Short Communication

In vitro evaluation of the efficacy of combined antimicrobial agents against β-lactamase negative ampicillin-resistant Haemophilus influenzae strains isolated from children with bacterial meningitis

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SUMMARY

Recently, Haemophilus influenzae (H. influenzae), the major causative organism of bacterial meningitis, has become increasingly resistant to β-lactam antibiotics. The design of new antimicrobial therapies against such resistant organisms is a pressing task. We measured the antimicrobial susceptibility of 8 β-lactamase-negative ampicillin-resistant H. influenzae (BLNAR) strains isolated from patients with meningitis between 1996 and 2003. Furthermore, the fractional inhibitory concentration index of combined antimicrobial agents against those strains was calculated. The minimum inhibitory concentrations of piperacillin (PIPC), ceftriaxone (CTRX), and meropenem (MEPM) showed almost identical cumulative distribution curves. In vitro, antimicrobial combinations involving CTRX + PIPC, CTRX + MEPM and PIPC + MEPM showed synergistic or additive effects against all strains. Among these combinations, the synergistic effects of CTRX + MEPM and PIPC + MEPM were apparent in most of the strains, suggesting that the choice of antibiotic treatment may be CTRX + MEPM or PIPC + MEPM in the event that the causative organism is ascertained to be a BLNAR strain.

Key words: CTRX, MEPM, PIPC, BLNAR, FIC index

The antibiotic resistance of Haemophilus influenzae (H. influenzae), the major causative organism of bacterial meningitis, has increased lately, and it is possible that conventional antimicrobial therapies may become inadequate in near future. Combined antimicrobial therapy is essential when antimicrobial monotherapy is inadequate, but in such circumstances, a synergistic or additive effect is desirable, and, at least, the individual drugs must not have antagonistic activity. However, there has been no report about the effects of combined antimicrobial agents against β-lactamase-negative ampicillin-resistant Haemophilus influenzae (BLNAR) strains in vitro. Regarding this background, we measured minimum inhibitory concentrations (MIC)

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会沢治朗, 石和田鉄彦, 太田節雄†, 河野陽一, 小児細菌性脳膜炎由来β-lactamase negative ampicillin resistant Haemophilus influenzae に対する抗菌薬併用療法の細菌学的検討.
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for BLNAR strains, and evaluated the effects of combined antimicrobial agents against this organism. Eight BLNAR strains isolated from meningitis patients treated between 1996 and 2003 were selected for study. These strains accounted for 8.7% (8/92) of all strains isolated from patients with *H. influenzae* meningitis in our laboratory during same period. All 8 strains were identified as serotype b by PCR method [1]. All strains were β-lactamase-negative and had ampicillin (ABPC) or sulbactam (SBT)/ABPC- MIC values of ≥1.56 μg/ml. The presence of the mutant *fts I* gene was confirmed for all strains using polymerase chain reaction with commercial reagents (Wakunaga Pharmaceuticals Co., Ltd., Osaka, Japan). Antimicrobial susceptibility was measured by the Agar Dilution Susceptibility Test according to the standard method prescribed by the Japanese Society of Chemotherapy [2].

The tested antimicrobial agents were ABPC, piperacillin (PIPC), ceftriaxone (CTRX) and meropenem (MEPM). The assay concentration ranges were 0.025-12.5 μg/ml for ABPC, 0.0016-12.5 μg/ml for PIPC, 0.0008-0.8 μg/ml for CTRX, and 0.0031-0.8 μg/ml for MEPM. Furthermore, to evaluate the effects of combined antimicrobial agents including CTRX + PIPC, CTRX + MEPM and PIPC + MEPM, we calculated the fractional inhibitory concentration (FIC) index for each of these combinations using the checkerboard technique. An FIC index of 0.5 or less was evaluated as a synergistic effect, of greater than 0.5 and less than 2 as an additive effect, of 2 as an indifferent, and of greater than 2 as an antagonistic effect[3].

Table 1 shows the MIC of each antimicrobial agent. The MIC of CTRX varied between 0.0125 and 0.2 μg/ml, that of PIPC from 0.025 and 0.2 μg/ml, and that of MEPM from 0.1 and 0.2 μg/ml. When *H. influenzae* strains were classified into slow-BLNAR and gBLNAR according to *fts I* gene mutations, the MICs of CTRX, PIPC and MEPM for slow-BLNAR strains varied between 0.0125-0.2 μg/ml, 0.025-0.2 μg/ml, and 0.1-0.2 μg/ml, respectively, and the MICs of CTRX, PIPC, and MEPM for BLNAR strains varied between 0.025-0.2 μg/ml, 0.05-0.1 μg/ml, and 0.1-0.2 μg/ml, respectively.

