular infiltrate of polymorphs, lymphocytes, monocytes and fibroblasts. The round window membrane was thickened and showed the presence of secretory cells. In the inner ear a mild hydrops of the cochlear duct and collapse of Claudius and Hensen cells, atrophy of the stria vascularis, mild fibrous precipitates in the perilymphatic space, blood congestion and mild ganglion cell losses throughout the cochlea were seen.

Histology of the pseudomonas infection differed a little from that of the pneumococcal infection. The mucosa of the bulla showed a chronic proliferative inflammation, however, the round window membrane did not show the same degree of inflammation (Fig. 2). Here a non-proliferative inflammation was present with resolution in some areas of the membrane. Despite these minor changes in the round window membrane, when compared with the pneumococcus infection, more severe changes were found in the cochlea (Fig. 3). The cochlear duct was collapsed, the Reissner's membrane fused with the atrophic stria vascularis. The organ of Corti with its hair cells and supporting elements was absent not only in the basal turn but throughout the cochlea. However, mild ganglion cell degeneration was present mainly in the basal portion of the cochlea. The cochlear aqueduct appeared closed with thick fibrous tissue, and at its entrance new bone formation could be seen.

DISCUSSION

Pneumococcus otitis media prevails in our younger population and an animal model had to be established to study the effects of infection in implanted cochleas prior to considering children for implantation. Despite the assumption that streptococcus pneumoniae would not create infection in the cat, this pilot study has shown that infection can be achieved as demonstrated...
in these cochleas. The high concentration of organisms directly inoculated into the bulla, their retention by Gelfoam within the bulla, and the enhancement of their virulence by intraperitoneal inoculation of mice are regarded as the keys to a successful streptococcus pneumoniae infection in the cat.

A successful inoculation with pseudomonas aeruginosa was expected in the cat and our results confirm previous findings in this animal (Sullivan, 1984). Although pseudomonas aeruginosa is not regarded as important as streptococcus pneumoniae, it appears worthy knowing that pseudomonas aeruginosa is in fourth place as a cause of middle ear infection in children in Europe (Fleischer, 1979).

Acute middle ear infection caused by pseudomonas aeruginosa is regarded as more damaging than by streptococcus pneumoniae (Fleischer, 1979). These findings are in agreement with the presented animal model. Thus more extensive ganglion cell losses, nerve fiber losses and destructions of the organ of Corti were found in pseudomonas aeruginosa infections. Apart from playing a more damaging role in acute otitis media, pseudomonas aeruginosa plays a dominant role in recurrent chronic middle ear infections, and is a common organism found in cholesteatoma.

The animal model presented here has established that both organisms are pathogenic to the inner ear of the cat. This finding has to be taken into account when implanted cat cochleas are to be evaluated especially in the attempt to separate the effects of trauma, infection or the combination of both. Although the number of animals in this pilot study was restricted the histological findings were confirmed in an extended investigation of this model (Berkowitz et al., 1985).

CONCLUSIONS

Experimental otitis media in the cat using pseudomonas aeruginosa and streptococcus pneumoniae can be achieved by direct inoculation of a highly concentrated suspension of microorganisms in the bulla; retention of the organisms with Gelfoam and virulence enhancement of the pneumococcus by intraperitoneal inoculation in mice.

ACKNOWLEDGEMENT

This study was funded by the Deafness Foundation of Victoria, The Channel 10 Deafness Appeal and the National Health and Medical Research Council of Australia. We would like to thank Dr. Robbins-Browne, Department of Microbiology, University of Melbourne, Dr. P. Cole, Veterinary Research Department, C.S.I.R.O., and Dr. N. Sullivan, Faculty of Veterinary Science, University of Melbourne, for their helpful advice and the supply of bacteria used in this study.

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Author/s:
Berkowitz, R. G.; Franz, B. K-H.; Shepherd, R. K; Clark, Graeme M.; Bloom, D.

Title:
Cochlear implant and otitis media: a pilot study to assess the feasibility of pseudomonas aeruginosa and streptococcus pneumoniae infection in the cat

Date:
1984/85

Citation:

Persistent Link:
http://hdl.handle.net/11343/27209

File Description:
Cochlear implant and otitis media: a pilot study to assess the feasibility of pseudomonas aeruginosa and streptococcus pneumoniae infection in the cat