THESIS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY (Ph.D.)

Role of the pathogenic oral flora in postoperative pneumonia in neurosurgical patients

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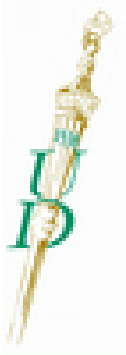

UNIVERSITY OF DEBRECEN
DOCTORAL SCHOOL OF PHARMACOLOGICAL SCIENCES

DEBRECEN, 2009

1. INTRODUCTION

Nosocomial bronchopneumonia is a threatening complication that increases mortality of hospitalized patients. Administration of cephalosporins to patients as prophylaxis for high-risk surgical procedures at the time of induction of general anaesthesia is routinely done in many institutes. To prevent post-operative infectious complications, first and second generation cephalosporins are generally administered because of their wide spectrum of anti-bacterial activities and the possibility of changing to third and fourth generation antibiotics if necessary.

Saliva contains many bacteria, and bad oral hygiene and periodontal diseases lead to the proliferation of pathogenic bacteria that can cause post-operative pneumonia. Since periodontal diseases are common in adults, antibiotic prophylaxis plays a significant role in the prevention of pneumonia in those medical interventions in which the possibility of aspiration exists. Surgical procedures under general anaesthesia are among such procedures, and the high mortality rates that accompany post-operative pneumonia, as well as the expense and treatment difficulties, underlie the significance of this problem. The etiologic role of pathogenic flora in post-operative pneumonia is of special concern for neurosurgical patients because the pharyngeal and coughing reflexes are weak or lacking, and the likelihood of long-term post-operative endotracheal intubation and immobilization are additional risk factors for infectious complications involving the lungs. In our experience, in spite of the specified antibiotic therapy, cessation of the production of abundant purulent bronchial secretion that contains pathogen bacteria and recovery from bronchopneumonia is a long-term process.

In the present study, the efficacy of pre-operative cephalosporin prophylaxis in controlling the growth of pathogenic oral flora in comatose patients undergoing neurosurgical procedures was investigated and the effectiveness of the applied antibiotic therapy in the sputum in case of postoperative pneumonia was also determined.

2.BACKGROUND

2.1. Nosocomial pneumonia

Bacterial pneumonia is one of the most common infectious diseases in adults, and is associated with a high morbidity and mortality. It has also been shown that the frequency of pneumonia in patients who are intubated in intensive care units correlates with the duration of intubation. Aspiration of oral bacteria is one of the main etiologic factors in developing pneumonia in hospitals. Since oral flora is dependent on periodontal status, impaired oral hygiene increases the incidence of respiratory tract infections.

During lengthy surgical procedures, saliva accumulates in the pharynx above the cuff of the tube used for endotracheal anaesthesia. Such secretions are periodically suctioned out, but this procedure cannot always be completely executed. The risk for post-operative aspiration is especially high in comatose patients because of the impaired function of the lower cranial nerves, resulting in impaired pharyngeal and coughing reflexes. Nevertheless, the likelihood of long-term intubation, combined with the immobilization period after brain surgery, also increases the chance of developing pneumonia. Since aspiration of pathogenic oral flora increases the incidence of pneumonia, administration of prophylactic antibiotics in high-risk patients is of enormous clinical importance.

In the 1970th nosocomial pneumonia was mainly determined as post-operative infection by the report of the CDC-NNIS (Centers for Disease Control and Prevention – National Nosocomial Surveillance System, since 75 % of nosocomial pneumonia was diagnosed after surgical interventions. Later, spread of using mechanical respirators on intensive care units highlighted the potential role of endotracheal intubation as a significant risk factor in developing nosocomial pneumonia. It was also established that pneumonia occurs frequently in very young and old patients.

2.2. Patomechanism of nosocomial pneumonia

Microorganisms can pass into the lower airways due to the following mechanisms: 1st aspiration of contaminated naso-oropharyngeal secretion, 2nd inhalation of the infected air, 3rd hematogene spread, 4th direct spread from the infected neighboring organs (gastro-intestinal tract, pleura, etc.). However, the most frequent mechanism is the aspiration of oropharyngeal secretion that contains pathogene bacteria.

