Antimicrobial efficacy of doripenem and its combinations with sulbactam, amikacin, colistin, tigecycline in experimental sepsis of carbapenem-resistant *Acinetobacter baumannii*

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SUMMARY

Acinetobacter baumannii is the most common species to have developed resistance to antibiotics. Due to increasing levels of drug resistance, the available therapeutic options are insufficient in *A. baumannii* infections. This study investigated the efficacy of doripenem monotherapy versus doripenem combination therapy with sulbactam, amikacin, colistin and tigecycline in experimental sepsis. A carbapenem-resistant *A. baumannii* was used to develop a sepsis model in 8-10-week-old Balb/c mice by intraperitoneal injection. Antibiotic therapies were initiated two hours after injection of bacterial suspension. Necropsy was performed at 24, 48 and 72 hours and cultures were made from heart, lung, liver and spleen samples. Bacterial loads of lung and liver were calculated as CFU/g. Combination therapies with doripenem were more effective than monotherapy at 24 and 48 hours of infection but no differences between groups were detected at 72 hours. The combination of doripenem with tigecycline and amikacin began to eradicate the bacterial load of lung and liver after 48 hours of infection, whereas doripenem+sulbactam and doripenem+colistin were started to eradication at 72 hours. The results of the study showed that combination therapies with doripenem are more effective than monotherapy and the combination of doripenem with tigeycline or amikacin has more rapid bactericidal effect than that with sulbactam or colistin.

KEY WORDS: Acinetobacter baumannii, Experimental sepsis, Antibiotic therapy.

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INTRODUCTION

In recent years, nosocomial infections caused by Gram-negative pathogens with multiple antibiotic resistance have become an important problem worldwide (Cisneros *et al*., 1995). *Acinetobacter baumannii* is an oppurtunistic pathogen that can lead to serious nosocomial infections in patients on long-term treatment with broad-spectrum antibiotics and immunosupressed patients (Poirel and Nordmann, 2006; Perez *et al*., 2007; Novak *et al*., 2012). Infections caused by multi-drug resistant *Acinetobacter* spp. are generally more complicated to treat than the infection caused by susceptible strains (Sheng *et al*., 2011; Deveci *et al*., 2012). Although carbapenems are the most popular antibiotics for these infections, carbapenem-resistant *A. baumannii* is a serious problem in many centers (Oliveira *et al.,* 2008*;* Marti *et al.,* 2009*;* Lambiase *et al*., 2012; Llaca-Diaz *et al.,* 2012). The deficiency of new antimicrobial agents with activity against multidrug-resistant Gram-negative microorganisms has led to the combination of alternatives to offer better therapeutic results (Kempf *et al*., 2012). Therefore, in recent years many

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in vitro and *in vivo* studies have investigated combination therapies for carbapenem-resistant *A. baumannii* infections (Pachon-Ibanez *et al*., 2010; Lee *et al*., 2008; Housman *et al*., 2013; Principe *et al*., 2013; Dinc *et al.,* 2013). For instance, our recent experimental study evaluated the therapeutic efficacy of sulbactam in monotherapy and combination with colistin, tigecycline and imipenem against a sepsis model with carbapenem-resistant *A. baumannii*. We found that the addition of sulbactam to imipenem showed better bactericidal activity than imipenem monotherapy (Dinc *et al*., 2013).

Doripenem is a newly marketed carbapenem with an *in vitro* activity against Gram-positive, Gram-negative, and anaerobic microorganisms and is also more stable against carbapenamases than other carbapenems. Thus, doripenem has been considered a valuable addition to the options available for the treatment of serious bacterial infections caused by multidrug-resistant Gram-negative infections in hospitalized patients (Keam, 2008; Dedhia and McKnight, 2009; Queenan *et al*., 2013). This study aimed to investigate the *in vivo* efficacy of doripenem monotherapy versus doripenem combination therapy with sulbactam, amikacin, colistin and tigecycline in an experimental sepsis model with a carbapenem-resistant *A. baumannii* strain.

MATERIALS AND METHODS

Challenge microorganism: This study used a carbapenem-resistant *A. baumannii* strain carrying OXA-51, OXA-58 and PER-1 genes isolated from a patient with nosocomial sepsis. The strain was provided by the Department of Microbiology, Faculty of Medicine, Istanbul University.

Antibiotics: The antimicrobial agents used in this study were the following: doripenem (Johnson & Johnson Industry, Turkey), amikacin (Zentiva, Turkey) sulbactam (Mustafa Nevzat Pharmaceutical, Turkey), colistimethate sodium (Koçak Farma, Turkey), tigecycline (Wyeth, UK).