Table 1 also shows the FIC index of combined use of CTRX + PIPC, CTRX + MEPM and PIPC + MEPM. The combined use of CTRX and PIPC showed a synergistic effect in 1 strain (12.5%) and an additive effect in 7 strains (87.5%). The combined use of CTRX and

<table>
<thead>
<tr>
<th>Strain No.</th>
<th>Classification According to <em>ftsI</em> mutations</th>
<th>CTRX MIC (μg/ml)</th>
<th>MEPM MIC</th>
<th>PIPC MIC</th>
<th>ABPC MIC</th>
<th>FIC index</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>CTRX+PIPC</td>
</tr>
<tr>
<td>1</td>
<td>glow BLNAR</td>
<td>0.025</td>
<td>0.2</td>
<td>0.05</td>
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<td>0.5625</td>
</tr>
<tr>
<td>2</td>
<td>glow BLNAR</td>
<td>0.0125</td>
<td>0.2</td>
<td>0.025</td>
<td>1.56</td>
<td>0.625</td>
</tr>
<tr>
<td>3</td>
<td>glow BLNAR</td>
<td>0.1</td>
<td>0.2</td>
<td>0.1</td>
<td>1.56</td>
<td>0.5625</td>
</tr>
<tr>
<td>4</td>
<td>glow BLNAR</td>
<td>0.2</td>
<td>0.1</td>
<td>0.2</td>
<td>1.56</td>
<td>0.5625</td>
</tr>
<tr>
<td>5</td>
<td>gBLNAR</td>
<td>0.025</td>
<td>0.2</td>
<td>0.05</td>
<td>1.56</td>
<td>0.5625</td>
</tr>
<tr>
<td>6</td>
<td>gBLNAR</td>
<td>0.2</td>
<td>0.2</td>
<td>0.1</td>
<td>1.56</td>
<td>0.375</td>
</tr>
<tr>
<td>7</td>
<td>gBLNAR</td>
<td>0.2</td>
<td>0.2</td>
<td>0.1</td>
<td>0.8</td>
<td>0.625</td>
</tr>
<tr>
<td>8</td>
<td>gBLNAR</td>
<td>0.2</td>
<td>0.2</td>
<td>0.1</td>
<td>0.8</td>
<td>0.625</td>
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</tbody>
</table>

MIC: minimum inhibitory concentration, FIC: fractional inhibitory concentration
BLNAR: β-lactamase negative ampicillin-resistant *Haemophilus influenzae*
CTRX: ceftriaxone, MEPM: meropenem, PIPC: piperacillin, ABPC: ampicillin
MEPM showed a synergistic effect in 7 of the 8 strains (87.5%) and an additive effect in 1 strain (12.5%). The combined use of PIPC and MEPM showed a synergistic effect in all strains. None of the combinations showed an antagonistic effect.

Recently, the proportion of BLNAR strains among clinical isolates of *H. influenzae* has increased,[4-5], and it has been noted that treatment may prove challenging when such strains of *H. influenzae* are implicated in the onset of meningitis.[6-7]. There are certain strains of this organism for which CTX shows high MIC values[5], and hence there is a possibility that conventional antimicrobials may prove inadequate for the treatment of meningitis in near future. In the present work, we evaluated the effect of combined antimicrobial agents including CTRX + PIPC, CTRX + MEPM and PIPC + MEPM. These 3 drugs were selected based on their MIC values. Since clinical isolates are difficult to obtain, we used only 8 strains in this study, but the use of each combination of 2 drugs resulted in an additive or synergistic effect, and not in an antagonistic effect, for BLNAR strains in *vitro*. Among these combinations, the frequency of synergy was high for combinations of CTRX + MEPM and PIPC + MEPM, and in the event that the causative organism is identified to be a BLNAR strain, combined treatment with CTRX + MEPM or PIPC + MEPM may be the appropriate antibiotic choice. Not only ABPC but also PIPC are not effective against β-lactamase positive *H. influenzae* strains. Tazobactam/PIPC, a 4:1 combination of PIPC and a β-lactamase inhibitor, could be a candidate for the treatment of meningitis caused by β-lactamase positive *H. influenzae* strain instead of PIPC. Among the antibiotics derived from carbapenems, MEPM has demonstrated several promising characteristics, including a low incidence of adverse events including convulsions, a low burden on the kidneys, and good penetration into cerebrospinal fluid. For these reasons, in the West, MEPM is widely used for the treatment of bacterial meningitis in children. Our study only entailed an *in vitro* experiment. To clarify the clinical effects of combined antimicrobial therapy, it is necessary to establish a clinical trial for evaluating the clinical aspects of patients receiving the combination therapy.

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**要 旨**

細菌性脳膜炎は小児科領域における代表的な重症感染症である。その起炎菌としてわが国で最多であるインフルエンザ菌において、近年βラクタム系薬に対する耐性化が進行し、治療に難渋する例が報告されている。このような耐性菌に対しては新たな治療薬を提案が急務である。今回インフルエンザ菌β-lactamase negative ampicillin resistant (BLNAR) 株を対象、BLNARに対して感受性良好な薬剤の併用効果について検討を行った。対象は1995年から2003年の間に脳膜炎症例より分離されたBLNAR8株とした。BLNAR株は、β-lactamase陰性かつampicillin（ABPC）あるいはsulbactam（SBT）/ABPCの最小発育阻止濃度が1.56μg/ml以上の株とし、インフルエンザ菌遺伝子検査検査（湯永製薬株式会社）を用い、耐性に関与するβ-lactamase遺伝子の変異を全株で確認した。これら8株に対し、感受性の比較的良好であったpiperacillin（PIPC）、ceftiraxone（CTRX）、meropenem（MEPM）それぞれ2剤の併用効果を検討する目的でfractional inhibitory concentration index（FIC index）を算出し、評価を行った。その結果、CTRX+PIPC、CTRX+MEPM、PIPC+MEPM全ての組み合わせで拮抗作用は認めなかった。特にCTRX+MEPM、PIPC+MEPMで高い相乗効果を認め、BLNAR性脳膜炎に対する選択肢のひとつになる可能性が示唆された。
References