2.3. The oral cavity as bacterial reservoir

Due to investigation of the etiological factors in postoperative pneumonia, the role of oral bacterial flora came to the front. It was established, that the oral cavity serves as bacterial reservoir and its anatomical connection to the airways bears a permanent risk for aspirating pathogene microorganisms into the lung.

The largest number of bacteria in the human organs can be found in the oral cavity; more than 500 bacterial species have been isolated until now. There are different bacteria that colonize predominantly the teeth, the mucosa or the gingival sulci. A normal gingival sulcus can contain about 10^3 bacteria, but in an inflammated parodontal pocket 10^8 microorganisms can be found. In healthy individuals there is a harmonic balance between the host and the oral flora. If this balance splits, mucosal infection, caries and periodontal diseases can develop. This can happen if new bacteria species appears in the dental plaque or the number of bacteria increases.

Klebsiellae can be detected in the oral cavity in 1-6% of healthy people, most frequently in consequence of caries and periodontal processes. Two species of Klebsiellae can cause bacteial pneumonia: *Klebsiella pneumoniae* and *Klebsiella oxytoca.*

Streptococcus viridans another member of the norma oral flora was thought to be innocent regarding pneumonia, but in recent studies its role in initiating pneumonia was emphasized.

The number of bacterial species and the absolute amount of microorganisms in the oral cavity is mainly influenced by the periodontal diseases, since dental surface has no own defensive immun activity and it provides an appropriate attaching area for the bacteria.

Microorganisms can attach to the gingiva or in the epithelial surface of the sulcus, but in these location detachment of epithelial cells can decrease the presence of bacteria and stimuli to inflammational processes.

3.4. Mechanisms of developing pneumonia by aspiration of the oral secretion

A. Aspiration of oral pathogens into the lungs

Bacteria existing in the supra- or subgingival plaque can get with the aspirated saliva into the lower airways and they may directly cause inflammation of the lung parenchyma.

B. Enzymes in saliva may modify mucosal surfaces to promote adhesion and colonization ba respiratory pathogens

There are adhesive receptors on the mucosal surface, that are normally hidden by glycoproteins. A lot of hydrolytic enzymes persists in the saliva that can uncover these receptors and increase the adhesion of Gram-negative bacteria to the mucosal surface.

C. Bacteria in the oral cavity can eliminate pellicles

Pellicles is a mucin-rich sheath in the saliva of healthy subjects that inhibits the attachment of bacteria to the mucosa. In case of poor oral hygiene hydrolitic enzymes accumulate in the saliva and digesting pellicula destroy this defensive mechamism.

D. Cytokines originating from periodontal tissues may alter respiratory epithelium

Periodontal disease is a localized chronic inflammation caused by infection of the periodontal tissues by bacteria in dental plaque. Oral pathogens stimulate cells of the oral tisues and periodontium to release a wide variety of cytokines, that entering the saliva upregulate the expression of adhesion receptors on the mucosal surface to promote colonozation of respiratory pathogens.

2.5. Risk factors

The main reason of bacterial pneumonia is aspiration of oropharyngeal secretion. Every disease that can increase possibility of aspiration the content of the oral cavity into the airways promotes the development of pneumonia: neurological diseases (cerebrovascular diseases, epilepsy, stroke, brain tumors, Parkinson-disease, Alzheimer disease, dementia, altered level of consciousness, neuro-degenerative disorders), gastric and eosophageal diseases (malignant tumors, gastroeosophageal reflux, eosophageal diverticulum, gastrectomy, use of antacids and H_2-receptor blockers), abnormalities in the the oral cavity (tumor, articulation disorder, xerostomia, mucosal diseases), drug or alcohol abuse, presence of nasogastric or endotracheal tube.

2.6. Use of cephalosporins in the therapy of pneumonia

Main therapy for bacterial pneumonia is antimicrobial therapy. Empiric antibiotics are choosen if exact pathogen is not clarified, but after microbiologica isolation of the bacteria causing the infection, specified antibiotic therapy is indicated. The therapeutic benefits of the different generations of cephalosporins have been often described and their wide spectrum of effectiveness often distinguishes them among the chosen antibiotics.