In vitro study: MIC determinations for doripenem, amikacin, sulbactam, colistin and tigecycline were performed by Etest (Diagnostic Liofilchem, Italy) according to the manufacturer's guidelines. *A. baumannii* suspension prepared to 0.5 MacFarland was cultured in Mueller Hinton Agar (Lab M Ltd, UK) and Etest strips were put in agar for 24 hours of incubation. *Escherichia coli* ATCC 25922 was used as the internal control. The MIC breakpoints for *A. baumannii* against to doripenem, amikacin, sulbactam, colistin and tigecycline were established according to CLSI M07-A9 guidelines (2012) and EUCAST Version 3.1 (2013).

In vivo experiments

Mice sepsis model and antibiotic therapies: Balb/c male mice, aged 8-10 weeks weighing 20-25 g were supplied by Hakan Cetinsaya Experimental and Clinical Research Center / Erciyes University, Kayseri, Turkey. The mice were sheltered in distinct cages and fed ad libitum food and water. Mice were housed in a room with light controlled in 12 h day-night periods. This study was approved by the Local Ethical Committee for Animal Studies of Erciyes University (No: 11/63).

Animals were randomised into control (positive and negative) and treatment groups (doripenem, doripenem+colistin, doripenem+tygecycline, doripenem+sulbactam and doripenem+amikacin). One hundred and five mice were included in this study and each group had 15 mice comprising 5 mice in subgroups for each time point of 24, 48 and 72 h. To create sepsis, except for the negative control group, mice were injected intraperitoneally (*ip*) with 0.5 ml 10^8 cfu/ml *A. baumannii* suspension which was determined in a prestudy. Antimicrobial therapies were started 2 hours after injection of bacterial suspension. Tigecycline 20 mg/kg, sulbactam 240 mg/kg, colistimethate sodium 5 mg/kg, amikacin 15 mg/kg and doripenem 150 mg/kg were divided into two doses and administered every 12 h by *ip*. Drug dosages were determined according to pharmacokinetic and pharmacodynamic information from previous experimental studies (Song *et al*., 2009; Bretonnière *et al*., 2010). Mice were killed at 24, 48 and 72 h post-infection with *ip* injection of overdose anesthetic (150 mg/kg ketamine hydrochloride, Pfizer, Turkey). The lung, liver, heart and spleen

samples were cultured on Tripticase Soy Agar (Merck, Germany) to verify whether sepsis developed or not. In addition, 10-fold dilutions of lung and liver homogenates were processed to obtain quantitative counts as CFU/g at each time point. The bacterial counts at 24, 48 and 72 h in lung and liver were compared between the control and study groups.

Statistical analysis: Results were analyzed using SPSS 15.0 (SPSS Inc., Chicago, IL, USA). Logarithmic transformations were used for statistical analysis. All bacterial counts were presented as mean ± standard error of mean. One-way analysis of variance (ANOVA) and T test were used when appropriate to compare differences between groups. Student-Newman-Keuls method was used to analyse intergroup differences. When the P-value was <0.05, differences were considered statistically significant.

RESULTS

In vitro study: The MIC values for OXA-51, 0XA-58, PER-1 positive *A. baumannii* were 6 µg/ml for doripenem, 48 µg/ml for amikacin, 1 µg/ml for colistin, 1 µg/ml for tigecycline, 16 µg/ml for sulbactam.

In vivo experiments

Efficacy of antibiotics: The bacterial counts in lung and liver tissues for all groups are shown in Table 1 and Figure 1. The lung and liver bacterial counts were statistically different between the control and treatment groups for all time points (P<0.05). At 24 h, combination therapies were more effective than doripenem monotherapy. While there were no significant differences between doripenem+sulbactam and doripenem+colistin efficacies, doripenem+amikacin

TABLE 1 - *Therapeutic effects of antibiotics on lung and liver bacterial counts.*

Groups	*Bacterial counts (Mean±SEM)*					
	Lung (Log_{10} CFU/g)			*Liver (Log_{10} CFU/g)*		
	24 h	*48 h*	*72 h*	*24 h*	*48 h*	*72 h*
Control (n=15)	7.76±0.10^{a}	7.78±0.06^{a}	5.12±0.28^{a}	8.14±0.19^{a}	7.43±0.32^{a}	5.54±0.44^{a}
Doripenem (n=15)	6.64±0.42^{a,b}	4.09±0.19^{b}	2.10±0.55^{b}	6.89±0.29^{a,b}	4.05±0.24^{b}	1.99±0.52^{b}
Doripenem+sulbactam (n=15)	4.84±1.19^{b,c}	1.69±0.54^{c}	0.84±0.52^{b}	4.79±1.23^{b,c}	1.88±0.33^{c}	0.98±0.60^{b}
Doripenem+colistin (n=15)	4.76±0.84^{b,c}	2.36±0.62^{c}	0.96±0.65^{b}	4.80±0.88^{b,c}	2.25±0.62^{c}	0.90±0.63^{b}
Doripenem+amikacin (n=15)	3.47±0.45^{c}	0.96±0.62^{c}	0.38±0.38^{b}	3.44±0.47^{c}	0.96±0.62^{c}	0.43±0.43^{b}
Doripenem+tigecycline (n=15)	2.47±0.51^{c}	1.11±0.69^{c}	0.97±0.6^{b}	2.21±0.66^{c}	1.05±0.67^{c}	0.94±0.62^{b}

