Effects of Colistin Sulphate, Tigecycline, and Cefoperazone-Sulbactam on the Multi-Drug Resistant Acinetobacter baumannii Experimental Mouse Sepsis Model

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Abstract

Objective: Incidence of *Acinetobacter* infections is increasing both in Turkey and worldwide. Based on their ability to develop resistance, *Acinetobacter* species can cause morbidity and mortality, particularly in newborns, children, immunocompromised patients, and critically ill intensive care patients. There are limited treatment options, particularly in carbapenem resistant *Acinetobacter* strains. In this study, we aimed to compare in vivo activities of colistin sulphate, tigecycline, and cefoperazone-sulbactam in an experimental mouse sepsis model.
Material and Methods: Each of the four study groups consisted of eight Wistar breed albino rats. In total, 107 colonies of the *Acinetobacter baumannii/calcoaceticus* complex were administered intraperitoneally to each rat after the presence of neutropenia with cyclophosphamide. Blood culture samples were taken from all rats after 24 h of treatment and colony count from lung, liver, and kidney specimens were taken after 72 h of treatment.
Results: In the tigecycline-treated group, the presence of positive blood culture results at 24 h were found to be lower than the control group (p=0.01). Presence of positive cultures from lung samples in the tigecycline (p<0.05), colistin (p<0.05), and cefoperazone-sulbactam (p<0.05) groups were found to be lower than the control group. Positive culture of liver samples were found to be significantly lower in colistin (p<0.05) and cefoperazone-sulbactam (p<0.05) groups than the control. Positive culture of kidney samples were found to be significantly lower in colistin (p<0.05), cefoperazone-sulbactam (p<0.05), and tigecycline (p<0.05) groups than the control. However, antibiotic groups did not differ among themselves with respect to positive culture. A comparison of colony counts in lung samples revealed a statistically significant decrease in tigecycline and colistin groups than the control group (p<0.01 and p<0.05, respectively).
Conclusion: In our study, tigecycline, colistin, and cefoperazone-sulbactam were found to be effective on culture positivity in lung, kidney, and liver specimens of rats and they may be a choice for treatment. Tigecycline was found to be more effective on colony counts in lungs and kidneys and is also more effective in reducing the 24 h bacteraemia than the control group. The results of the ongoing clinical trials about tigecycline use in children with severe infection due to resistant microorganisms will give us an idea about this situation. *(J Pediatr Inf 2015; 9: 25-33)*

Keywords: Tigecycline, colistin, cefoperazone-sulbactam, *Acinetobacter baumannii*, carbapenem resistant

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Introduction

Nosocomial infections are among the most important causes of morbidity and mortality in neonatal and pediatric intensive care units (1-3). As a result of the exhaustive use of antibiotics and extended stays in intensive care units in recent years, there has been an increase in the number of resistant infections. Infections that are caused by factors related to increased multiple antibiotic resistance in intensive care units are among the most frequent infections (3, 4). *Acinetobacter* species were shown to be among the frequent infection factors in neonatal and

pediatric intensive care units; they usually cause opportunistic nosocomial infections, increase multiple antibiotic resistance and have a high mortality rate. Immune deficiency or chronic disease in the host, surgery attempts, catheter and urethral sounding, and the use of a mechanical ventilator or an extended stay in the ventilator with an extended period of broad-spectrum antibiotic use in the intensive care units have been described as related risk factors (5-11). Carbapenems, aminoglycosides and combinations with sulbactam are the most frequently used antibiotics for treating antibiotic-resistant infections. Tigecycline, colistin and cefoperazone-sulbactam are among the treatment options that can be used in cases when carbapenem resistance may develop. In our study, we planned to compare the in vivo efficiency of colistin sulfate, tigecycline and cefoperazone-sulbactam against a multiple antibiotic-resistant *Acinetobacter* strain that was isolated in our clinic and studied within an experimental sepsis model.

