Table 2: Surface local anesthetic activity of diastereoisomeric hexyloxyderivative mixtures expressed as concentrations evoking anesthesia lasting 20 min (with their ranges) and relative indices of anesthetic activity in comparison to cocaine

cis : *trans* molar ratio (%)	$EC_{20\,min}$ ($mol \cdot l^{-1}$)	Efficiency index (SLLA)
100 : 0	7.04×10^{-5} (6.80–7.22)	142.2
84 : 16	8.77×10^{-5} (8.38–8.96)	114.0
66 : 34	6.58×10^{-5} (6.20–6.96)	152.0
50 : 50	5.40×10^{-5} (5.24–5.53)	185.2
34 : 66	5.21×10^{-5} (5.08–5.34)	192.0
16 : 84	4.50×10^{-5} (4.38–4.71)	222.2
0 : 100	6.0×10^{-5} (5.81–6.13)	166.8
cocaine	1.0×10^{-2}	1.0

Each value was obtained from 3–6 separate measurements in the least three different concentrations

In the present study the observed indices of LAA of individual *cis*- and *trans*-isomers did not differ significantly (142.8 vs. 166.8). However, when the mixtures of both isomers in various molar concentrations were applied, the intensity of LAA has changed (Table 2). When the molar concentration ratio of *cis*- and *trans*-isomers was 84 : 16 (%) the index of local anesthetic efficiency was the lowest (114.0). When the ratio was reversed, the efficiency index was the highest (222.2). Dependence of LAA on molar ratios of the mixtures of the isomers could be expressed by a sinusoidal curve (Fig. 2). On the other hand a qualitatively different, two peaks curve was found in our previous experiments for phenylcarbamates with the six carbon ring. In this case the highest index of LAA was found when *cis* : *trans* percentual mixture ratio was 16 : 84, i.e. reversed [8].

It seems that the problems of diastereoisomerism in a series of phenylcarbamates are complex and connected mainly with conformational changes at the regions of sodium channels that bind to the tertiary amine moieties of local anesthetic. Although we cannot answer these specific questions now, the existing synergism between individual stereoisomers intimate some attribute which could influence the mechanism of local anesthetic efficiency.

3. Experimental

The surface local anesthetic activity of all compounds was estimated on rabbits cornea according to the method of Vrba and Sekera [11]. Different concentrations of the compound were applied into the conjuctival sac for 30 min. Then, corneal sensitivity was tested by hair esthesiometer repeatedly in 3 min intervals. Full anesthesia occurred if no response was elicited by 6 consecutive stimulations. Each compound was tested in 3–6 separate measurements with a least three different concentrations. Calculated $EC_{20\,min}$ express the time of full local anesthetic lasting 20 min and/or efficiency indices the relative anesthesia activity to the standard drug cocaine (cocaine = 1).

Acute toxicity was estimated on mice after s.c. administration of 1% solutions of tested compounds. The mortality of the animals was recorded 24 h after the application and expressed as LD_{50} in $mg \cdot kg^{-1}$ [12].

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In vitro effect of imipenem on *Acinetobacter baumannii*

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Imipenem at suprainhibitory concentrations (2×, 4× or 8× MIC) induced postantibiotic effects (PAEs) (suppression of bacterial growth after a short time exposure of bacteria to antimicrobials) against two of three *Acinetobacter baumannii* strains. The highest concentration tested demonstrated the longest delay of bacterial regrowth (1.7 h (strain 5570) or 3.9 h (strain 6070)). All. *A. baumannii* strains showed changes in surface hydrophobicity and serum sensitivity after treatment with imipenem. The antibiotic at 8× MIC reduced hydrophobicity of the strains most significantly (from 42.3%–72.0%) as compared to controls (without antibiotic). Susceptibility of the treated bacteria to serum bactericidal activity has also been lowered. Though imipenem suppressed bacterial growth and decreased surface hydrophobicity of the bacteria, it increased survival of bacteria after incubation with serum. These different alterations observed in the studied strains should be taken into account when evaluating the effects of imipenem.