SEM: Standard error of mean. ^{a,b,c}Statistically significant differences between groups indicated by different letters in same column (P<0.05).

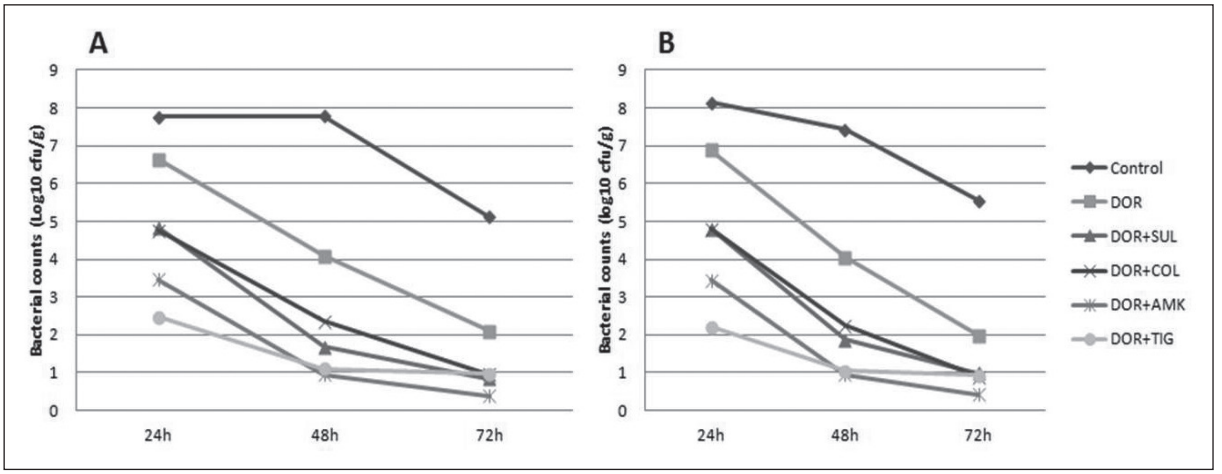

FIGURE 1 - *Results of colony counts in lung (A) and liver (B) at 24, 48 and 72 h of therapy. DOR, doripenem; SUL, sulbactam; COL, colistin; AMK, amikacin; TIG, tigecycline.*

showed better bactericidal activity than these treatments but the tigecycline combination did not. There were no statistical differences in the bacterial loads of lung and liver among combination therapies (P>0.05). However, all combination groups showed a better efficacy than the monotherapy group at 48 h. At 72 h, monotherapy and combination therapies showed the same results on the bacterial counts of lung and liver. Doripenem combinations with tigecycline and with amikacin showed faster bactericidal activity than with colistin and with sulbactam. The doripenem+tigecycline combination was the most effective therapy decreasing bacterial counts in all groups.

DISCUSSION

Multidrug resistant (MDR) *Acinetobacter* spp. strains threaten successful treatment of infections, especially in critical care patients worldwide. Carbapenems are the most active agents against MDR *Acinetobacter* spp. strains. However, due to the overuse of carbapenems, many centers have faced the emergence of carbapenem-resistant *Acinetobacter* spp. and the incidence is reported to be over 50% (Garza-Gonzáles *et al*., 2010; Alp *et al*., 2012; Hou *et al*., 2012; Lemos *et al*., 2013). In our recent study, this rate was detected to be 92% in intensive care units with a high crude mortality (Alp *et al*., 2012). Due to the emergence of resistance against different classes of commercially available antimicrobials, colistin has been reconsidered as a therapeutic option. However, the clinical effectiveness of colistin is still doubtful and very low rates have been reported by different researchers (Montero *et al*., 2004; Falagas and Kasiakou, 2005; Motaouakkil *et al*., 2006; Kalin *et al*., 2013). Although combination therapies may be alternative therapeutic options for better clinical efficacy, there are limited data on these therapies. (Roberts *et al*., 2001; Pachón-Ibánez *et al*., 2010; Lemos *et al*., 2013). In our very recent study, sulbactam efficacy in mono and combined therapies was evaluated in a carbapenem-resistant *A. baumannii* sepsis model (Dinc *et al*., 2013). In that study, colistin monotherapy was found to be the most effective therapy and only sulbactam+imipenem had better efficacy than imipenem monotherapy. However, Pantopoulou *et al.* (2007) reported that the combination of colistin and rifampicin was more effective than colistin monotherapy in experimental infection with multiresistant *A. baumannii*. Another experimental study found that colistin monotherapy was not the best choise for carbapenem-resistant strains (Montero *et al.,* 2002). In the studies of Montero *et al.* (2004) and Pachón-Ibánez *et al.* (2010), imipenem, sulbactam or colistin combinations with rifampin or rifampicin were found to be more efficient than colistin monotherapy. In addition, our very recent clinical study investigating the efficacy of colistin and colistin+sulbactam therapies for patients with ventilator-associated pneumoniae due to MDR *A. baumannii* found that bacterial clearance was better in the combination group (Kalin *et al*., 2013).