Material and Methods

In this study, we planned to assess the efficiency of cefoperazone-sulbactam, colistin, and tigecycline against an experimental *Acinetobacter baumannii/calcoaceticus* complex infection. These experimental attempts were made at the Pharmacology Laboratory and Medical and Surgery Experimental Research Center of the Osmangazi University Medical School, and the microbiological tests were performed at the Microbiology Research Laboratory of Osmangazi University Medical School. Before the study, the protocol was approved by the Animal Research Ethical Committee of Osmangazi University Hospital (06.05.2010-159). All the trials that took place during the study were implemented by participants who had certificates in performing animal experiments. Thirty-two Wistar breed albino rats weighing between 230 and 250 grams were used in this study. The rats were randomly divided into four groups by the researchers. The first groups were given colistin, the second group tigecycline, and the third group cefoperazone sulbactam. The group that was not included in the study was the control group. All the rats were isolated and maintained in special cages with clean water and appropriate pellet food. The *Acinetobacter baumannii/calcoaceticus complex* strain used in our study was chosen from among the imipenem-resistant and tigecycline, cefoperazone-sulbactam and colistin-sensitive strains after we examined strains that were obtained from blood cultures from the pediatric neonatal and intensive care units in 2010, from the medical school at Osmangazi University. The following values were present in the antibiogram of the *Acinetobacter baumannii/calcoaceticus* complex isolate; (together with the MIC values) amikacin (>32), aztreonam (>16), cefepime (>16), cefoperazone-sulbactam (≤8), cefotaxime (>32), ceftazidime (>16), ciprofloxacin (>2), colistin (≤0.5), gentamycin (>8), imipenem (>8), levofloxacin (>4), meropenem (>8), piperacillin (>64), piperacillin-tazobactam (>64/4), tetracycline (>8), tigecycline (<12), and trimethoprim-sulfamethoxazole (>2/38). The strain that was selected for the study was preserved in skim milk at -70°C at the Microbiology Laboratory of Osmangazi University Medical School. The organism was plated on blood agar during the study period and allowed to reproduce for 24 hours in an incubator. An inoculum was prepared for the study, and it included a 0.5 Mc Farland standard in ID broth. The culture was made into a $1x10^7$ cfu/mL isolate in SF.

***Acinetobacter baumannii/Calcoaceticus* Complex Experimental Sepsis Model:** Ninety-six hours before the *Acinetobacter baumannii/calcoaceticus* complex inoculum-inclusive suspension was given to all the rats, the animals were sedated with an injection of 100 mg/kg/dose (0.25 mg/dose) of intraperitoneal pantocaine sodium. After an evaluation of their sedation state, they were given cyclophosphamide (Endoxan flacon) intraperitoneally at 150 mg/kg/dose. The cyclophosphamide dose was repeated 48 hours before the inoculum was given. One rat in the control group out of 32 died after receiving anesthesia. The presence of neutropenia was supported by peripheral smears from the remaining 31 rats. All the rats in the study were given *Acinetobacter baumannii/calcoaceticus* complex intraperitoneally at $1x10^7$ colony, including a 0.5 mL suspension. Following the *Acinetobacter baumannii/calcoaceticus* complex inoculation, antibiotic injections were administered, starting at the 8th hour. All the rats were given the following antibiotics intraperitoneally at the given doses: cefoperazone-sulbactam (Sulperazon, Pfizer®) 150 mg/kg/day (2x75 mg/kg), Tigecycline (Tygacil, Pfizer®) 20 mg/kg/day (2x10 mg/kg), and Colistin 5 mg/kg/day (2x2.5 mg/kg). Intraperitoneal applications were implemented at 12-hour intervals. All the rats developed hemorrhagic cystitis before *Acinetobacter* inoculation, which was regarded as a cyclophosphamide-driven side effect. This hemorrhagic cystitis was minimized within 48 hours and then totally disappeared. During the rat follow-up, at the end of 24 hours following the *Acinetobacter* inoculation, the rats exhibited a visible decrease in their movements. This observation might be related to the transmission of the infection.

