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Comparison in a rat thigh abscess model of imipenem, meropenem and cefoperazone-sulbactam against *Acinetobacter baumannii* strains in terms of bactericidal efficacy and resistance selection

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Abstract

Background: We compared imipenem, meropenem and cefoperazone-sulbactam against hospital originated *A. baumannii* strains in terms of bactericidal efficacy and selection of resistant mutants during treatment in a rat thigh abscess model.

Methods: A total of 18 strains were inoculated in 54 animals (one strain for three animals). Randomly selected 10 among these 18 strains were inoculated in another 10 rats as the control group. Imipenem, meropenem and cefoperazone-sulbactam were the antibiotics compared. After four days of treatment, Wistar albino rats (200 to 250 g) were sacrificed and the abscess materials were processed for mean colony counts and for the presence of resistant mutants.

Results: The mean CFUs per gram (mean \pm (std. deviation) [$\times 10^4$]) of the abscess were: 9,14 (25,24), 2,11 (3,78), 1,20 (1,70) in the imipenem (n = 17), meropenem (n = 18) and cefoperazone-sulbactam (n = 17) groups, respectively. The differences were not significant. On the other hand, no resistant mutant was detected in abscess materials.

Conclusion: This study indicated; first, cefoperazone-sulbactam is comparable to carbapenems in bactericidal efficacy in this particular abscess model and second, emergence of resistance due to spontaneous mutations is not at least a frequent phenomenon among *A. baumannii*.

Background

Acinetobacter species are associated with fatal infections in hospitals, particularly in intensive care units [1]. Severe underlying conditions like head trauma or head surgery that cause gross aspiration are major risk factors for *Ac-*

netobacter infections [2,3]. The relation between *Acinetobacter spp* and an ominous outcome do not, however, solely depend on the fact that the members of this genus tend to cause infections in patients with severe underlying

conditions. Another determining feature is these bacteria often appear as multiply resistant to antibiotics [4].

Multiple resistance in *Acinetobacter* is believed -in most instances- to be related to the abnormal expression of chromosomally encoded, inherited mechanisms, like porins, penicillin bounding proteins and chromosomal beta-lactamases [5-8].

Resistance to beta-lactams due to altered intrinsic mechanisms is not unique to *Acinetobacter*. Down regulated porins co-operate with over expressed chromosomal beta-lactamases and confer resistance to beta-lactams in some other bacteria, as well. *Pseudomonas aeruginosa* and *Enterobacter cloacae* are well known examples [9].

Normally, in resting conditions, these intrinsic mechanisms are under strict control in *P. aeruginosa* and *E. cloacae*. During replication, certain mutations push these systems out of control as to give a resistant phenotype to the mutants. In the presence of antibiotics these highly resistant mutants are selected. This phenomenon is called "emergence of resistance during antibiotic treatment", which is of great concern in medical practice [10].

Since, *Acinetobacter* species bear similar systems; emergence of resistance during treatment might be a significant problem for this genus as well. As soon as we know, however, "emergence of resistance during treatment" has never been tested in *Acinetobacter* in vivo conditions.

Here, we compared three most effective antibiotics against *Acinetobacterbaumannii* in a rat thigh abscess model in terms of bactericidal efficacy and selection of resistance.

Methods

Bacterial strains

To ensure the clonal variability, we obtained a total of 18 *A. baumannii* strains from four university hospitals of different regions. Strains were susceptible to those studied antibiotics. MICs were obtained by E-test method. The MICs of the strains ranged as for imipenem 0.25 to 1 mg/L, meropenem 0.03 to 4 mg/L and cefoperazone-sulbactam 1 to 8 mg/L.

In our institute, we first re-identified the strains by classical methods. Later, glucose non-fermenting, oxidase negative, non-motile isolates those able to grow at 44 °C were further identified by Sceptor System non-fermenter ID panel (Becton Dickinson Diagnostic Instrument Systems, USA).

Animal model

Male Wistar albino rats of 200 to 250 g in weight were inoculated by 6 log 10 colony-forming units (CFUs) of the

test strain in one thigh. The method has been explained in details elsewhere [11]. Briefly, fresh overnight broth cultures of the strains were adjusted to 8 log 10 CFUs per ml and three Whatman disks per strain were saturated each by 10 µl with the adjusted broth culture.

Following local ketamine and xylazine infusion approximately one cm incision were made in the medial side of right thighs of the rats. Whatman disks -one per animal- were implanted deep in the muscles and later the skin closed by metal clips. Antibiotics were applied by intra-peritoneal route two hours after the inoculation. Consequently, 54 animals were implanted with that of 18 *A. baumannii* strains. An additional 10 rats, as the control group, were implanted with randomly selected strains among these 18 strains. The control group did not receive antibiotic.

Antibiotic daily amounts and dosing were as follows: imipenem, 120 mg/kg every 8 h; meropenem 120 mg/kg every 8 h; cefoperazone 400 mg/kg/day (with a fixed ratio of [1:1] cefoperazone to sulbactam).

Antibiotic bioassay, Colony count and resistance selection

On the fourth day, 30 minutes after the last dose of the antibiotic infusion, animals were sacrificed. Following this, without any delay, 0.5 to one ml blood was aspirated by appropriate needle from cardiac compartments. Sera was separated and stored at minus 80 °C until the antibiotic bioassay test.

