A Novel Nonpsychotropic Cannabinoid, HU-211, in the Treatment of Experimental Pneumococcal Meningitis

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Typical features of pneumococcal meningitis have been demonstrated in rats inoculated with Streptococcus pneumoniae. HU-211, a novel noncompetitive N-methyl-D-aspartate antagonist recently demonstrated to inhibit tumor necrosis factor-α production under various conditions, improves recovery in some experimental models of brain injury. The present study tested the efficacy of HU-211 in combination with antimicrobial therapy in reducing brain damage in experimental pneumococcal meningitis. S. pneumoniae–infected rats were treated with saline alone, ceftriaxone alone, or with a combination of ceftriaxone and HU-211 18 h after inoculation of the bacteria. Brain edema and blood-brain barrier impairment 48 h after infection were significantly (P < .05) reduced in rats treated with ceftriaxone–HU-211 compared with rats in other treatment groups. The results suggest that HU-211 when given concomitantly with antibiotics attenuates brain damage in the rat model of pneumococcal meningitis.

Bacterial meningitis due to Streptococcus pneumoniae remains an important cause of mortality and morbidity despite advanced intensive care and effective antibiotic therapy [1–2]. The key elements in improving the outcome lie not only in killing the bacteria but also in preventing brain damage (i.e., the development of blood-brain barrier [BBB] alterations, brain edema, cerebrovascular complications, and increased intracranial pressure) [3].

Experimental research has shown that calcium and excitatory amino acids (glutamate and aspartate) play a role in the pathogenesis of brain injury and bacterial meningitis [3–5]. Studies of closed head injury [6] and cerebral ischemia [7] revealed that the neurotoxic effects associated with these insults are attenuated by HU-211, a synthetic cannabinoid, (+)-(3S,4S)-7-hydroxy-Δ 6 tetrahydrocannabinol 1,1-dimethylheptyl. HU-211 does not have any cannabimimetic effects and it exhibits pharmacologic properties characteristic of N-methyl-D-aspartate (NMDA)–receptor antagonists. HU-211 exhibits cerebroprotective effects in both in vivo [6–8] and in vitro [9, 10] models of brain injury. Recently, we demonstrated that HU-211 effectively inhibited tumor necrosis factor (TNF)-α production after traumatic brain injury and endotoxic shock. This inhibition occurs at the posttranscriptional level (unpublished data).

The present study was designed to evaluate the efficacy of combining HU-211 with antibiotics as therapy for experimental pneumococcal meningitis. We measured the effect of the combination on the clinical manifestations of the disease, brain edema formation, BBB permeability, and histopathology.

Materials and Methods
Induction of Meningitis

The experimental pneumococcal meningitis model described by Quagliarello et al. [11] was used. Male Sabra rats (Hebrew University strain) weighing 300–350 g were anesthetized with chloral hydrate (350 mg/kg). Pneumococcal meningitis was induced by percutaneous intracisternal injection of a 70-μL inoculum containing 10^6–10^7 cfu of S. pneumoniae type 14, which had been isolated from the cerebrospinal fluid (CSF) of a patient with pneumococcal meningitis.

Experimental Design

Four groups of rats were investigated. Group I (sham treatment group) consisted of noninfected rats intracisternally injected with 70 μL of saline. Groups II–IV comprised the infected rats, who were randomly assigned to 1 of the 3 experimental groups 18 h after inoculation with S. pneumoniae: group II rats (controls) received saline, group III received one intravenous (iv) dose of ceftriaxone (100 mg/kg), and group IV received a combination of iv ceftriaxone (100 mg/kg) and iv HU-211 (5 mg/kg; donated by Pharmos [Rehovot, Israel]).

Experimental Variables

Clinical examination. Rats were clinically evaluated 18 h after induction of pneumococcal meningitis and again at 48 h. At 18 h, all infected rats exhibited at least two of the following disease symptoms: tremor, lethargy, pilo-erection, and hypothermia.
(<36°C). The clinical status of the rats was scored as 1 point for the copresence of two or more clinical symptoms, regardless of severity, and 3 additional points for impaired motor skills (i.e., inability to walk on 8- and 5-cm-wide beams and to balance on a 1.5-cm-wide beam for 30 s). Thus, the maximum total score was 4. Sham-treated, group I, rats had a score of 0.

