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Effectiveness of Cephalosporins in the Sputum of Patients with Nosocomial Bronchopneumonia

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Nosocomial bronchopneumonia is a frequent complication in patients with chronic intratracheal intubation. Despite targeted antibiotic treatment, production of abundant bronchial secretion containing pathogen bacteria often tends to be chronic, and so mortality drastically increases. This problem led to an investigation of the penetration of five cephalosporin antibiotics into the sputum. Serum and sputum were collected from 24 chronically intubated patients having purulent nosocomial bronchopneumonia treated in an intensive care unit (ICU). Patients received the following doses intravenously every 24 h: five received 70 mg/kg of body weight cefuroxime, four received 110 mg/kg cefamandole, six received 80 mg/kg ceftriaxone, four received 80 mg/kg ceftazidime, and five received 80 mg/kg cefepime. Antibiotic concentrations in the serum and sputum were evaluated by capillary electrophoresis. MICs were determined for bacteria isolated from the purulent bronchial secretions. The mean levels of the cephalosporins in the sputum did not reach the MICs for the bacteria isolated from the same samples. Ceftriaxone was the only one of the investigated five cephalosporins that had a measurable concentration in the sputum (1.4 ± 1.2 mg/liter). The low concentration of antibiotics in the purulent tracheobronchial secretion can be one of the many reasons for ineffective therapy of nosocomial bronchopneumonia in intubated patients in the ICUs. In the case of intubated or mechanically ventilated patients having chronic bronchopneumonia, determination of drug concentration in the bronchial secretion might be considered when selecting an antibiotic for treatment.

Nosocomial bronchopneumonia is a threatening complication that increases the mortality of hospitalized patients. However, many risk factors are known that worsen the prognosis to critical form, especially in comatose patients treated in intensive care units (ICU) (9, 19, 24). The majority of patients lying unconscious in the ICU are intubated, and some of them are on respirators. In many cases, these patients undergo one or more operations, which also increase their susceptibility for infections. Finally, chronic intratracheal intubation, the possibility of aspiration due to the weakness or lack of the pharyngeal and coughing reflex prior to intubation, the reduced immune system of the exhausted host, and long-term immobility lead to bronchopneumonia (14, 21, 32, 33, 39). Since the causes of the disease in general cannot be eliminated rapidly, chronic forms tend to develop and progression to purulent form usually appears. The basis of therapy is antibiotic treatment, and the drug is chosen according to the culture results from the blood or sputum. However, antibiotic therapy is not always effective (8, 29). The situation is often worsened by nosocomial infections, when polyresistant bacteria make therapy much more difficult (7, 13, 22, 36). In our experience, in spite of the specified antibiotic therapy, cessation of the production of abundant purulent bronchial secretion that contains pathogenic bacteria and recovery from bronchopneumonia are long-term processes. This problem gave rise to our investigation of the concentrations of five cephalosporins (cefuroxime, cefamandole, ceftriaxone, ceftazidime, and cefepime) in the sputum from patients suffering from nosocomial purulent bronchopneumonia in our ICU. To evaluate the effectiveness of the applied antibiotic therapy in the sputum, the MICs of the investigated cephalosporins for the cultured bacteria from the sputum were also determined.

Sample collection and evaluation. Serum and sputum samples were collected from 24 chronically intubated patients who suffered from nosocomial bronchopneumonia by procedures that are in accordance with Hungarian ethical rules. Fourteen females and 10 males were investigated (mean age, 55.2 ± 12.0 years; range, 44 to 68 years; mean body weight, 67.6 ± 14.5 kg; range, 53 to 82 kg). All patients were treated in the ICU because of serious intracranial lesion, and 16 of them were mechanically ventilated. The average duration of endotracheal intubation at diagnosis of pneumonia was 5 days; the range was 3 to 7 days. As soon as pneumonia was diagnosed by X-ray, laboratory investigations, and clinical symptoms, antibiotic therapy was started, but later it was promptly modified as the bacterial culture results from blood or sputum arrived. Our investigations were performed at the beginning of therapy, when, in each 24-h period, five patients received 70 mg/kg of body weight cefuroxime, four patients received 110 mg/kg cefamandole, six patients received 80 mg/kg ceftriaxone, four patients received 80 mg/kg ceftazidime, and five patients received 80 mg/kg cefepime intravenously. Ceftriaxone was administered in two equal doses per day; cefuroxime, ceftazidime, and cefepime were administered in three equal doses per day; and

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TABLE 1. Mean concentrations of five cephalosporins in the serum and sputum 6 hours after intravenous drug administration