Six of the rats whose intra-cardiac blood cultures were taken during anesthesia with ether on the 24th hour died as soon as the procedure ended, and 5 of them died

in the following 12 hours. During the sample dissections, these rats were revealed to have developed cardiac tamponade. No tamponade was found in the two rats that died 18 hours after the procedure. Following antibiotherapy, one more rat was found dead on the last day. After the 72-hour therapy, the remaining 17 rats were terminated with pantocaine anesthesia and then sampled. After the sampling, which was performed under sterile conditions with a 1 cm vertical incision in the hypogastric area after cleaning the body and making an incision in the abdomen to open up the chest wall, the liver, kidneys and right lower lobe of the liver were placed in individual sterile containers. To prevent the organs from drying out during the waiting period, the samples were placed in the containers together with 0.5 cc of SF. The tissue samples that were not investigated immediately were preserved at +4°C for less than 12 hours before the examination. The blood samples that were taken on the 24th hour were placed inside of BD BactecTM Peds PlusTM/F culture bottles and left on an automatic culture device. Upon detecting a growth signal in all the samples, the cultures were added to microbiological food containers for verification and growth. After the pieces that were taken from the samples were weighed on precision scales, they were dissected with a tissue homogenizer. The homogenate was diluted to different concentrations (10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, and 10^{-6}) and spread over each plate with 50 microliters of blood agar. The samples were then placed in an incubator at 35-37°C, and after 48 hours of incubation, the microorganism typology, an antibiogram and colony counts were analyzed for the plates that exhibited growth. The N X D X F X 20/W=cfu/g formula was used for the colony count (N: number of colonies on the plate, D: dilution coefficient: 10^{-1}=1/10, F: dilution factor (V+W)/W, V: bouillon volume (1 cc), W: tissue weight (g), and 20: constant coefficient (0.05 ml plate planting)).

Statistical analysis

An SPSS for Windows 16.0 program was used for statistical analysis (SPSS Inc.; Chicago, IL, United States). Fisher's exact test (two-tailed) was used to compare the growth of the organisms in the blood culture and liver, and the kidney and lung tissue cultures. A Mann-Whitney U test was used to compare the colony numbers across tissue cultures. A $p<0.05$ value was considered to be statistically significant.

Results

In this study, the results for eight rats in the tigecycline, cefoperazone-sulbactam and colistin groups and seven in the control group were evaluated. Positive results were found on the 24th hour, and the positive result rates in the tissue cultures (Table 1) and colony numbers in the tissue cultures were found for the blood cultures from all the groups.

Colistin Group: No growth was observed in the blood culture on the 24th hour for the five rats out of eight that were treated with colistin, and three rats exhibited continued growth in their blood cultures. Two rats that had growth in their blood cultures died on the 24th hour, but the other six rats lived for 72 hours, and they were terminated under the 72 hour study protocol. Although growth was found in the liver and lung tissues of one of the rats, growth was observed in the kidney of one rat.

Tigecycline Group: The growth in the blood culture did not continue on the 24th hour in only one rat that received tigecycline treatment. Two rats died without displaying any growth in their blood culture on the 24th hour, and one rat that had growth in its blood culture and five other rats were terminated on the 72nd hour. Although growth was observed in the liver culture from three rats and in the lung culture from one rat, the rat with growth in its blood culture was found to have liver, lung and kidney *Acinetobacter* colonies on the 72nd hour.

Cefoperazone-Sulbactam Group: Four (50%) of the eight rats that received cefoperazone-sulbactam treatment displayed a continuation of growth in their blood cultures on the 24th hour. Growth along with substantial numbers of colonies in the liver and kidneys were observed on the 72nd hour in the two rats from the cefoperazone-sulbactam group. No growth was found in the clinical samples from the other six rats.

Control Group: Growth was found in the blood culture of all seven untreated rats in the control group on the 24th hour, and growth was found in at least one of the tissue cultures.

Given the evaluation that was based on the blood culture growth from the 24th hour of this study, and given that less growth was found in the colistin group in comparison with the control group, no significant difference was found ($p=0.282$). Positive culture results that were found in the liver, lung and kidney samples of the colistin group were clearly lower than the results from the control group (1/8 for all the tissue isolates in the colistin group and 6/7 in the control group; $p=0.01$) (Table 1).

When the tigecycline and control groups were compared with one another in terms of *Acinetobacter* growth in the liver, lung, kidney and blood culture mediums, the growth frequency in the blood culture of the tigecycline group was shown to visibly decrease on the 24th hour ($p=0.01$). No significant difference was found even though the growth in the liver samples from the tigecycline group was lower ($p=0.119$). The growth frequency in liver and kidney samples from the tigecycline-treated group was

statistically lower than that of the control group (in lung samples, it was 2/8 in the tigecycline group and 6/7 in the control group; p=0.04; and in kidney samples, it was 1/8 in the tigecycline group and 6/7; p=0.01 in the control group) (Table 1).