Brain edema. Eight rats from each group were sacrificed 48 h after induction of pneumococcal meningitis. The brain was removed, and a cortical segment was taken for determination of wet weight (WW). Dry weight (DW) was measured after drying the segment at 95°C. Percent tissue water (TW) content was calculated according to the formula \(\text{TW} = ([\text{WW} - \text{DW}] \times 100)/\text{WW}\).

BBB integrity. Forty-eight hours after pneumococcal meningitis induction, BBB integrity was evaluated as previously described [6]. Evans blue dye was injected and allowed to circulate for 60 min, and then animals were perfused with saline through the left ventricle. Their brains were removed and homogenized, and the level of the extracted dye was determined by spectrofluorometry (Perkin-Elmer Cetus, Norwalk, CT). The amount of Evans blue was quantified and is expressed per brain weight.

Histologic examination. Forty-eight hours after induction of pneumococcal meningitis, 3 rats were anesthetized and perfused via the left ventricle with paraformaldehyde dissolved in 0.1 M phosphate buffer. The brains were removed 4 h later, and sections were stained with hematoxylin-eosin and examined under a light microscope.

TNF-α activity. The titer of TNF-α was determined by cytotoxicity assay using BALB/c CL.7 cells as described previously [12]. In brief, destruction of CL.7 cells, to which diluted brain extracts had been added, was assessed using an MR700 microplate reader (Dynatech, Farmingdale, NY). TNF-α titer \((S_{50})\) is defined as the reciprocal of the dilution of the test extracts required to destroy 50% of the target cell monolayer.

Statistical Analysis

The percentage of water and amount of Evans blue are expressed as the mean ± SE. The clinical-neurologic score is expressed as the median and range. One-way analysis of variance followed by Bonferroni post-test or \(t\) test were done to compare the groups for water content and Evans blue. The intergroup comparison of the clinical score was made using the nonparametric Mann-Whitney test (corrected for multiple comparison). Mortality was compared using Fisher’s exact probability test.

Results

Model of Pneumococcal Meningitis in Rats

Within 18 h after inoculation, 40% of the infected rats died, and all survivors showed typical signs of the disease and impaired motor functions. All surviving rats had CSF and blood cultures positive for \(S. pneumoniae\) and CSF pleocytosis (>1200 white blood cells/mm³). Forty-eight hours after induction of pneumococcal meningitis, the median clinical score of the control rats (group II) was 4 (range: 3–4), while that of the sham-treated rats (group I) was 0 (table 1). Rats with meningitis had edema, as shown by an increase in water content (79.90% ± 0.11%) compared with the sham treatment group (group I: 78.2% ± 0.14%, \(P < .001\)). Extravasation of Evans blue was found in both hemispheres. The amount of Evans blue extracted from the brains of infected control rats (group II) was 191 ± 20.3 ng/g of tissue, compared with 46.9 ± 5.3 ng/g of tissue in the sham-treated rats (\(P < .001\)).

After 48 h of infection, infected rats showed pronounced infiltration of granulocytes into the subarachnoid space. In some animals, inflammation was also evident in the ventricles, with occasional ventricular hemorrhage. TNF-α assay of brain extracts \((n = 7 \text{ rats/group})\) revealed that TNF-α activity was nondetectable in uninfected rats, but \(S_{50}\) was 59.7 ± 7.9 in infected animals.

Effect of Drug Therapy

The next set of experiments was designed to test the effect of the novel drug HU-211 coadministered with ceftriaxone (group IV) on the outcome of experimental pneumococcal meningitis and to compare the effect with that of ceftriaxone alone (group III). Eighteen hours after inoculation, rats were randomized to the 3 experimental groups (II–IV). At this time, all rats had a similar degree of disease (i.e., clinical score of 4, positive CSF culture, and 1200–2500 white blood cells/mm³). The results of these experiments are summarized in table 1.

Mortality. Eighteen to 48 h after inoculation, 70% of the group II rats (untreated) died, compared with only 48% in group III \((P = .05)\) and 40% in group IV \((P = .012)\). There was no statistically significant difference between mortality in groups III and IV.

Clinical outcome. Both treatment regimens improved clinical scores of treated rats compared with scores of untreated animals (table 1). Rats that received HU-211 and antibiotics had a marginally better outcome than those treated with antibiotics alone \((P = .08, \text{Mann-Whitney})\).

Edema formation. Concomitant treatment with HU-211 and ceftriaxone (group IV) resulted in a significantly lower accumulation of water in brain tissue than did treatment with ceftriaxone alone (group III, \(P < .05\)) or no treatment (group II, \(P = .001\)). Ceftriaxone treatment alone did not reduce edema formation in the infected rats (table 1).