Due to increased resistance among *A. baumannii* strains against commonly used carbapenems, doripenem being the newest carbapenem has become the drug of choice for clinicians. However, there few studies have reported on the activity of doripenem mono and/or combination therapies on MDR-resistant bacterial infection around the world. Housman *et al.* (2013) compared mono and combination therapies with ampicillin+sulbactam, doripenem and tigecycline against MDR *A. baumannii* in an *in vitro* pharmacodynamic model, demonstrating that aggressive doses of ampicilin+sulbactam combined with tigecycline or doripenem may be good option for these infections. Principe *et al.* (2013) investigated the *in vitro* activity of doripenem and its combinations with colistin, tigecycline and amikacin. Doripenem showed *in vitro* synergistic activity in combined forms against doripenem-resistant *A. baumannii* strains, suggesting that these results may offer *in vivo* novel combination therapies. Queenan *et al.* (2013) evaluated the *in vitro* and *in vivo* synergy of doripenem+levofloxacin or ciprofloxacin and reported that doripenem+levofloxacin combination may provide a clinical advantage for treatment of non-susceptible *A. baumannii* infections. The present study investigated the efficacy of doripenem, which is more stable against beta-lactamases and shows broad-spectrum activity than imipenem, in monotherapy and in combination with current

therapeutic options (sulbactam, colistin, tigecycline, amikacin). As a result, the combination therapy had a better efficacy on bacterial clearance than doripenem monotherapy at 48 h. Among the combination groups, the combination of doripenem with tigeycline or amikacin had more rapid bactericidal effect than sulbactam or colistin.

Several studies reported that tigecycline and sulbactam show *in vitro* activity to carbapenem-resistant *A. baumannii*, but there are still doubts about combination therapy in clinical studies (Rodríguez-Hernández *et al*., 2001; Song *et al*., 2007; Sheng *et al*., 2011; Deveci *et al*., 2012; Kalin *et al*., 2013). Sulbactam has been reported to show synergistic activity with tigecycline, colistin and carbapenems (Montero *et al*., 2002; Song *et al*., 2009; Pongpech *et al*., 2010; Sheng *et al*., 2011; Deveci *et al*., 2012; Kempf *et al*., 2012). In addition, some experimental infection models have shown that imipenem, meropenem or rifampicin combinations with sulbactam are more effective than monotherapy (Ko *et al*., 2004; Pachón-Ibánez *et al*., 2006). Regarding tigecycline, in previous experimental studies combination therapies with tigecycline have been reported to decrease the bacterial load of tissues (Entenza *et al*., 2009; Yilmaz *et al*., 2012). The present study showed similarities with these previous studies. In the case of amikacin, Bernabeu-Wittel *et al*. (2005) reported that although amikacin and imipenem had synergistic effects on experimental multiresistant *A. baumannii* pneumonia, the *in vivo* effects of combination therapy were not superior to imipenem monotherapy. However in this study, the combination with amikacin had a rapid bactericidal effect at 48 h.

In conclusion, although doripenem is the newest addition to the carbapenems and it shows activity against Gram-positive bacteria, Gram-negative bacteria, including extended-spectrum β-lactamase-producing strains, and anaerobic pathogens (Keam, 2008; Dedhia and McKnight, 2009), the present study indicated that combination therapies with doripenem were better than doripenem monotherapy in terms of bacterial clearance of lung and liver at 48 h. Amikacin and tigecycline appear to be the most effective agents in combination with doripenem. However, these data have to be supported by advanced clinical research to determine the best therapeutic choice in serious carbapenem-resistant *A. baumannii* infections.

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