2.7. Detection of cephalosporins by capillary electrophoresis

Analysis of cephalosporins was made dominantly by high-performance liquid chromatography (HPLC). Recently a novel technic has come in the front: the capillary electrophoresis. It has two main technics: the micellar electrokinetic chromatography (MECC) and capillary zone-electrophoresis. Both methods are appropriate for fast and economical separation of chemical compounds, but they are not routinely used for determination of drug levels in human samples in clinical investigations.

In this study capillary zone-electrophoresis was applied for direct measurement of cephalosporins in the serum, saliva and sputum for providing useful information for the clinicians in monitoring drug efficacy.

3.10. Role of dental prevention

Oral hygiene: Aspiration of oropharyngeal bacteria into the lungs play a significant role in developing bacterial pneumonia. In high risk patients oral hygiene is one of the most important factor to reduce aspiration of pathogens in the airways.

Use of topical antiseptic drugs: There are many antiseptic solution or intraoral pills for desinfection the oral cavity, but their exact role in inhibition of colonization of oral pathogens into the lung is not yet clarified in all details. The most known oral antiseptic solution is the chlorhexidine gluconate, that inhibits proteases produced by the subgingival bacteria and it also has bactericid effect by destroying the membrane of the microorganisms.

3. OBJECTIVES

Since aspiration of pathogenic oral flora increases the incidence of pneumonia, administration of prophylactic antibiotics in high-risk patients is of enormous clinical importance. Usage of prophylactic cephalosporin for neurosurgical procedures is done routinely worldwide, the drug selected or the dosage used may differ.

Medicinal treatment of bronchopneumonia of chronic intubated patients on intensive care units always means a considerable effort for the clinicians. Pulmonary complications in unconscious patients on neurosurgical ICU can also decrease satisfactory outcome. The high mortality rate and the long-lasting treatment period of nosocomial bronchopneumonia despite the specified antibiotic therapy led to the investigation of the presence of antibiotics in bronchial secretions after intravenous drug administration.

Direct monitoring of the effectiveness of antibiotics is not yet included in any clinical practice. In this study we tested the prophylactic efficacy of three different cephalosporin antibiotics and the applicability of five other cefalosporins in the therapy of purulent bronchopneumonia by direct determination of capillary electrophoresis (CE).

<u>Specific points of the study:</u>

1. Is the concentration of prophylactic cephalosporin in the saliva high enough for satisfactory effectivity?
2. What is the relation of the actual antibiotic concentration in the saliva and the minimal inhibitory concentration (MIC) of the pathogen bacteria isolated from the same saliva sample?
3. What is the concentration of the cephalosporins in the sputum of patients suffering from purulent bronchopneumonia?
4. Further, the prophylactic effect of a single dose preoperative cefazolin on the oral bacteria in relation to postoperative pneumonia was investigated.
5. It was hypothesized that impaired periodontal status and pathogenic oral bacteria significantly contribute to development of postoperative pneumonia following neurosurgical operations. We investigated if there are any statistically verifiable connections between the seriousness of the periodontal diseases and the frequency of postoperative pneumonia in neurosurgical patients?
6. Is the capillary electrophoresis a suitable method for routine determination of actual antibiotic concentration in clinical investigations?

4. PATIENTS AND METHODS

Patients were selected from the neurosurgical patients of the Department of Neurosurgery, University of Debrecen MHSC. All procedures were approved by the Ethical Committee of the University of Debrecen, Hungary.

4.1. Patient reqruitment

The efficacy of pre-operative cephalosporin prophylaxis in controlling pathogenic oral bacterial growth in comatose patients

Investigations were performed during surgical procedures on 30 comatose patients (Glasgow coma scale [GCS] < 7). At the time of induction of general anaesthesia, 10, 11, and 9 patients received 1 g of cefazolin, 1.5 g of cefuroxime, or 2 g of cefamandole intravenously, respectively. Age, gender, body weight, and the duration of surgery are detailed in Table 1. At the completion of the surgical procedure, saliva samples were obtained from above the cuff of the endotracheal tube and residual serum samples from routine blood testing were simultaneously collected by procedures that were approved by the Ethical Committee of the University of Debrecen. The actual antibiotic concentrations in the serum and saliva samples were determined by capillary electrophoresis (CE). The bacteria from the saliva were isolated, and the minimal inhibitory concentrations (MIC) of the cephalosporins against the specific bacteria were also determined.

