Introduction

Despite efficient antibiotic treatment overall mortality of adults suffering from bacterial meningitis has remained unchanged at approximately 25% over the past three decades [5]. Several strategies of adjunctive immunomodulatory therapy have been investigated in bacterial meningitis for decreasing mortality and the frequency of persistent neurological or audiological sequelae [20]. Only dexamethasone administered prior to or at the beginning of antibiotic treatment has gained acceptance in clinical practice for pediatric patients suffering from *Haemophilus influenzae* meningitis [9, 12]. Its use in children and adults with meningitis caused by *Streptococcus pneumoniae* is still controversial. In experimental meningitis dexamethasone has been shown to decrease the entry of β-lactam antibiotics into the CSF [13] and to increase neuronal damage in the dentate gyrus of the hippocampal formation [24]. A recent study conducted under the medical conditions of a developing country found no beneficial effect of dexamethasone on morbidity or mortality of bacterial meningitis [15].

Neuronal and glial damage in bacterial meningitis is caused not only by viable bacteria but also by bacterial cell wall products such as teichoic and lipoteichoic acids, proinflammatory cytokines such as tumor-necrosis factor (TNF) α, interleukin (IL) 1β and 8, free radicals, and excitatory amino acids [20, 21]. The filtration of CSF by filters with the ability to bind proinflammatory cytokines and endotoxin appears a promising approach to modulate subarachnoid space inflammation and reduce neuronal damage. This technique was first successfully used as an adjuvant treatment in critically ill patients suffering from

Abstract The effect of CSF filtration on inflammation and neuronal damage was studied in experimental *Streptococcus pneumoniae* meningitis. New Zealand white rabbits received either antibiotic therapy alone (ceftriaxone i.v., 20 mg/kg bolus, 10 mg/kg maintenance dose; n = 10) or ceftriaxone plus CSF filtration (n = 11) 12 h after intracisternal infection. Immediately after the onset of antibiotic therapy 300 μl cisternal CSF was removed, passed through a miniaturized CSF-1 filter at a constant flow of 20 μl/min, and then reinjected. This procedure was repeated six times at intervals of 20 min. Antibiosis plus CSF filtration caused a transient reduction in CSF bacterial titers and leukocyte counts compared with antibiosis alone (P = 0.04 and 0.02 5 h after initiation of therapy). CSF lipoteichoic acid concentrations were not reduced. The concentration of neuron-specific enolase in CSF and the density of apoptotic neurons in the dentate gyrus were almost equal 12 h after the onset of treatment. Adjuvant CSF filtration accelerated the elimination of viable bacteria from CSF in comparison to antibiotic treatment alone. Parameters of neuronal destruction, however, were not reduced.

Key words Cerebrospinal fluid · Filtration · Meningitis · Immunomodulation · Neuronal damage
polyradiculitis [23], multiple sclerosis [6, 22], and cerebral lupus erythematosus [14]. The filter membrane (CSF-1; Pall, Dreieich, Germany) removes bacteria, endotoxin, TNF-α, IL-6, IL-2, interferon-γ, and C5a from artificial CSF in vitro [16]. For this reason CSF filtration has been used as an adjunct to antibiotic therapy in humans with bacterial meningitis. In these case reports it decreased parameters of inflammation in the subarachnoid space [2, 14], and in one case of S. pneumoniae meningitis clinical improvement was documented in close temporal relationship to CSF filtration [2]. Since S. pneumoniae, the most frequent pathogen in adult bacterial meningitis, often causes neurological sequelae, these reports prompted us to investigate the effect of CSF filtration on subarachnoid space inflammation and parameters of neuronal damage in a rabbit model of pneumococcal meningitis. For this study a miniaturized version of the only filter presently available for CSF filtration in humans was employed.

Materials and methods

Rabbit model of S. pneumoniae meningitis

After intramuscular induction of anesthesia with ketamine (25 mg/kg) and xylazine (5 mg/kg) New Zealand white rabbits (weighing approx. 2.5 kg) were anesthetized with intravenous urethane for the entire duration of the experiment (24 h). Blood was drawn from the ear artery contralateral to the ear vein in which drugs and saline were infused. All animals received the same amount of fluid (160 ml saline/24 h i.v.).

