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Hearing loss and cochlear damage in experimental pneumococcal meningitis, with special reference to the role of neutrophil granulocytes

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Hearing loss is a well-known sequelae from meningitis, affecting up to 25% of survivors. However, the principal components of the infectious and inflammatory reaction responsible for the sensorineural hearing loss remain to be identified.

The present study aimed to investigate the impact of an augmented neutrophil response on the development of hearing loss and cochlear damage in a model of experimental pneumococcal meningitis in rats.

Hearing loss and cochlear damage were assessed by distortion product oto-acoustic emissions (DPOAE), auditory brainstem response (ABR) and histopathology in rats treated with ceftriaxone 28 h after infection. Rats were treated with Granulocyte Colony Stimulating Factor (G-CSF) initiated prior to infection, 28 h after infection or with ceftriaxone only. Rats were followed for 7 days, and assessment of hearing was performed before infection and 24 h and day 8 after infection.

Pretreatment with G-CSF increased hearing loss 24 h after infection and on day 8 compared to untreated rats (Mann-Whitney, $P = 0.012$ and $P = 0.013$ respectively). The increased sensorineural hearing loss at day 8 was associated with significantly decreased spiral ganglion cell counts ($P = 0.0006$), increased damage to the organ of Corti ($P = 0.007$), increased areas of inflammatory infiltrates ($P = 0.02$) and increased white blood cell (WBC) counts in cerebrospinal fluid on day 8 after infection ($P = 0.0084$). Initiation of G-CSF 28 h after infection did not significantly affect hearing loss or cochlear pathology compared to controls.

In conclusion, the inflammatory host reaction contributes significantly to the development of hearing loss in experimental meningitis.

Keywords: Experimental meningitis; Hearing loss; Oto-acoustic emissions; Inflammation; Neutrophil granulocyte; Cochlear pathology

Introduction

Hearing loss is the most common sequelae from bacterial meningitis with up to 25% of survivors suffering from a uni- or bilateral hearing loss ranging from partial hearing loss to deafness (Yeat et al., 1997; de Gans and van de Beek, 2002; Molyneux et al., 2002; Rasmussen et al., 1991; Ostergaard et al., 2005). Hearing loss affects quality of life and is associated with both social and learning disabilities. The development of hearing aids and cochlear implants has reduced some of the severe consequences of post-meningitis hearing loss although destruction of the inner ear followed by tissue remodeling and bone formation may compromise the efficacy of these applications (Baldwin et al., 1985; Callanan and Poje, 2004; Karino et al., 2004).

The pathway of which the infection and inflammatory infiltration spreads from the meninges to the inner ear and even the middle ear has been debated, although the communication between cerebrospinal fluid (CSF) and the cochlear perilymphatic space through the cochlear aqueduct is the obvious route (Kastenbauer et al., 2001; Klein et al., 2003b; Kay, 1991; Kesser et al., 1999). Experimental studies of hearing loss in pneumococcal meningitis have described meningeal inflammatory infiltrates extending to the cochlear perilymphatic space—most prominent at the base of the cochlea (Kesser et al., 1999; Bhatt et al., 1993; Klein et al., 2003b). Additional spreading of infection to the cochlea from other sites, i.e., bloodstream has also been suggested (Kastenbauer et al., 2001; Klein et al., 2003b; Kay, 1991; Kesser et al., 1999). This is in agreement with the observation of inflammation in the endolymphatic space which is separated from the perilymphatic space by Reissner's membrane (Kay, 1991).

This presence of neutrophil granulocytes and pneumococci in the cochlea has been suggested to be mediators of injury to the

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inner and outer hair cells, leading to a loss of hearing. Experimentally, the development of hearing loss has been shown to progress as CSF inflammation increases (Bhatt et al., 1993) and the scavenging of reactive nitrogen species (RNSs) and reactive oxygen radicals (ROIs) shown to reduce hearing loss in experimental pneumococcal meningitis (Kastenbauer et al., 2001; Klein et al., 2003a; Ge et al., 2004; Winter et al., 1997). Also, bacterial endotoxin mediated loss of outer and inner hair cells has been shown to be diminished by anti-inflammatory treatment with dexamethasone (Tarlow et al., 1991). However, the contribution of the individual components of the meningeal infection to the development of hearing loss remains to be clarified.