Cephalosporin	No. of cases	Mean concn (mg/liter) ± SD in:	
		Serum	Sputum
Cefuroxime	5	26.9 ± 4.9	<0.5
Cefamandole	4	41.5 ± 10.6	<0.5
Ceftriaxone	6	64.8 ± 20.8	1.4 ± 1.2
Ceftazidime	4	9.1 ± 2.0	<0.5
Cefepime	5	28.2 ± 25.3	<0.5

cefamandole was administered in four equal doses per day. Drug concentrations were measured in serum and sputum samples collected on the second day of treatment 6 hours after the last dose of antibiotics. Bronchial secretion was sucked out from the trachea by a sterile suction pipe through the nasotracheal tube.

Bacteria from cultures of the sputum were also isolated, and the MICs of the cephalosporins for the bacterial strains were evaluated.

Analysis using CE. The concentration of cephalosporins was determined by capillary electrophoresis (CE). Serum and sputum samples were stored at −18°C until analysis was performed, 24 h after sample collection. Optimized separation conditions for different cephalosporin antibiotics using CE were investigated in our earlier work (16, 17). The developed method gave satisfactory precision with respect to migration times (relative standard deviation < 1%) and gave linear responses ($R^2 > 0.998$) over a concentration range of about 1 to 100 mg/liter for all studied compounds. These concentration ranges cover the normal therapeutic concentrations of these antibiotics. The capillary electrophoresis instrument was an HP^{3D} model (Agilent, Waldbronn, Germany). In all measurements hydrodynamic sample introduction (10 kPa) was used for injecting samples. The sample solutions were introduced at the anodic end of the capillary. Separations were performed using fused-silica capillaries coated on the outside with polyimide (Polymicro Technology, Phoenix, AZ). Capillaries were 48.5 cm with an inside diameter of 50 μm and an effective capillary length of 40 cm.. The applied voltage was 25 kV. The temperature of the capillary holder was kept constant at 25°C. Detection was carried out by on-column photometric measurement at 270 nm. The electropherograms were recorded and processed by the ChemStation computer program, version 7.01 (Agilent). Before use, the capillaries were preconditioned with the buffer electrolyte for 10 min. During analysis of biofluid samples, the capillaries were also postconditioned after each run by flushing with 0.3 M sodium dodecyl sulfate (2 min), 0.5 M NaOH (6 min), and distilled water (2 min) to remove all the proteinaceous components, which have a high tendency to stick to the capillary walls.

The serum samples were defrosted immediately before analysis. Since the highly viscous sputum sample could not be injected directly into the capillary, 1 g of these samples was lyophilized and then dissolved in 500 μl methanol-water (1:1) prior to analysis. The suitability of sample pretreatment of sputum and other highly viscous biological samples using lyophilization and dissolution before the CE analysis was investigated and validated in detail. Electrophoretic runs were performed as quickly as possible and not later than 4 h after sample preparation.

Determination of MICs. Pure cultures of the bacteria were tested. The antibiotic was dissolved in double-distilled water. The stock solutions were stored at −30°C for 2 weeks; working solutions were stored at 4°C for a maximum of 24 h. The inoculum was prepared as follows. Isolated colonies from blood agar were grown in Mueller-Hinton broth (Oxoid) for 18 h at 37°C. The strains were diluted in Mueller-Hinton broth to yield an inoculum of 1.5×10^5 CFU per ml. The stock solutions of antibiotics were serially twofold diluted in Mueller-Hinton broth to obtain working concentrations of 0.016, 0.032, 0.064, 0.128, 0.25, 1.0, 2.0, 4.0, 8.0, 16.0, 32.0, and 64.0 μg/ml. Ninety-microliter amounts from broth containing twofold concentration increments of antimicrobial agents were added to 96-well microdilution trays. In general, the plate was prepared on the day before inoculation. Each well was inoculated with 10 μl bacterial suspension. Thus, the final inoculum was 1.5×10^4 CFU/ml. The strains were also inoculated on antibiotic-free control plates. Growth (turbidity) of aerobic strains was recorded after 18 h at 37°C. The MIC was reported as the lowest concentration of the antibiotic at which no growth was recorded (34).

Observations. The concentrations of the cephalosporins in the serum 6 hours after intravenous drug administration were as follows: cefuroxime, 26.9 ± 4.9 mg/liter; cefamandole, 41.5 ± 10.6 mg/liter; ceftazidime, 9.1 ± 2.0 mg/liter; ceftriaxone, 64.8 ± 20.8 mg/liter; cefepime, 28.2 ± 25.3 mg/liter.