Meningitis was induced by intracisternal injection of 10^6 CFU of a S. pneumoniae type 3 strain originally isolated from a patient with meningitis (minimal inhibitory concentration for ceftriaxone: 0.03 mg/l; gift from M.G. Täuber, Department of Infectology, University of Berne, Switzerland). Blood (3 ml in a serum tube) and CSF were drawn before (0.3 ml), 12 h (0.5 ml), 14 h (0.3 ml), 17 h (0.3 ml), 20 h (0.3 ml), and 24 h (0.5 ml) after infection. Antibiotic treatment was initiated 12 h after infection with 20 mg/kg ceftriaxone (Rocephin; kindly provided by Hoffmann-LaRoche, Grenzach-Wyhlen, Germany) as an intravenous bolus followed by 10 mg/kg/h maintenance dose in all animals. Eleven rabbits were subjected to CSF filtration; a miniaturized CSF-1 filter (pore size 0.2 μm, priming volume 250 μl, developed by Pall) was filled with sterile saline. CSF (300 μl) drawn 12 h after infection was passed through this filter by a high-precision Harvard perfusion pump (model 22) at a constant flow rate of 20 μl/min and then reinjected intracisternally (duration of the filtration 15 min + 5 min withdrawal and injection of CSF). Immediately before reinjection of the filtered CSF, 300 μl CSF was drawn for the next filtration cycle. This procedure was repeated six times during the first 2 h of antibiotic therapy, i.e., 1.8 ml CSF was filtered. The volume of CSF space of a rabbit weighing 2.5 kg was approximately 1.5–2 ml. The CSF turnover in rabbits is about 0.7 ml/h [4]. The same filter was used throughout the six cycles. Ten rabbits served as controls without adjunctive treatment. At the end of the first filtration cycle the control animals received 250 μl 0.9% NaCl once to compensate for the void volume of the filter filled with saline.

In three uninfected rabbits the CSF remained sterile at 6, 12, 18, and 24 h. The median CSF leukocyte densities at these times in those rabbits were 0, 41, 16, 45, and 38/μl.

After coagulation, blood was centrifuged at 3000 g for 5 min, and the supernatant was immediately frozen at −80°C. CSF white blood cells were counted in a Fuchs-Rosenthal hemocytometer. Pneumococcal CSF titers were determined by plating 10 μl of serial tenfold dilutions on blood agar plates, which were then incubated overnight at 37°C with 5% CO2. Bacterial titers at 12 h, 14 h, 17 h, 20 h, and 24 h served for log-linear regression analysis as previously described [11]. The remaining CSF was immediately centrifuged at 3000 g for 5 min, and the supernatants were stored at −80°C. Of the 300 μl CSF drawn at each time point, 5 μl was needed for the measurement of TNF, 10 μl for leukocyte count, bacterial density, lipoteichoic acid, protein content, and lactate concentration, 50 μl for neuron-specific enolase (NSE) concentration, and 25 μl for ceftriaxone. TNF activity in CSF was measured by a cytolytic assay using the L929 fibroblast cell line [11]. CSF drawn 24 h after infection was analyzed for NSE concentrations by an immunoluminometric method (LIA-mat, NSE Proliferin, Byk-Sangtec, Dietzenbach, Germany). Lactate was measured enzymatically (Riusen; Dreieich, Germany), and the CSF protein concentration was measured photometrically with the BCA protein test (Pierce; Rockford, Ill., USA). The concentrations of ceftriaxone in serum and CSF were determined by the agar-well diffusion technique with Escherichia coli no. 108 (collection of Prof. Dr. H. Hof, Department of Medical Microbiology, University of Heidelberg, Klinikum Mannheim, Germany).

The concentrations of lipoteichoic and teichoic acids were measured by a sandwich enzyme-linked immunosorbent assay (ELISA) using the mouse monoclonal antibody TEP28-15 (Sigma; Deisenhofen, Germany) directed against phosphorylcholine as capture and a polyclonal rabbit antisem raised against lipoteichoic acids as detector antibody [18].

The rabbits were killed 12 h after the beginning of therapy by intravenous injection of 75 mg thiopental (Trapanal, Byk Gulden; Constance, Germany). Brains were removed immediately, fixed in paraformaldehyde 5%, and embedded in paraffin. To determine the density of apoptotic neurons in the dentate gyrus, adjacent sections containing the dorsal part of the hippocampal formation were stained by hematoxillin and eosin and by in situ tailing [24]. The number of apoptotic neurons in the dentate gyrus was divided by the area of the granular cell layer measured by a microscopic planimeter (Contron Videoplan, Grundig; Nuremberg, Germany). The density of apoptotic neurons in the granular layer of the dentate gyrus of uninfected rabbits after 24 h of anesthesia has been reported to be 35 ± 27 cells/mm² [19]. Therefore no uninfected rabbits where killed in this experiment.

Measurement of binding of lipoteichoic acids to the CSF-1 filter in vitro

Lipoteichoic acids, purified as described previously [1], were diluted in trypsin soy broth and phosphate-buffered saline supplemented with 5% fetal calf serum to yield final concentrations of 50–2000 μg/ml. The solutions were pumped through the filters by a high precision Harvard perfusion pump (model 22) with a constant flow rate of 20 μl/min. The lipoteichoic acid concentrations were measured by ELISA (see above).

Statistics

Data are presented as mean ± standard deviation when normally distributed. Differences between groups are compared by the unpaired two-tailed t test. In the absence of normal distribution, median, 25th and 75th percentiles are used to describe the data, and differences between groups are compared by the Mann-Whitney U test.