The effectiveness of cephalosporins in the sputum in nosocomial bronchopneumonia

Serum and sputum samples were collected from 24 chronic intubated patients who suffered from nosocomial bronchopneumonia by procedures that are in accordance with Hungarian ethical rules. Fourteen females and ten males were investigated; mean age 55.2 ± 12.0 years, range 44-68 years, body weight 67.6 ± 14.5 kgs, range: 53-82 kgs. All patients were treated on the ICU because of serious intracranial lesion and sixteen of them were mechanically ventilated. Average duration of endotracheal intubation at diagnose of pneumonia was 5 days; range: 3-7 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 five patients received 70 mg/kg/24h cefuroxime, four patients 110 mg/kg/24h cefamandole, six patients 80 mg/kg/24h

ceftriaxone, four patients 80 mg/kg/24h ceftazidime and five patients 80 mg/kg/24h cefepime. Ceftriaxone was intravenously administered in two equal doses, cefuroxime, ceftazidime and cefepime in three equal doses and cefamandole in four equal doses per day. Drug concentrations were measured in serum and sputum samples collected on the second day of treatment six 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 minimal inhibitory concentrations (MIC values) of cephalosporins for the bacteria strains regarding to the four investigated antibiotics were evaluated.

Role of pathogenic oral flora in postoperative pneumonia following brain surgery:

A matched cohort of 18 patients without postoperative lung complications (controll group) was compared to 5 patients who developed pneumonia (pneumonia group) within 48 hours after brain surgery.

Patients recruited into the study were nonsmokers, aged older than 60 years and were diagnosed with the presence of an intracranial solitary extraaxial supratentorial tumor, Glasgow Coma Scale (GCS) 15; scheduled for elective routine craniotomy and tumor removal on the Neurosurgical Department of the University of Debrecen. Morbidly obese patients, smokers and who had diabetes mellitus, alcohol abuse, any immune deficiency, preexisting pulmonary conditions (e.g. chronic obstructive pulmonary disease, COPD), heart insufficiency and in whom prompt postoperative extubation could not be anticipated or neurological complications appeared, were excluded from the study. The recruited patients had normal preoperative chest X-ray and their laboratory parameters were in the physiological range. Patients had no lower cranial nerve palsy, so they had normal gag and coughing reflexes. Only those patients were recruited finally in the study that had no changes in the neurological status after the operation.

Preoperative dental examinations were performed During dental examination saliva samples were collected for bacterial cultures. Bacterial strains were isolated preoperatively from the saliva of the patients and the minimal inhibitory concentrations (MIC) of cefazolin for each of the saliva-derived bacterial strains were determined.

During extubation, serum samples were obtained and saliva samples were collected from the pharynx above the cuff of the endotracheal tube. The concentrations of cefazolin in the serum, saliva and bronchial secretion were determined and the levels of cefazolin were compared to the MIC values of the bacteria isolated preoperatively from the saliva.

Bacteria were isolated again from the sputum of the five patients in whom postoperative pneumonia developed 48 hours after brain surgery. Sputum samples were carefully collected before ceftriaxone administration by a pulmonologist with a sterile suction-tube to avoid oral contamination. The isolated bacteria were compared to the bacteria isolated before the operation from the saliva of the same patients.

4.2. Methods

Microbiological cultures and determination of the minimal inhibitory concentration

Microbiological cultures were made accordingly to the standard routine clinical investigation for isolating bacteria in human samples.

For determination of MIC values pure cultures of the bacteria were tested. The antibiotic was dissolved in double distilled water. The stock solutions were stored at –30 ^{o}C for 2 weeks; working solutions were stored at 4 ^{o}C for a maximum of 24 hours. The inoculum was prepared as follows: isolated colonies from blood agar were grown in Mueller-Hinton Broth (Oxoid) for 18 hours at ^{o}37 C. The strains were diluted in Mueller-Hinton Broth to yield an inoculum of 1.5 x 10^5 colony forming units (CFU) per ml. The stock solutions of antibiotics were serially two-times diluted in Mueller-Hinton Broth to obtain the 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, 64.0 μg/ml. 90 μl amounts from broth containing two-fold 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 x 10^4 CFU/ml. The strains were also inoculated on antibiotic-free control plates. Growth (turbidity) of aerobic strains was recorded after 18 hours at 37 ^{o}C. The MIC was reported as the lowest concentration of the antibiotic at which no growth is recorded.