1. Introduction

Acinetobacter spp. have been more and more frequently responsible for hospital infections over the past three decades [1, 2]. *Acinetobacter baumannii*, as one member of this genus, is an opportunistic pathogen causing pneumonia with high mortality [3, 4]. Treatment of these serious infections is being made complicated by the widespread multidrug resistance of these organisms [5–7]. Literature data documented that imipenem remains the most active antibiotic for *Acinetobacter* isolates [5, 8], though also strains being resistant to imipenem have emerged [9, 10].

Aminoglycosides, quinolones and carbapenems of beta-lactam antibiotics can induce a postantibiotic effect (suppression of bacterial growth after a short time exposure of organisms to antimicrobials) against Gram-negative bacteria [11–13]. In many cases, the suppression of bacterial growth was associated with alterations of some bacterial properties [14–16].

In the present study, suppression of bacterial growth, changes in cell surface hydrophobicity and serum sensitivity of three *A. baumannii* strains treated with suprainhibitory concentrations of imipenem were evaluated.

2. Investigations and results

Imipenem showed PAEs only against two (5570 and 6070) of three *A. baumannii* strains (Table 1). Suppression of growth of treated strains lasted 0.3 h and 1.7 h (2× MIC); 0.9 h and 2.2 h (4× MIC) and 1.9 h and 3.7 h (8× MIC). Suprainhibitory concentrations of imipenem affected some properties of all strains studied. Surface hydrophobicity of the treated strains was decreased. Most effective was the highest antibiotic concentration. Imipenem at 8× MIC reduced cell hydrophobicity to 42.3% (5570), to 49.2% (6070) and to 72.0% (16246) as compared to controls. Also sensitivity of exposed bacteria to the bactericidal activity of serum was lowered. Survival of treated bacteria was in the range from 112.4%–186.2% (2× MIC), from 158.8%–326.6% (4× MIC) and from 139.3%–283.0% (8× MIC). The viability of nontreated bacteria was 95.2% (5570), 141.4% (6070) and 116.0% (16246).

3. Discussion

The pharmacodynamic parameter postantibiotic effect (PAE) may potentially lead to better timing of antimicrobial doses in clinical practice [17]. In the present study, imipenem demonstrated PAEs only against two of three *A. baumannii* strains tested. The results supported the studies of some other authors, who showed a PAE of imipenem for gramnegative bacteria, mainly for *Pseudomonas aeruginosa* [11, 18, 19]. In contrast, no *in vitro* PAE of imipenem against *A. baumannii* and *P. aeruginosa* ATCC 27853 was observed [20, 21] corresponding with our results.

Hydrophobicity as one of the important properties of bacterial surface, is associated with the adhesion, which may influence the amount and distribution of bacteria, contributing to the development of infection. Virulent strains in some pathogens have been found to be more hydrophobic than avirulent ones [22, 23]. Though imipenem had induced PAE only against two strains out of three tested, bacterial populations of all strains has been physiologically altered. Treated strains showed lowered surface hydrophobicity. The most efficient antibiotic concentration was the highest one. Literature data documented that hydrophobicity of bacteria was studied after the effect of antibiotics mainly at subinhibitory concentrations. The published results are various and it is suggested that antimicrobials may selectively affect cell hydrophobicity. Similarly to our results, a decreased hydrophobicity of some gramnegative bacteria – *P. aeruginosa*, *Escherichia coli* and *Klebsiella pneumoniae* treated with antimicrobials was reported [24–26]. On the contrary, some studies showed an increased bacterial hydrophobicity after exposure to antimicrobials [27, 28].

Resistance of bacterial strains to the bactericidal activity of human serum is useful in determining the clinical significance of the strains [29]. Several authors found a correlation between the virulence of some bacteria and the serum resistance [30, 31]. The presented study showed a decrease in serum sensitivity of *A. baumannii* strains treated with suprainhibitory concentrations of imipenem mainly at 4× or 8× MIC, which means, that the viability of the exposed strains was significantly increased after incubation with serum. Numerous literature data mention the alterations in sensitivity of bacteria to bactericidal activity of serum after exposure to imipenem, but preferentially at subinhibitory concentrations. In agreement with our observation, a reduced serum sensitivity of other bacteria, e.g. *K. pneumoniae* after treatment with imipenem in most subinhibitory concentrations tested, was also found [26]. On the other hand, Adinolfi and Bonventre [32] similarly to Darveau and Cunningham [33] published an increased serum sensitivity of *P. aeruginosa* and *E. coli* after exposure to imipenem. No changes in the serum sen-