On this background, the present study aimed to investigate experimentally the contribution of leukocytes on hearing loss and cochlear pathology during pneumococcal meningitis. To study the effects of an altered systemic and local inflammatory response, we administered the cytokine hormone Granulocyte Colony Stimulating Factor (G-CSF), which stimulates bone marrow production and release of neutrophil granulocytes and further boosts the antibacterial capacities of these cells (Roilides et al., 1991). The full hearing frequency spectrum was investigated, since hearing loss may vary accordingly to frequencies as related to the anatomical distribution of the cochlear infiltration during meningitis.

Materials and methods

Animals

The experimental protocol was approved by the Danish Animal Inspectorate (Dyreforsoegstilsynet) and based on a well-described model of pneumococcal meningitis (Kastenbauer et al., 2001; Brandt et al., 2004). Young adult male Wistar rats were used for the meningitis experiments. Normal day/night cycles and free access to food and water were provided.

Experimental study design

Pilot study

A study in 16 infected rats (antibiotic treated $n = 13$, untreated $n = 3$) was performed to determine the time course for the development of a sensorineural hearing loss. Hearing assessment with DPOAE (se details below) was performed before infection and 20 h, 44 h, 6, 7 and 9 days after infection to follow the loss or conversely recovery of hearing. CSF and blood samples were obtained 20 h, 44 h and 68 h after infection.

G-CSF pre- and late treatment study

A total of 66 rats were included in this study. Fifty-four infected rats all treated with ceftriaxone (Rocephalin®, F. Hoffmann la Roche, Basel, Switzerland, 125 mg/kg subcutaneously every 24 h for 6 days) from 28 h after infection were included in the study in the following groups: G-CSF pretreatment group (treatment initiated 2 days prior to infection, $n = 16$), G-CSF late treatment group (treatment initiated 28 h after bacterial inoculation, $n = 16$), and control group ($n = 22$). Twelve other rats were inoculated with saline without bacteria and either treated with G-CSF ($n = 6$) or no additional treatment ($n = 6$). Three days prior to inoculation, assessment of hearing, as described below under Experimental procedures, was performed to ensure normal and equal hearing in rats. Based on results from the pilot study, showing only incipient hearing loss after 20 h and no recovery of hearing between days 7 and 9, hearing assessments in the main experiment were performed at 24 h and on day 8 after infection.

ABR was evaluated at 53 kHz since the pilot study showed great dynamics in this area concerning hearing loss and eventual recovery of hearing.

An acoustic startle response was assessed with 105 and 130 dB SPL "click" sounds every 8 h for the first 72 h after infection, thereafter every 24 h until day 8. Hearing assessment was only performed in 12 controls after 24 h since G-CSF late treated rats could be included as an additional control group for comparisons at 24 h, prior to administration of G-CSF treatment in this group.

Eligibility

Rats surviving to the predetermined times for measurement of DPOAE (20 h, 44 h, days 6, 7 and 9 after infection in the pilot study and 24 h and day 7 days after infection in the main study).

Experimental procedures

Bacterial strain

A *Streptococcus pneumoniae* type 3 strain (68034, Statens Serum Institute (SSI), Copenhagen, Denmark) was used for the experiments. A frozen stock of the bacteria was thawed and grown on 5% blood agar plates (SSI) for 18 h, suspended in beef broth (SSI) and grown to mid-log-phase. The bacteria were washed and centrifuged (3509 × *g*, 4 C° in 10 min) diluted in cold saline to a final concentration of $\sim 1 \times 10^5$ CFU/ml, as confirmed by quantitative cultures.

Infection

On the day of infection, rats were anesthetized with Hypnorm©/Dormicum© diluted in sterile water (1:1:2), 1.3 ml/kg subcutaneously and were infected by intracisternal injection of 30 μl of a bacterial suspension using a 25-gauge butterfly syringe.

Experimental treatments

G-CSF treatment (10 μg/kg × 2 daily subcutaneously) was administered either as a pretreatment initiated 2 days prior to infection for a total of 5 days (G-CSF pretreatment group) or from 28 h after infection for a total of 2 days (G-CSF late treatment group) as previously described (Brandt et al., 2004).

CSF and blood samples

A blood tap was performed before infection, 24 h after, and on day 8. A CSF tap was performed 24 h after infection and on day 8. WBC counts in CSF and blood were measured on an automatic cell counter (Swelab Autocounter AC 920, Swelab Instruments, Sweden) using 20 μl of CSF or blood. Bacterial counts in CSF were determined by plating 10-fold serial dilutions of 20 μl CSF.