Determination of drug concentration in serum, saliva and sputum using capillary electrophoresis

Serum and sputum samples were stored at -18 ^{o}C until analysis was performed, 24 hours after sample collection. The capillary electrophoresis instrument was an HP^{3D} model (Agilent, Waldbronn, Germany). 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 metanol-water (1:1) prior to analysis 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, USA) of 48.5 cm x 50 µm i.d., whose effective length was 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 ChemStation computer program version 7.01 (Agilent).

Dental examination

Preoperative dental examinations were performed by the same experienced dentist on each patient awaiting neurosurgical procedures. In order to be able to quantify the severity of the periodontal disease in patient populations, a numeric scoring system based on commonly accepted disease severity definitions for evaluation of periodontal diseases was established. Periodontal conditions of the patients were categorized in five main diagnoses that were each given a numeric score.

The "Disease Score" was then calculated as the sum of the scores of co-existing periodontal diseases in a patient. In addition, patients received a "Severity Score", which equaled the number of their co-existing periodontal diseases (possible range.

5. RESULTS

5.1. Results of investigation of the efficacy of pre-operative cephalosporin prophylaxis in controlling pathogenic oral bacterial growth in comatose patients

Twenty-one bacteria were isolated from the saliva in patients who received 1 g of cefazolin prior to surgery. The MIC values of 3 bacteria (*Acinetobacter baumannii, Citrobacter freundii és Pseudomonas aeruginosa*) were higher than the mean drug concentrations in the serum (68.4 ± 14.4 mg/l), but the MIC values of 16 bacteria exceeded the level of cefazolin in the saliva (< 0.5 mg/l).

Twenty bacterial species were isolated from the saliva of patients who received 1.5 g of cefazolin prophylactically. Two bacteria (*Citrobacter freundii és Pseudomonas aeruginosa*) had higher MIC values than the mean antibiotic level in the serum (50.3 ± 18.2 mg/l). The concentration of cefuroxime in the saliva (< 0.5 mg/l) remained less than the MIC values of 15 bacteria.

In the patients who received 2 g of cefamandole, 24 bacteria were isolated from the saliva. Four bacteria (*Acinetobacter baumannii, Citrobacter freundii, Enterobacter cloacae és Pseudomonas aeruginosa*) had higher MIC values than the mean drug level in the serum (42.4 ± 9.9 mg/l). The MIC values of 18 bacteria exceeded the levels of cefamandole in the saliva (< 0.5 mg/l).

The concentration of antibiotic in each patient group is given as the mean ± standard deviation (SD).

5.2. Results of testing the effectiveness of cephalosporins in the sputum in nosocomial bronchopneumonia

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

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

Nine bacteria strains were isolated from the sputum cultures of the investigated 24 patients. In ten cases, two bacteria were found in the tracheobronchial secretion. The MIC values for the investigated five cephalosporins exceed the mean concentration of cephalosporins in the sputum. In two cases, the level of ceftriaxone in the sputum was higher than 2.0 mg/l (2.2 and 3.8 mg/l), which is theoretically high enough for treating 6 bacteria strains from nine, but in both cases MIC values of the actual bacteria were over 4 mg/l *(Acinetobacter baumanii and Staphylococcus aureus).*

5.3. Role of the pathogenic oral flora in postoperative pneumonia following brain surgery

Both the Disease Score (Figure 2A) and the Severity score (Figure 2B) was significantly elevated in patients who developed postoperative pneumonia ($p = 0.0018$ and $p = 0.031$, respectively).

To calculate the risk of pneumonia, we divided the patients into a "High periodontal score" (Disease Score ≥ 15, Severity score ≥ 3) and a "Low periodontal score" group (Disease Score < 15, Severity score < 3). Analysis of the relative risk revealed that the "High periodontal score" patients had a significantly greater chance to develop pneumonia than the "Low periodontal score" patients.