When the cefoperazone-sulbactam and control groups were compared with one another to determine the *Acinetobacter* growth in the liver, lung, kidney and blood culture mediums, no difference was found in the growth frequency in the 24th hour blood culture between those two groups (5/8 in the cefoperazone-sulbactam group and 6/7, p=0.569 in the control group). The growth that occurred in the liver, lung and kidney samples of the cefoperazone-sulbactam group was visibly lower from a statistical perspective in comparison with the control group (for the liver, lung and kidney samples, 2/8 in the cefoperazone-sulbactam group, and 6/7 in the control group; p=0.04 for all three) (Table 1).

In addition to the control and treatment group comparison, the three treatment options were compared amongst themselves with regards to the growth in the blood culture and tissue vulture.

When the colistin and tigecycline groups were compared with one another in terms of efficiency, no significant difference was found even though the growth frequency in the 24th hour blood culture was less in the tigecycline group (17 in the tigecycline group and 4/7 in the colistin group, p<0.282). Although the growth frequency in the liver samples from the colistin group was less than that of the tigecycline group, no significant difference was found (p=0.569). No difference in the growth for the lung and kidney cultures between the colistin and tigecycline groups was found.

When the colistin and cefoperazone-sulbactam groups were compared in terms of efficiency, no difference was found with regards to the 24th hour blood growth, or in the growth frequency in the liver, lung and kidney samples.

When the tigecycline and cefoperazone-sulbactam groups were compared in terms of efficiency in the 24th hour blood culture evaluation, no significant difference was found (p=0.119), even though the efficiency in the tigecycline group was lower than that of the cefoperazone-sulbactam group. No significant difference was found in the growth frequency between the tigecycline and cefoperazone-sulbactam groups in the liver, lungs and kidneys.

For the colony numbers in the rat livers from the study group, no significant difference was found between the three groups that received antibiotic treatment and the non-treated control group, or for the antibiotic groups among themselves. The *Acinetobacter* colony number found in the lung tissue samples in the tigecycline group was found to be lower than that of the control group (p=0.006). Similarly, the *Acinetobacter* colony number in the lung tissue samples in the colistin group was lower than it was in the control group (p=0.021). Although the *Acinetobacter* colony number in the lung tissue samples in the cefoperazone-sulbactam group was lower than it was in the control group, no significant difference was found (p=0.054). However, no significant difference was found between the colistin, tigecycline and cefoperazone-sulbactam groups in terms of colony numbers in the lung samples (Table 2).

Given the comparison of colony numbers in the kidney, and even though there was a significant difference between the colistin and tigecycline groups in comparison with the control group (p=0.032), no significant difference was found between the cefoperazone-sulbactam group and the control group. A comparison of the colistin, tigecycline and cefoperazone-sulbactam groups among themselves revealed no difference in the number of colonies in the kidney (Table 2).

Discussion

The frequency of *Acinetobacter* infections in Turkey and all over the world has been gradually increasing. Because of their ability to develop resistance quickly,

Table 1. Comparison of growth frequency in the blood culture and tissue samples of the groups that received colistin, tigecycline and cefoperazom-sulbactam treatment and the control group

	Tigecycline n=8	Colistin n=8	Cefoperazom -Sulbactam n=8	Control n=7
Blood Culture	1/8[a]	4/8	5/8	6/7
Liver	3/8	1/8[b]	2/8[d]	6/7
Lung	2/8[c]	1/8[b]	2/8[d]	6/7
Kidney	1/8[c]	1/8[b]	2/8[d]	6/7

a; Tigecycline vs. control p=0.01
b; Colistin vs. control p=0.01
c; Tigecycline vs. control p<0.05
d; Cefoperazom-sulbactam vs. control p<0.05

Acinetobacter species lead to infection scenarios that are difficult to treat, especially in specific patient groups (patients that are immunosuppressed, neonatal and of infant age, and intensive care unit patients), and they cause significant mortality and morbidity. The resistance that is developed against the carbapenem group of antibiotics, which is used for treating *Acinetobacter* infections, and the ever-increasing frequency of resistance will eventually minimize the number of treatment options and complicate the treatment of this infection. There has been an increase in the number of carbapenem-resistant *Acinetobacter* infections in our clinic as well, and they cause serious problems during treatment. There are few studies on the cefoperazone-sulbactam antibiotic, which