BBB breakdown. The maximum amount of Evans blue was extracted from the brain tissue of rats in group II (untreated rats); the concentration was significantly decreased in group III rats (ceftriaxone alone; \(P < .001\) vs. group II). Since the main question addressed in the present study was whether the combined therapy of HU-211 and ceftriaxone is more effective than ceftriaxone alone, we compared the effect of this combination on BBB disruption with the effect of ceftriaxone alone. The \(t\) test (uncorrected comparison) that compared Evans blue extravasation in groups III and IV revealed a further significant decrease in group IV, which received HU-211 and ceftriaxone.
(P = .03 vs. group III), indicating an additive effect of the two drugs.

**Discussion**

Our findings confirm that the procedure used to inject *S. pneumoniae* into rat cisterna magna induced the features of meningitis: positive cultures, pleocytosis, and typical histology [11]. Typical symptoms, including motor function deficits, edema, and BBB alteration, were evident in all infected rats, and elevated levels of TNF-α were demonstrated.

Treatment of infected rats with ceftriaxone alone effectively reduced mortality and ameliorated the clinical status and the BBB permeability defect (table I). However, edema formation was not affected by this treatment. Brain edema observed during bacterial meningitis may be of both vasogenic and cytotoxic origin. Antimicrobial agents can liberate large quantities of toxic substances released by activated leukocytes and astrocytes (inflammatory cytokines and excitatory amino acids) may contribute to direct neuronal damage and cytotoxic edema. Saukkonen et al. [13] and Tuomanen [14] demonstrated that components of the pneumococcal cell wall induced meningeal inflammation accompanied by brain edema. Peptidoglycan and lipoteichoic acid are the two main constituents of the gram-positive bacterial cell wall responsible for stimulating the release of cellular and humoral protagonists of inflammation. The use of anti–TNF-α monoclonal antibodies combined with dexamethasone has been shown to reduce the severity of experimental inflammation to a greater extent than either agent alone [15].

In a recent series of experiments on the effect of HU-211 on cytokine levels in models of closed head injury in the rat and septic shock in the mouse, we found that this drug inhibits the production of TNF-α by 80%-90% and that inhibition

**Table 1.** Mortality between 18 and 48 h and clinical status, edema, and blood-brain barrier (BBB) permeability at 48 h in sham-treated rats (group I) and rats with pneumococcal meningitis treated with saline (group II), ceftriaxone alone (group III), or ceftriaxone with HU-211 (group IV).

<table>
<thead>
<tr>
<th>Group</th>
<th>Mortality, no. died/no. total (%)</th>
<th>Clinical score, median (range)</th>
<th>Edema, % water</th>
<th>BBB, Evans blue (ng/g of tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>0/16 (0)</td>
<td>0</td>
<td>78.2 ± 0.14</td>
<td>46.9 ± 5.3</td>
</tr>
<tr>
<td>II</td>
<td>35/51 (70)</td>
<td>4 (3–4)</td>
<td>79.9 ± 0.11</td>
<td>191.0 ± 20.3</td>
</tr>
<tr>
<td>III</td>
<td>13/28 (46)*</td>
<td>3 (2–4)*</td>
<td>79.54 ± 0.10</td>
<td>60.3 ± 9.6*</td>
</tr>
<tr>
<td>IV</td>
<td>10/25 (40)*</td>
<td>2 (2–4)*</td>
<td>79.08 ± 0.09**</td>
<td>33.6 ± 4.96**</td>
</tr>
</tbody>
</table>

* P < .05 vs. group II. Mann-Whitney test corrected for multiple comparisons.
† P = .08 vs. group III.
‡ P < .0001. analysis of variance.
§ P < .001 vs. group II.
** P = .03 vs. group III, t test.
occurs at the posttranscriptional level (unpublished data). This finding may suggest that in addition to the anti-NMDA properties of HU-211, another mechanism related to cytokine inhibition is responsible for the observed synergistic effect of HU-211 in combination with ceftriaxone. This mechanism may also play a role in the cerebroprotection observed in trauma and ischemia. Future studies will shed more light on the mechanism(s) of action of this unique compound.

Our results demonstrate that the novel compound HU-211 administered concomitantly with antibiotics affects the pathophysiologic processes involved in pneumococcal meningitis. We hypothesize that the addition of HU-211 to antibiotics may improve the clinical outcome of pneumococcal meningitis by reducing brain edema and BBB disruption and, thus, may improve clinical outcome.

References