ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Sept. 2010, p. 3985–3987 Vol. 54, No. 9
0066-4804/10/$12.00 doi:10.1128/AAC.01799-09

Serum and Cerebrospinal Fluid Levels of Colistin in Pediatric Patients▽

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Received 18 December 2009/Returned for modification 28 March 2010/Accepted 8 June 2010

Using a liquid chromatography-tandem mass spectrometry method, the serum and cerebrospinal fluid (CSF) concentrations of colistin were determined in patients aged 1½ months to 14 years receiving intravenous colistimethate sodium (60,000 to 225,000 IU/kg of body weight/day). Only in one of five courses studied (a 14-year-old receiving 225,000 IU/kg/day) did serum concentrations exceed 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 to 67% of serum levels).

Colistin is administered parenterally as colistimethate sodium (CMS), a less toxic inactive prodrug (4, 7, 9). The recommended daily pediatric CMS doses range between 50,000 and 75,000 IU/kg/day (4 to 6 mg/kg of body weight/day) in Europe and 83,000 to 166,000 IU/kg/day (6.6 to 13.3 mg/kg/day) in the United States (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 the 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 old, hospitalized from March 2008 until February 2009, who (i) were on intravenous CMS treatment for Gram-negative bacterial infections and had already received at least four doses or (ii) carried an external ventricular drainage (EVD) system due to hydrocephalus. Exclusion criteria were (i) cystic fibrosis, (ii) renal impairment, or (iii) additional administration of CMS through other routes (intraventricular, intrathecal, or per inhalation).

CMS (Norma Pharmaceuticals, Athens, Greece) was dissolved in 30 to 50 ml of normal saline and administered every 8 hours by intravenous 20-min infusion. One milligram 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 to 225,000 IU/kg/day [4.8 to 18 mg/kg/day]).

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 h 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.

The concentrations of colistin A and colistin B in serum were determined by the LC–MS-MS method already mentioned (5, 11). The 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 and containing 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 interday coefficient of variation (CV) and accuracy for serum were <4.4% and within ±1.9%, respectively, and for the CSF-serum mix were <3.0% and within ±2.7%, respectively. The standard preparations covered the whole range of measured colistin concentrations in each assay subset. All samples were assayed at the same time.

The 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. Sulfuric acid (1 M) was mixed with the serum or CSF-serum sample, and after 15 to 20 min, sodium hydroxide (1 M) was added. Thereafter, proteins were precipitated with acetonitrile containing 0.1% trifluoroacetic acid. CMS concentrations were determined by subtracting the amount of colistin determined in the samples before hydrolysis from the amount of colistin determined after hydrolysis. CMS controls in the range of 0.25 to 18.5 μg/ml (colistin methanesulfonate sodium salt; Sigma-Aldrich, St. Louis, MO) were analyzed together with the study samples, and the interday CV and accuracy were <8.2% and within ±3.2%, respectively.

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 adminis-

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▽ Published ahead of print on 28 June 2010.

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

CMS course	Patient	Sex	Age	Weight (kg)	Body surface area (m^2)	CMS dose [IU/kg/day (mg/kg/day)]	Duration of treatment (days)	Medications administered concomitantly
1	Pt1	M	1½ mo	6.2	0.30	60,000 (4.8)	7	Amikacin, metronidazole, vancomycin, midazolam, dopamine, dobutamine, ranitidine
2			2½ mo	6.4	0.31	130,000 (10.4)	46	Meropenem, phenytoin, dexamethasone, ranitidine
3			5½ mo	7.8	0.38	200,000 (16)	93	Meropenem, phenytoin, domperidone
4	Pt2	F	5½ yr	15	0.65	200,000 (16)	51	Isoniazid, pyrazinamide, levofloxacin, amikacin, voriconazole, liposomal amphotericin B, phenytoin, levetiracetam, dopamine, domperidone, ranitidine
5	Pt3	M	14 yr	40	1.33	225,000 (18)	42	Vancomycin, amikacin, piperacillin-tazobactam, phenytoin, gabapentin, midazolam, dopamine, domperidone, omeprazole

tration of other medications, and 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 the purposes of this study, 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 the 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 test results. CSF analysis indicated meningeal inflammation only during course 3 in Pt1.

