Serum and Cerebrospinal Fluid Levels of Colistin
in Pediatric Patients

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Running title: Colistin concentrations in children
Abstract

Using a Liquid Chromatography tandem Mass Spectrometry method serum and cerebrospinal fluid concentrations of colistin were determined in patients aged 1½ months-14 years receiving intravenous colistimethate sodium (60,000-225,000 IU/kg/d). Only in one of five courses studied (14 years-225,000 IU/kg/d) serum concentrations exceeded the 2 µg/ml CLSI/EUCAST breakpoint defining susceptibility to colistin for *Pseudomonas* and *Acinetobacter*. CSF colistin concentrations were <0.2 µg/ml but increased in the presence of meningitis (~0.5 µg/ml or 34-67% of serum levels).
Colistin is administered parenterally as colistimethate sodium (CMS), a less toxic inactive prodrug (4, 7, 9). Recommended daily pediatric CMS doses range between 50,000-75,000 IU/kg/d (4-6 mg/kg/d) in Europe and 83,000-166,000 IU/kg/d (6.6-13.3 mg/kg/d) in USA (7, 9). There is a paucity of pharmacokinetic data regarding colistin administration in children. Most older studies have determined colistin levels using microbiological assays, which are problematic due to degradation and diffusion problems (7, 9). In the present study we used a recently published Liquid Chromatography tandem Mass Spectrometry (LC-MS/MS) method (5, 11) to determine concentrations of colistin and CMS in the serum and cerebrospinal fluid (CSF) of infants and children treated intravenously with CMS for gram-negative bacterial infections.

The study included patients < 18 years, hospitalized from March 2008 until February 2009, who: a) were on intravenous CMS treatment for gram-negative bacterial infections and had already received at least four doses; b) carried an external ventricular drainage (EVD) system due to hydrocephalus. Exclusion criteria were: a) cystic fibrosis; b) renal impairment; c) additional administration of CMS through other routes (intraventricular, intrathecal, per inhalation).

CMS (Norma Pharmaceuticals, Athens, Greece) was dissolved in 30-50 ml of normal saline and administered 8-hourly by intravenous 20-min infusion. 1 mg of CMS equals 12,500 IU. Initially, doses in the lower range of those recommended were used. Subsequently, reported evidence on dose escalation from critically ill adult and cystic fibrosis patients (9) prompted us to use higher doses [range of doses used: 60,000-225,000 IU/kg/d (4.8-18 mg/kg/d)].

Blood samples were collected immediately before and 30 min after the end of CMS infusion. Following centrifugation serum was collected and immediately stored
at -80°C. CSF samples were collected from EVD systems concomitantly with the blood samples, within 1 hour prior to CMS infusion and after its end, and stored at -80°C. For each patient and CMS regimen, paired blood and CSF sampling was repeated on a different day.

Concentrations of colistin A and B in serum were determined by the LC-MS/MS method already mentioned (5, 11). Determination of colistin concentrations in CSF samples was performed using the LC-MS/MS method for culture medium (5), where the samples were diluted with an equal volume of serum before protein precipitation in order to avoid unspecific binding of colistin. Standards and quality controls (QCs) were prepared in a modified Ringer solution mimicking CSF composition, that contained NaCl (147 mM), KCl (2.7 mM), CaCl$_2$ (1.2 mM), MgCl$_2$ (0.85 mM) and D-glucose (16.7 mM). The solution was buffered with sodium phosphate to pH 7.4 and diluted with an aliquot of serum. The inter-day CV and accuracy for serum was <4.4% and <±1.9% and for the CSF-serum mix <3.0% and <±2.7%. Standard preparations covered the whole range of measured colistin concentrations in each assay subset. All samples were assayed at the same time.

Serum and CSF concentrations of CMS were determined by hydrolysis of CMS to colistin using the method described by Li et al. (8) with some modifications. Sulphuric acid (1M) was mixed with the serum or CSF-serum sample and after 15-20 minutes sodium hydroxide (1M) was added. Thereafter proteins were precipitated with acetonitrile containing 0.1% trifluoroacetic acid. CMS concentrations were determined by subtracting the colistin determined in the samples before hydrolysis from the colistin determined after the hydrolysis. CMS controls in the range of 0.25-18.5µg/ml (colistin methanesulfonate sodium salt, Sigma-Aldrich, St Louis, MO) were
analyzed together with the study samples and the inter-day CV was <8.2% and the accuracy was <±3.2%.

On the day of sampling the following data were recorded for each patient: age, sex, weight, height, body surface area, liver function enzymes, blood urea and creatinine, CSF cytochemistry, CMS dosage and day of therapy, concomitant administration of other medications, reason for admission and treatment with CMS as well as species identification and colistin MIC for the microorganism implicated using the VITEK-2 automated system (bioMerieux, Marcy l’Etoile, France). For study purposes changes in the dosing regimen of CMS for a particular patient defined different courses of CMS therapy. The study was approved by the Hospital Ethics Committee. Informed consent was obtained from patient’s parents.