Table 2. Colony numbers in the rats in the antibiotic group and the control group

	N	N	N	W	W	W	cfu/g		
Group	**KC**	**AC**	**BB**	**KC**	**AC**	**BB**	**KC**	**AC**	**BB**
TIG 1	5	1	1	0.289	0.173	0.187	$1.5x10^4$	$0.7x10^4$	$0.6x10^4$
TIG 2	0	1	0	0.170	0.199	0.198		$0.6x10^4$	
TIG 3	9	0	0	0.176	0.177	0.188	$6.8x10^4$		
TIG 4	0	0	0	0.154	0.156	0.218			
TIG 5	0	0	0	0.210	0.150	0.170			
TIG 6	0	0	0	0.200	0.165	0.190			
TIG 7	0	0	0	0.222	0.197	0.181			
TIG 8	1	0	0	0.473	0.195	0.147	$0.1x10^4$		
CONT 1	1	0	0	0.205	0.157	0.187	$0.5x10^4$		
CONT 2	8	2	5	0.185	0.157	0.178	$5.5x10^4$	$0.9x10^4$	$3.7x10^4$
CONT 3	10	>100	10	0.153	0.226	0.228	$9.2x10^4$	$5.1x10^5$	$0.5x10^4$
CONT 4	0	10	3	0.187	0.161	0.184		$8.9x10^4$	$2.0x10^4$
CONT 5	9	1	5	0.210	0.165	0.178	$5.7x10^4$	$0.8x10^4$	$3.5x10^4$
CONT 6	12	>100	9	0.183	0.226	0.228	$9.8x10^4$	$4,8x10^5$	$0,4x10^4$
CONT 7	0	9	3	0.142	0.167	0.187		$9.1x10^4$	$1.9x10^4$
COL 1	0	0	1	0.243	0.218	0.255			$0.3x10^4$
COL 2	0	0	0	0.275	0.276	0.253			
COL 3	0	0	0	0.153	0.147	0.174			
COL 4	0	0	0	0.160	0.155	0.196			
COL 5	0	0	0	0.211	0.187	0.160			
COL 6	0	0	0	0.159	0.173	0.227			
COL 7	0	0	0	0.195	0.229	0.218			
COL 8	10	20	0	0.169	0.228	0.180	$8.1x10^4$	$9.4x10^4$	
CEF 1	0	0	0	0.182	0.112	0.115			
CEF 2	0	0	0	0.212	0.217	0.216			
CEF 3	30	17	>100	0.261	0.193	0.214	$1.1x10^5$	$1.0x10^5$	$5.3x10^5$
CEF 4	3	10	20	0.185	0.178	0.261	$2.0x10^4$	$7.4x10^4$	$7.4x10^4$
CEF 5	0	0	0	0.160	0.174	0.196			
CEF 6	0	0	0	0.201	0.167	0.188			
CEF 7	0	0	0	0.140	0.182	0.156			
CEF 8	0	0	0	0.205	0.154	0.166			

N: colony number on the plate; D: dilution coefficient: $10^{-1 = 1/10}$; F: dilution factor (V+W)/W; V: bouillon volume (1 cc); W: tissue weight (g); 20: constant coefficient (0.05 mL plate planting)

Following formula was used for the counting of colonies.
Formula: N x D x F x 20/W=cfu/g
TIG: Tigecycline; COL: Colistin; CEF: Cefoperazom-sulbactam; CONT: Control

is one of the treatment options. The use of tigecycline in children is limited, and phase studies are still on-going. Colistin was used frequently before the 1980s, but because of the development of new antibiotics as well as its nephrotoxicity, its use was suspended, although it came to the fore again because of the sensitivity of the *Acinetobacter* species to this drug. There are a few studies that have addressed the treatment of resistant strains via monotherapy or combined therapies of cefoperazone-sulbactam, colistin and tigecycline, which we used in our study as well, and different results have been reported (12-17). In a study published by Livermore et al. (18) in 2010, carbapenem-resistant and MDR *Acinetobacter baumanii*-infected and/or colonized patients were evaluated, and only colistin and tigecycline were found to have high in vitro efficiency against the isolated strains (99.4% and 81.9%).