Pt1 suffered from posthemorrhagic hydrocephalus, complicated by multiresistant *Acinetobacter baumannii* infection (colistin MIC, ≤0.5 μg/ml, and meropenem MIC, 8 μg/ml) that resulted in the formation of multiple epidural abscesses. During course 3, he had meningitis following replacement of his EVD system (CSF cells, 200/μl, and protein, 90 mg/dl; *A. baumannii* was isolated). Resolution of the abscesses occurred after long-term treatment with CMS (200,000 IU/kg/day) and meropenem.

Pt2 had hydrocephalus due to a 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 a CSF sample. 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* (the colistin MIC for both isolates was ≤0.5 μg/ml). He received the adult CMS dose (9,000,000 IU/day), corresponding to 225,000 IU/kg/day (Table 1), and had a favorable outcome.

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

A 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

TABLE 2. Concentrations of colistin and CMS in serum and CSF obtained from pediatric patients before and after intravenous CMS administration

CMS course	Time of measurement	Mean concn (range)[a] of drug or prodrug in serum or CSF (μg/ml) or ratio of corresponding mean CSF/serum concn					
		Colistin			CMS		
		Serum	CSF	CSF/serum (%)	Serum	CSF	CSF/serum (%)
1[b]	Before	0.19	0.05	25.2	0.28	0.06	20.6
	After	0.29	0.06	19.3	2.86	0.06	2.0
2	Before	0.52 (0.20–0.85)	0.06 (0.05–0.06)	11.1	0.84 (0.12–1.57)	0.03 (0.02–0.03)	3.1
	After	1.46 (1.00–1.92)	0.05 (0.03–0.07)	3.4	7.90 (4.22–11.57)	0.02 (0.02–0.02)	0.2
3[c]	Before	0.73 (0.56–0.90)	0.50 (0.49–0.50)	67.7	0.75 (0.31–1.19)	0.38 (0.27–0.49)	50.9
	After	1.33 (1.11–1.55)	0.46 (0.46–0.46)	34.8	8.41 (8.11–8.71)	0.26 (0.16–0.36)	3.1
4	Before	0.97 (0.62–1.32)	0.15 (0.13–0.17)	16.1	0.93 (0.66–1.20)	0.09 (0.08–0.09)	9.9
	After	1.60 (1.39–1.82)	0.11 (0.07–0.15)	7.2	9.89 (9.86–9.91)	0.09 (0.07–0.12)	0.9
5	Before	2.29 (1.87–2.71)	0.07 (0.07–0.07)	3.0	6.11 (2.47–9.74)	0.04 (0.03–0.04)	0.6
	After	2.20 (1.80–2.59)	0.07 (0.06–0.07)	3.2	7.98 (7.78–8.18)	0.03 (0.03–0.03)	0.4

[a] The mean and range of two measurements obtained on different days are presented.
[b] For course 1, single measurements were performed because the CMS dose was increased after the first sampling.
[c] The results of the CSF analysis in course 3 were suggestive of meningitis (cells, 200/μl, and protein, 90 mg/dl).

demonstrated that, with CMS doses in the range of those recommended and even higher (200,000 IU/kg/day), 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; however, more data are 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 3), penetration was significantly higher, reaching 34 to 67% of the levels in serum. 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 the *S. maltophilia* isolate 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, which yielded variable CSF/serum ratios. Similarly, the CMS concentrations in CSF did not follow the changes in peak/trough serum levels. CMS exhibited lower CSF/serum concentration ratios than colistin (Table 2).

Therefore, CMS doses higher than previously recommended 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, the concentrations achieved may be inadequate for the treatment of bacterial infections, and intraventricular/intrathecal administration remains an option. Pharmacokinetic studies of higher (>200,000 IU/kg/day) dosing regimens of CMS are needed for pediatric patients.

This work was supported in part by a research grant from the Hellenic National Institute of Scholarships.

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