When serum cefazolin concentrations were compared between control and pneumonia patients we found no significant difference suggesting that there was no relationship between circulating cefazolin levels and presence of pneumonia after brain surgery.

The same cefazolin resistant Gram negative bacteria were isolated from the saliva pre-operatively and the sputum of the patients who developed postoperative pneumonia. In order to determine whether adequate serum cefazolin concentrations were achieved against the pathogenic bacteria in the pneumonia patients, we calculated the serum cefazolin/MIC ratio (using the MIC that was determined for the bacteria isolated from the sputum) in each of the patients. To provide adequate antibacterial protection, this value should ideally be greater than 1. Although circulating cefazolin/MIC ratios appeared adequate, the same ratios for the saliva of the patients were significantly below 1.

6. DISCUSSION

Bacterial bronchopneumonia is one of the most common infectious diseases in adults, with a well-known high morbidity and mortality, especially in old patients. Accurate information of the general incidence of postoperative nosocomial pneumonia however is lacking, as it is influenced by many factors including for instance the case mix studied, and prior exposure to antibiotics. In a large-scale European study pneumonia accounted for approximately 50% of all intensive care unit (ICU) infections which significantly increased ICU death. Although multiple risk factors are identified, the single most important one has been considered to be intubation. The frequency of pneumonia in patients with endotracheal intubation in intensive care units correlated closely with the duration of intubation. The clinical course of up to 28% of patients receiving long-term mechanical ventilation was complicated by an episode of pneumonia and intubated patients have rates of pneumonia up to 21 times higher than patients without an artificial airway. Pneumonia also often appears as a postoperative complication after various surgical procedures. Pneumonia can develop in surgical patients without previous lung disease or brain damage, but risk for aspiration is higher in older patients. Further, age, sex, obesity, preoperative hospital stay, smoking, chronic obstructive pulmonary disease, other preexisting pulmonary conditions, type and duration of surgery and anesthesia, and nasogastric tube placement would each be considered a significant risk factor. While aspiration of oral bacteria is one of the main etiologic factors in developing pneumonia in hospitals the significance of existing periodontal disease is not completely clarified.

Patients, who waited for or underwent neurosurgical operations, were selected for investigation. As opposed to abdominal surgery, brain surgery is considered “clean”, without open exposure to enteral pathogens. Therefore this surgical population is particularly suitable to investigate the importance of enteral bacteria from the oral cavity and upper airways, in development of postoperative pneumonia. During long surgical procedures, saliva and oropharyngeal secretions accumulate in the pharynx above the cuff of the tube used for intratracheal ventilation. These secretions may then be aspirated because function of the lower cranial nerves can be impaired and so pharyngeal and coughing reflexes return slowly in this patient population. Additionally, the low level of consciousness and prolonged immobilization period after cranial surgery are all important factors in aspiration of saliva and oropharyngeal secretions and can increase the likelihood of developing pulmonary complications. Thus, verification of the importance of aspiration etiology and the ability to

identify patients who are at increased risk to develop postoperative pneumonia has a particularly high clinical importance in neurosurgical patients.

In this study, as the first step the efficacy of prophylactic cefazolin, cefuroxime, and cefamandole in controlling the growth of oral pathogenic bacteria was evaluated by monitoring the actual concentrations of antibiotics in the serum and saliva. The detected drug levels in the serum proved to have a significant effect against bacteria isolated from the saliva, since in the case of cefazolin, only 3 of 21 bacteria were resistant *(Acinetobacter baumannii, Citrobacter freundii,* and *Pseudomonas aeruginosa);* in the case of cefuroxime, only 2 of 20 bacteria were resistant (*Citrobacter freundii* and *Pseudomonas aeruginosa);* and in patients that received cefamandole, 4 of 24 bacteria were resistant *(Acinetobacter baumannii, Citrobacter freundii, Enterobacter cloacae,* and *Pseudomonas aeruginosa).* Unfortunately, the concentrations of every cephalosporin tested was very low in the saliva; cefazolin, cefuroxime, and cefamandole did not reach the MIC values of 76.2%, 75%, and 75% of the bacteria, respectively, and therefore had only a very moderate effect on the oral flora, and thus the potential prevention of postoperative pneumonia.