A total of 5 courses of CMS treatment in 3 patients (Pt) were studied, with Pt1 receiving 3 courses (Table 1). None of the patients had abnormal liver or renal function tests. CSF analysis indicated meningeal inflammation only in course No 3 of Pt1.

Pt1 suffered from post-hemorrhagic hydrocephalus, complicated by multiresistant Acinetobacter baumannii infection (colistin MIC: ≤ 0.5 µg/ml, meropenem MIC: 8 µg/ml) resulting in formation of multiple epidural abscesses. In course No 3 he had meningitis following replacement of EVD system (CSF cells: 200/µl, protein 90 mg/dl, A. baumannii isolated). Resolution of the abscesses occurred after long-term treatment with CMS (200,000 IU/kg/d) and meropenem.

Pt2 had hydrocephalus due to prior history of tuberculous meningitis and Aspergillus fumigatus ventriculitis; both had subsided at the time of sample collection. She was started on CMS because of multiresistant Stenotrophomonas maltophilia...
isolated from CSF. The isolate’s colistin MIC was initially 2 µg/ml but during therapy increased to >16 µg/ml. The infection resolved after replacement of the EVD system.

Pt3 had hydrocephalus following head trauma and received CMS because of bloodstream infection with *Klebsiella pneumoniae* and *S. maltophilia* (colistin MIC for both isolates was ≤ 0.5 µg/ml). He received the adult CMS dose (9,000,000 IU/d) corresponding to 225,000 IU/kg/d (Table 1) and had a favorable outcome.

The concentrations of colistin (sum of colistin A and B concentrations) and CMS determined in serum and CSF during courses 1-5 are presented in Table 2.

The MIC breakpoint of 2 µg/ml is used by CLSI and EUCAST to define susceptibility to colistin for *Pseudomonas aeruginosa* and *A. baumannii* (1, 2). Using a novel LC-MS/MS method we demonstrated that, with CMS doses in the range of those recommended and even higher (200,000 IU/kg/d), the concentrations of colistin achieved in the serum and, furthermore, the CSF of pediatric patients may not reach the level of 2 µg/ml. This appears to be more likely for infants and younger children, as the levels in Pt3 were higher than those of Pt1 (course 3) and Pt2 (Table 2). Age-related differences in colistin clearance may explain these findings; more data are however needed to support this hypothesis.

In the absence of meningeal inflammation, colistin penetration in the CSF appears to be minimal (levels <0.2 µg/ml, Table 2) in agreement with previous studies (10). In the presence of meningitis (course No 3), penetration was significantly higher, reaching 34-67% of serum levels. Still, colistin concentrations in the CSF did not exceed 0.5 µg/ml (Table 2). These sub-MIC concentrations may explain the development of resistance in *S. maltophilia* isolated from patient Pt2. Previous studies using bioassays suggested no enhancement of colistin penetration in the case of meningeal inflammation (3) or a 25% peak CSF/serum ratio (6).
Markantonis et al demonstrated that colistin concentrations in CSF paralleled those in serum, yielding similar CSF/serum ratios at all sampling times (10). This differs from our findings, where CSF colistin concentrations were rather stable before and after intravenous administration in each course, in contrast to serum levels, yielding variable CSF/serum ratios. Similarly, CMS concentrations in CSF did not follow changes in peak/trough serum levels. CMS exhibited lower CSF/serum concentration ratios than colistin (Table 2).

Therefore, higher than previously recommended CMS doses may be needed for pediatric patients to treat bloodstream infections caused by gram-negative bacteria, particularly if these exhibit borderline susceptibility to colistin (MIC of 2 µg/ml).

Colistin penetration in CSF appears to increase significantly in the presence of meningitis; still, concentrations achieved may be inadequate for treatment of bacterial infections and intraventricular/intrathecal administration remains an option.

Pharmacokinetic studies of higher (>200,000 IU/kg/d) dosing regimens of CMS are needed for pediatric patients.

**Acknowledgements**

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References


TABLE 1. Characteristics of pediatric patients, CMS doses and medications concomitantly administered during intravenous CMS treatment.