The levels of cefuroxime, cefamandole, ceftazidime, and cefepime in the sputum remained under 0.5 mg/liter, but the concentration of ceftriaxone was 1.4 ± 1.2 mg/liter (Table 1.).

TABLE 2. MICs for nine bacterial strains from sputum of five cephalosporins

Bacterial species	No. of cases	MIC (mg/liter) of:				
		Cefuroxime	Cefamandole	Ceftriaxone	Ceftazidime	Cefepime
Klebsiella pneumoniae	3	4	4	2	2	2
Acinetobacter baumannii	5	16	64	16	2	8
Citrobacter freundii	2	64	64	2	8	2
Pseudomonas aeruginosa	3	1,024	64	64	2	2
Escherichia coli	4	4	4	2	2	2
Proteus mirabilis	1	2	4	2	4	8
Enterobacter cloacae	3	4	32	2	4	2
Staphylococcus aureus	5	8	2	8	8	8
Staphylococcus epidermidis	4	2	2	2	4	2

Nine bacterial strains were isolated from the sputum cultures of the investigated 24 patients. In 10 cases, two bacteria were found in the tracheobronchial secretion. The results of the MICs for the bacteria are detailed in Table 2. The MICs of the investigated five cephalosporins exceed the mean concentrations of cephalosporins in the sputum. In two cases, the level of ceftriaxone in the sputum was higher than 2.0 mg/liter (2.2 and 3.8 mg/liter), which is theoretically high enough for treating six of the nine bacterial strains, but in both cases MICs for the bacteria were over 4 mg/liter (*Acinetobacter baumannii and Staphylococcus aureus*).

Discussion. Medicinal treatment of bronchopneumonia for chronically intubated patients in intensive care units always means a considerable effort for the clinicians (12, 19, 23). Pulmonary complications in unconscious patients in neurosurgical ICU can also decrease the chances for a satisfactory outcome (15, 31). If production of purulent bronchial secretion becomes chronic in spite of the antibacterial therapy, the likelihood of developing polyresistant bacterial strains increases. Nosocomial infections especially impede treatment, although there are some therapeutic guidelines in the literature (3–5, 28, 39).

The therapeutic benefits of the different cephalosporins have been often described, and their wide spectrum of effectiveness often distinguishes them among the chosen antibiotics (11, 26–28). Cefuroxime (6, 18), cefamandole (38), ceftriaxone (20, 25, 35), ceftazidime (3, 11, 37), and cefepime (5) also are recommended antibiotics for treating pneumonia. The abundant bronchial secretion from purulent bronchopneumonia in chronically intubated comatose patients serves as a suitable medium for bacteria, which makes therapy many times more difficult. Some tests of the antibiotic concentrations in sputum have already been carried out (10, 20, 25, 35, 37). Although antibiotics could be detected in the majority of the measurements, the concentration of the investigated drug did not always reach the MIC of the bacterial strains found in the samples (6). In some other studies, the determined drug levels were low (1, 18, 25), but there are reports of appropriate antibiotic concentration in the sputum, too (2, 11, 30, 37). In consideration of the wide spectrum of the reported results and the low number of measurements directly in purulent secretion, further pharmacodynamical investigations are still needed.

The high mortality rate and the long-lasting treatment period for patients with nosocomial bronchopneumonia despite the specified antibiotic therapy led to the investigation of the presence of antibiotics in bronchial secretions after intravenous drug administration. The level of antibiotics in serum corresponded to the reported results in the literature, but only the mean concentration of ceftriaxone exceeded the 0.5-mg/liter detectability level in the sputum while cefuroxime, cefamandole, ceftazidime, and cefepime remained under this level, and the mean concentrations of all investigated antibiotics did not reach the MICs for the bacteria isolated from the purulent bronchial secretion (Tables 1 and 2).

Conclusions. In spite of the targeted treatment based on the culture from the copious sputum from nosocomial bronchopneumonia in intubated comatose patients, the therapy seems to be often ineffective. One of the main reasons for this clinical experience can be the low concentrations of the drugs—in this case five cephalosporins—in the purulent bronchial secretion. The fast and economical method for detection of drug concentration in the sputum by capillary electrophoresis can help to check the real effectivity of the applied antibiotic. In the case of intubated or mechanically ventilated patients that have chronic bronchopneumonia caused by nosocomial infections, drug monitoring by determination of its concentration in the bronchial secretion might be a method to consider to facilitate appropriate drug selection.

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