The abscess were totally excised with its capsule and put in pre-weighted sterile tubes with one ml Mueller-Hinton (MH) broth, all in aseptic conditions. After homogenization of the abscess by sterile glass rods, 10 and 100 µl of these suspensions were inoculated on two separate MacConkey agar plates for colony count and 100 µl each on three MH agar plates supplemented with 4 mg/L imipenem, 4 mg/L meropenem or 16 mg/L cefoperazone-sulbactam. Before evaluation, plates were over-night incubated at 37 °C in an incubator. Colonies grown on the antibiotic supplemented media were re-tested by agar disk diffusion for resistance to these antibiotics.

Antibiotic bioassays were performed on MH agar plates. MH agars were supplemented with 6 to 7 log 10 CFUs of *E. coli* ATCC 25922 at 55 °C, just before pouring to the plates. Afterwards, under strict sterile conditions, five wells (2–3 mm diameter) per plate were made and each well was inoculated by 20 µl of serum. Plates were evaluated after an over-night incubation at 37 °C.

Statistical analysis

Statistical analysis was performed by a software package SPSS (ver 7.5). Significance was defined as $p < 0.05$. Continuous variables were compared by ANOVA test.

Results

Abscess from 17 rats in each the imipenem and cefoperazone-sulbactam groups and from 18 rats in the meropenem group were eligible. Results from two rats, one in the imipenem and one in the meropenem groups were excluded because of the technical problems during antibiotic bioassay test.

All of the control animals were alive and the abscesses were well developed. The CFUs per 100 μ l of samples in the control group were higher than the countable limits. Antibiotic bioassays, abscess weights and CFUs per 100 μ l of samples were shown in the table. Standard deviations in the treated group are apparently lower than the control group. Hence, it is not wrong to say that the treated groups have significantly lower bacterial counts. On the other hand, there was no statistical significance either between the weights of abscess materials or between mean CFUs obtained from 100 μ l of these samples. The mean CFUs per gram ($\times 10^4$) of the abscess were: 9,14 ($\pm 25,24$) in the imipenem group, 2,11 ($\pm 3,78$) in the meropenem group and 1,20 ($\pm 1,70$) in the cefoperazone-sulbactam group. Although the mean CFUs per gram of abscess was lower in the cefoperazone-sulbactam group this was not significant. Antibiotic bioassays were also similar and adequate for all the groups. Important is, in this study we were not able to detect any resistant isolate in the abscess materials.

Discussion

Acinetobacter spp has a tendency to develop resistance to multiple antibiotics [12]. The co-operation of down regulated outer membrane porins with chromosomal beta-lactamases and/or PBPs have been already proposed to explain the multiple resistance in *Acinetobacter* [7,8,13].

In Turkish hospitals, resistance to expanded-spectrum beta-lactams among *Acinetobacter* is not less than 80% and only half of this resistance has been shown to be related to extended-spectrum beta-lactamases [14,15]. Although the resistance mechanisms in the remaining half have not been studied, it would not be unwise to accuse above mentioned intrinsic mechanisms here, as well.

Here, we studied among *A. baumannii* strains from different regions of Turkey. We selected *A. baumannii*, because this species is proposed as more common in nosocomial infections and probably more virulent relative to the other members of the genus [2,12]. On the other hand, the strains were selected from different regions to ensure the clonal variability. In these conditions and up to four days

we could not detect any resistant mutant under the treatment of commonly used three antibiotics among these *A. baumannii* isolates. Nevertheless, resistance might emerge with prolonged time of exposure to these antibiotics [16].

Conclusions

Results of this study convince to think that "emergence of resistance during treatment" with the above antibiotics is not at least a frequent phenomenon for *A. baumannii* and cefoperazone-sulbactam is as effective as carbapenems in the abscess model.

Authors' contributions

Kolayli Fetiye: Take place in planning the study plus worked in the animal model

Aynur Karadenizli: Take place in planning the study plus worked in the animal model

Erdem Okay: Worked in the animal model

Sarpkaya Oz: Worked in the animal model

Fatma Budak: Worked in the bioassay and MIC tests

Sibel Gundes: Worked in the bioassay and MIC tests

Haluk Vahaboglu: Planning and writing the manuscript

Table 1: Comparison of variables between groups

	Imipenem (n = 17)	Meropenem (n = 18)	mean \pm (std. dev) Cefo-sulb ¹ (n = 17)	Control (n = 10)	<i>p</i> ²
CFUs ³	166.71 (285.87)	109.00 (165.38)	156.41 (229.48)	> 1000 -	0.73
Abscess Weight (grams)	0.071 (0.049)	0.125 (0.253)	0.138 (0.037)	0.120 (0.058)	0.41
CFUs/gm abscess ($\times 10^3$)	9,14 (25,24)	2,11 (3,78)	1,20 (1,70)	>100	0.23
Bioassay ⁴	19.88 (2.45)	19.33 (2.63)	20.76 (4.42)	0 -	0.43

¹ Cefo-sulb, cefoperazone-sulbactam ² *p* value obtained by ANOVA test. Comparisons were between treatment groups. ³ CFUs; colony forming units in 100 μ l of sample ⁴ Bioassay: zone diameters in mm

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