In addition to its efficiency in minimizing liver and kidney colony numbers, tigecycline also had more significant efficiency in reducing the bacteremia that occurred on the 24th hour. Despite being a bacteriostatic agent, tigecycline had a positive effect on this bacteremia when used at appropriate doses. In their experimental study, Crandon et al. (19) showed that the tigecycline concentrations in *Acinetobacter baumanii*-infected lung tissue were higher than the concentrations in non-infected lung tissues (19). Although the tigecycline concentrations in the lung tissues have not been investigated in our study, that *Acinetobacter* growth rate in the lung tissues was found to be lower than that of the control group, and the colony number in the lungs was significantly lower than it was in the control group. In a pneumonia-modeling study by Pichardo et al. (20), tigecycline was found to be more effective at minimizing the colonies in the lung tissues in comparison with the control group, but it was less effective in comparison with imipenem. Therefore, carbapenem treatment was preferred in imipenem-sensitive cases, and tigecycline was preferred in imipenem-resistant cases. Because an imipenem-resistant strain was used in our study, tigecycline was clearly effective in the lung tissues, and it could be considered as a treatment option. Although the tigecycline in our study seems to be effective in comparison with the control group for minimizing colonies in the lung tissues, it does not have any superiority over colistin and cefoperazone-sulbactam.

With regards to the group that was given colistin in our study, the colistin was more effective in comparison with the control group at minimizing colonies in the lung, kidney and liver. Kasiakou et al. (21) stated that using colistin for treatment enabled clinical improvement or full recovery in 67% of the 50 patients with serious *Acinetobacter* and *Pseudomonas* infections. Similarly, Sobieszczyk et al. (22) reported colistin79% survival in 25 patients with *Acinetobacter* or *Pseudomonas*-driven pneumonia when using the colistin combination. In a study by Katragkou et al. (23) on the efficiency of colistin in the MDR *Acinetobacter* meningitis, the cure rate was 93%; however, the figures were reportedly not explicit because the study took the form of a systematic compilation in which successful studies were published. In their rat-pneumonia model study, Montero et al. (17) used two different carbapenem-resistant *Acinetobacter baumanii*, and colistin was revealed to be ineffective at reducing mortality and eradicating the bacterial colonization of the blood and lung. Levin et al. (24) found that the efficiency of colistin in patients with MDR *Pseudomonas aeruginosa* and *Acinetobacter baumanii* infections (pneumonia, UTIs, catheter-related infection, otitis media, and peritonitis) was 58%, but the lowest rate of recovery was in the lung infection, at 25%. This specific result was elucidated by the negative transfer of colistin into the lungs.

Although no difference was found with regards to the blood culture growth in the cefoperazone-sulbactam group in comparison with the control group, it had a more visibly positive effect on the lung-liver and kidney tissue colonies in comparison with the control group. The efficiency of the *Acinetobacter* infection treatment is related to the use of sulbactam, and there are limited numbers of studies about this drug in the literature. Betrosian et al. (25) investigated the efficiency of two different high-dose ampicillin-sulbactam treatments in MDR *Acinetobacter*-driven critical intensive care patients on ventilator treatments, and they concluded that the clinical improvement from high-dose ampicillin-sulbactam treatments was 69.2%. No difference was reported between these two groups in terms of both bacteriological eradication and 14th day mortality; and it was eventually concluded that the high-dose use of this medicine could be effective.

No difference was found in our study between the uses of colistin and cefoperazone-sulbactam. In their study consisting of 28 MDR-resistant *Acinetobacter*-related critical intensive care unit patients, Betrosian et al. (26) compared colistin and cefoperazone-sulbactam and found no significant difference between the improvement in symptoms, bacteriological eradication and 14th and 28th day mortality rates. In performing their studies in a rat-pneumonia model, Montero et al. used two different carbapenem-resistant *Acinetobacter baumanii* strains (with medium and high resistance) (15), and they showed that sulbactam was totally ineffective at treating pneumonia when used as a monotherapy. Many studies concluded that ampicilin-sulbactam and cefoperazone-sulbactam combinations did not exert significant control over *Acinetobacter*-agent bacteremia and ventilator-attached