Medicinal treatment of bronchopneumonia of chronic intubated patients on intensive care units always means a considerable effort for the clinicians. 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/l detectability level in the sputum while cefuroxime, cefamandole, ceftazidime and cefepime remained under this level and the mean concentration of every investigated antibiotics did not reach the MIC values of the bacteria isolated from the purulent bronchial secretion.

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.

In our third study we investigated the significance of periodontal diseases in postoperative pneumonia and the effects of preoperative cefazolin prophylaxis on the oral pathogen flora in older patients after neurosurgical operation. Alhough several methods for evaluation of oral hygiene were reported problems in reliability and validity have also been noted. The currently used methods to assess periodontal status range from simple visual evaluation of presence or absence of tongue coating through manual probing to elaborate bacterial cultures or sophisticated molecular biology techniques. In our study a quantitative

scoring assessment was established using generally accepted visual diagnosis and manual probing .Based on these scores we divided the patients into a "High periodontal score" and a "Low periodontal score" group.Our results showed that the "High periodontal risk" patients had a significantly greater chance to develop pneumonia than the "Low periodontal risk" patients indicating that presence of severe periodontal diseases predisposed to postoperative pneumonia in our patient population. Regarding the low n however, further validation of our scoring system is needed in a larger clinical trial. Nevertheless, these results confirmed that evaluation of periodontal status using a relatively simple visual scoring system may identify patients with high risk for developing postoperative pneumonia.

Indeed, Gram negative bacteria isolated from the saliva of patients with high periodontal score before surgery, were also grown from the sputum after they developed pneumonia post-operatively, further suggesting that the infectious organisms originated from the oropharyngeal region.

In this study capillary electrophoresis was applied for direct determination of antibiotic concentration in human samples. This method currently is not used for such purposes in routine clinical investigations. We tested the clinical applicability of CE that was originally developed for analytical chemistry. During recent investigations CE has been applied and shown to be an inexpensive, simple, and creditable method that requires very small samples and offers the opportunity for clinicians to evaluate the actual efficacy of antibiotics for promoting optimisation of individual antibiotic therapies.

7. SUMMARY, NEW STATEMENTS

1. Preoperative prophylactic administration of cefazolin, cefuroxim and cefamandol provided an adequate drug concentration in the serum against bacteria isolated from the saliva of comatose patients during the neurosurgical operation.
2. The concentrations of the tested cephalosporins were very low in the saliva; cefazolin, cefuroxime, and cefamandole did not reach the MIC values of the majority of the oral bacteria. These antibiotics had only a very moderate effect on the oral flora, and thus their role in potential prevention of postoperative pneumonia could not be supported.
3. In case of purulent bronchopneumonia, the low penetration rate of the investigated cephalosporins (cefuroxim, cefamandol, ceftazidim, ceftriaxon és cefepim) into the sputum does not ensure an appropriate drug concentration for elimination the pathogen bacteria. So, their effectiveness in mono-therapy against purulent pneumonia in high risk patients seems to be doubtful.
4. The MIC values of the bacteria isolated from the sputum of patients having purulent bronchopneumonia proved to be higher than the actual antibiotic concentration. Thus, bacteria existing in the bronchial secretion can easily recolonize the lung parenchyma after finishing antibiotic treatment, so the role of expectorant physiotherapeutical technics in treating pneumonia seems to be revaluated.
5. In some patients that had postoperative pneumonia, the same bacteria could be isolated from the preoperative saliva and later in the sputum. In these patients, the ineffectiveness of prophylactic cefazolin regarding postoperative lung complications could be established.
6. Prevention of postoperative pneumonia is especially important by surgery of high risk patients. In this population decrease of pathogen oral flora by preoperative dental treatment and use of oral antiseptic pills or solutions would be expedient.
7. During recent investigations capillary electrophoresis been applied and proved to be an inexpensive, simple, and creditable method that requires very small samples and offers the opportunity for clinicians to evaluate the actual efficacy of antibiotics for promoting optimisation of individual antibiotic therapies.

9. PUBLICATIONS

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