<table>
<thead>
<tr>
<th>CMS course</th>
<th>Patient</th>
<th>Sex</th>
<th>Age (yr)</th>
<th>Weight (kg)</th>
<th>Body surface area (m²)</th>
<th>CMS dose (IU/kg/d)</th>
<th>Duration of treatment (d)</th>
<th>Medications administered concomitantly</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Pt1 M</td>
<td>1½ mo</td>
<td>6.2</td>
<td>0.30</td>
<td>60,000</td>
<td>(4.8 mg/kg/d)</td>
<td>7</td>
<td>amikacin, metronidazole, vancomycin, dopamine, dobutamine, ranitidine</td>
<td></td>
</tr>
<tr>
<td>2 Pt1 F</td>
<td>2½ mo</td>
<td>6.4</td>
<td>0.31</td>
<td>130,000</td>
<td>(10.4 mg/kg/d)</td>
<td>46</td>
<td>meropenem, phenytoin, dexamethasone</td>
<td></td>
</tr>
<tr>
<td>3 Pt1 M</td>
<td>5½ mo</td>
<td>7.8</td>
<td>0.38</td>
<td>200,000</td>
<td>(16 mg/kg/d)</td>
<td>93</td>
<td>meropenem, phenytoin, domperidone</td>
<td></td>
</tr>
<tr>
<td>4 Pt1 F</td>
<td>5½ yr</td>
<td>15</td>
<td>0.65</td>
<td>200,000</td>
<td>(16 mg/kg/d)</td>
<td>51</td>
<td>isoniazid, pyrazinamide, levofloxacin, voriconazole, liposomal amphotericin, levetiracetam, phenytoin, gabapentin, midazolam, domperidone</td>
<td></td>
</tr>
<tr>
<td>5 Pt1 M</td>
<td>14 yr</td>
<td>40</td>
<td>1.33</td>
<td>225,000</td>
<td>(18 mg/kg/d)</td>
<td>42</td>
<td>vancomycin, amikacin, piperacillin, phenytoin, omeprazole</td>
<td></td>
</tr>
</tbody>
</table>


<table>
<thead>
<tr>
<th>CMS course</th>
<th>Before (B) / after (A)</th>
<th>Colistin, serum (^a)</th>
<th>Colistin, CSF (^a)</th>
<th>Colistin, CSF/serum (%)</th>
<th>CMS, serum (^a)</th>
<th>CMS, CSF (^a)</th>
<th>CMS, CSF/serum (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1(^b)</td>
<td>B 0.19</td>
<td>0.05</td>
<td>25.2</td>
<td>0.28</td>
<td>0.06</td>
<td>0.06</td>
<td>20.6</td>
</tr>
<tr>
<td></td>
<td>A 0.29</td>
<td>0.06</td>
<td>19.3</td>
<td>2.86</td>
<td>0.06</td>
<td>0.06</td>
<td>2.0</td>
</tr>
<tr>
<td>2</td>
<td>B 0.52 (0.20-0.85)</td>
<td>0.06 (0.05-0.06)</td>
<td>11.1</td>
<td>0.84 (0.12-1.57)</td>
<td>0.03 (0.02-0.03)</td>
<td>3.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>A 1.46 (1.00-1.92)</td>
<td>0.05 (0.03-0.07)</td>
<td>3.4</td>
<td>7.90 (4.22-11.57)</td>
<td>0.02 (0.02-0.02)</td>
<td>0.2</td>
<td></td>
</tr>
<tr>
<td>3(^c)</td>
<td>B 0.73 (0.56-0.90)</td>
<td>0.50 (0.49-0.50)</td>
<td>67.7</td>
<td>0.75 (0.31-1.19)</td>
<td>0.38 (0.27-0.49)</td>
<td>50.9</td>
<td></td>
</tr>
<tr>
<td></td>
<td>A 1.33 (1.11-1.55)</td>
<td>0.46 (0.46-0.46)</td>
<td>34.8</td>
<td>8.41 (8.11-8.71)</td>
<td>0.26 (0.16-0.36)</td>
<td>3.1</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>B 0.97 (0.62-1.32)</td>
<td>0.15 (0.13-0.17)</td>
<td>16.1</td>
<td>0.93 (0.66-1.20)</td>
<td>0.09 (0.08-0.09)</td>
<td>9.9</td>
<td></td>
</tr>
<tr>
<td></td>
<td>A 1.60 (1.39-1.82)</td>
<td>0.11 (0.07-0.15)</td>
<td>7.2</td>
<td>9.89 (9.86-9.91)</td>
<td>0.09 (0.07-0.12)</td>
<td>0.9</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>B 2.29 (1.87-2.71)</td>
<td>0.07 (0.07-0.07)</td>
<td>3.0</td>
<td>6.11 (2.47-7.94)</td>
<td>0.04 (0.03-0.04)</td>
<td>0.6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>A 2.20 (1.80-2.59)</td>
<td>0.07 (0.06-0.07)</td>
<td>3.2</td>
<td>7.98 (7.78-8.18)</td>
<td>0.03 (0.03-0.03)</td>
<td>0.4</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) The mean (range) of two measurements obtained on different days is presented.

\(^b\) For course No 1 single measurements were performed as CMS dose was increased after first sampling.

\(^c\) CSF analysis in course No 3 was suggestive of meningitis (cells: 200/µl, protein 90 mg/dl).