pneumonia patients in comparison with imipenem monotherapy. However, because of the problems caused by the use of tigecycline in childhood and colistin nephrotoxicity, cefoperazone-sulbactam can be used as a treatment option in carbapenem-resistant cases. Song et al. (27) used *Acinetobacter* strains that are resistant to all antibiotics, including imipenem, similar to the *Acinetobacter* strain in our study, and these strains only displayed colistin and tigecycline in vitro sensitivity. One difference noted in this study is that the *Acinetobacter* strains used here were classified as those with OXA-51, IMP-1 and VIM-2-type β-lactamase, and the results were evaluated accordingly. In conclusion, these researchers found that only rifampicin was effective against the OXA-51 strain; tigecycline was totally ineffective against the IMP-1 strain despite its in vitro efficiency. The colistin and rifampicin combination did not increase the rifampicin monotherapy efficiency, and its synergistic efficiency could only be seen in the rifampin-imipenem combination. For the VIM-2 strain, rifampicin alone was ineffective, but the rifampin-imipenem combination had bacteriostatic efficiency. The study conclusively emphasized that the rifampin-colistin and rifampin-imipenem combinations could be effective, but the data should be supported by more clinical studies. Our study did not include combination treatments. However, because the resistance profile of the strain used in our study has not been demonstrated genetically, data collection is among the limitations of this study with regards to efficiency evaluation. Other studies reveal similar models when performed with similar resistant strains, or with very different or opposite results when performed in clinics. In recent years, it has become possible to explain the reasons for these differences better by identifying the *Acinetobacter* and resistance mechanisms together with genetic characteristics. The genetic differences that occur between bacteria directly determine the enzyme in advance that will be used in cases of resistance, and they determine the ability to influence the other intracellular changes. This determination causes us to believe that the genetic resistance potential should be determined as well as the drug resistance that is observed in the antibiogram to predict the success of the agents that will be used for treatment. Moreover, it is possible that genetic characteristics also have a role in determining which previously used antibiotherapies will reveal a resistant sub-group in due course.

As in all experimental studies, our study has some limitations as well. In our study, during which a limited numbers of subjects were used (as permitted by the ethical committee), only eight subjects were investigated in each group. The implementation of the invasive procedures was thought to have a possible impact on the results of immune suppressive treatment in forming the sepsis model, but because all the groups went through similar initiatives, the results would not be affected. As in all experimental studies, it should be kept in mind that the results of animal studies may differ from the results of human studies. Depending on the transmission of the infection in relation to different infection types, it is possible to say that the antibiotic with seemingly little efficiency in our study may, in practice, have similar, equal or better efficiency. Similarly, because of the brief period of the study, the resistance that is likely to be developed against these antibiotics has not been considered in this study, and there is a need for further studies in which the possible changes in the efficiency will be evaluated with random culture samples.

Conclusions

In our study, tigecycline, colistin and cefoperazone-sulbactam were found to be more effective for treating *Acinetobacter* infections in the lung, kidney and liver in comparison with the control group. Tigecycline had a more positive effect in that it minimized the spread of bacteremia as well as the spread of kidney and lung infections in comparison with the control group. The existence of significant and similar results within the same group when comparing other treatments with one another may be related to the scarcity of the number of subjects and/or to the fact that each treatment has its own degree of effects. There have not been many comprehensive studies on tigecycline, colistin and cefoperazone-sulbactam in recent years, and there is a need for similar studies to support the efficiency of these medicines. The results of phase studies with respect to the use of tigecycline, especially in children, will enlighten us about the use of tigecycline for serious infections that are associated with especially resistant microorganisms. Despite the abundance of *Acinetobacter*-related studies, problems in treating this infection are still on-going. Although there are efficient treatment modes that are currently available for the fight against this infection, the more efficient, low-cost method with fewer side effects is to follow infection control measures categorically. For strains that are multiple drug-resistant, each and every clinic must keep their own *Acinetobacter* strains under control as part of their treatment services.

Ethics Committee Approval: This study has been approved by the Local Ethical Commitee of Eskisehir Osmangazi University for Experimental Studies (06.05.2010-159).

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