Table 1: MICs and PAEs of imipenem against *A. baumannii*

Strain	MIC (mg/l)	Conc. (mg/l)	PAE (h)
5570	3.12	2× MIC	1.7
		4× MIC	2.2
		8× MIC	3.7
6070	3.12	2× MIC	0.3
		4× MIC	0.9
		8× MIC	1.9
16246	3.12	2× MIC	NM
		4× MIC	NM
		8× MIC	NM

NM: not manifested PAE

Table 2: Hydrophobicity and serum sensitivity of *A. baumannii* strains (mean ± SD) treated with imipenem

Strain	Conc. (mg/l)	Hydrophobicity*	Serum sensitivity***
5570	0	90.3 ± 0.2 (100) **	95.2 ± 4.2
	2× MIC	75.7 ± 2.0 (83.8)	112.4 ± 4.9
	4× MIC	68.2 ± 1.4 (75.5)	158.8 ± 18.7
	8× MIC	38.2 ± 2.3 (42.3)	139.3 ± 13.7
6070	0	91.7 ± 0.5 (100)	141.4 ± 14.3
	2× MIC	88.9 ± 0.3 (96.9)	186.2 ± 14.9
	4× MIC	87.2 ± 0.7 (95.1)	326.6 ± 35.6
	8× MIC	45.1 ± 2.0 (49.2)	283.0 ± 17.3
16246	0	82.9 ± 0.6 (100)	116.0 ± 5.5
	2× MIC	73.9 ± 1.0 (89.1)	124.2 ± 8.4
	4× MIC	72.8 ± 0.9 (87.8)	213.6 ± 15.2
	8× MIC	59.7 ± 1.3 (72.0)	190.9 ± 6.6

* Percentage decrease in absorbance of the lower aqueous phase compared with that of the original suspension
** Percentage hydrophobicity in parentheses
*** Percentage of viable bacteria after incubation with serum

sitivity of treated bacteria have been observed [24, 32, 33].

The results showed that imipenem at suprainhibitory concentrations demonstrates various physiological effects on *A. baumannii* strains and that its effects may be broader than only inhibition of growth. Also, these additional effects may be useful in the judgement of clinical significance of the bacteria.

4. Experimental

4.1. Materials

Bacterial strains: three strains of *Acinetobacter baumannii*, isolates 5570 and 6070 from cases of urinary tract infections and isolate 16246 from a respiratory tract infection, were examined.

Antibiotic: Imipenem (Tienam®, Merck-Sharp and Dohmechibret AG, Germany) was used in the experiments.

Serum: Serum obtained from human blood of three healthy volunteers was diluted in physiological saline and stored at −70 °C until needed.

4.2. Methods

4.2.1. Minimal inhibitory concentration (MIC)

Macrodilution broth method using serial two-fold dilutions of antibiotic was applied. The medium used was Mueller-Hinton broth supplemented with 25 mg of $CaCl_2$ and 12.5 mg of $MgSO_4$ per liter (MHB). The MIC was defined as the lowest concentration of antibiotic which prevented visible growth after an incubation at 37 °C for 24 h.

4.2.2. Postantibiotic effect (PAE)

Bacterical suspensions were treated with imipenem (2×, 4× or 8× MIC) for 30 min. Control cultures were left untreated. After 30 min, the antibiotic was eliminated by the dilution method and regrowth of the control as well as the exposed bacteria was measured for 24 h [34]. The bacterial suspensions obtained after regrowth were centrifuged and bacterial pellets were washed, adjusted for optical density and used for serum sensitivity and cell surface hydrophobicity assays. The PAE was defined as the difference between the time required for the treated and the corresponding untreated cultures to grow to a chosen point (A_{25}) on the absorbance curve. A_{25} was defined as 25% of the maximum absorbance of the control culture (A_{25} represented approximately the growth of 1 $\log_{10}$ CFU) [13].

4.2.3. Serum bactericidal assay

The method described by Siegfried et al. [35] was applied. The adjusted bacterial suspensions (0.05 ml) with 10% serum (0.05 ml) in microplates were rotated on a roller at 45° at 37 °C for 180 min. Then the samples were withdrawn and spread on agar plates to determine the viable count. Susceptibility of bacteria to serum bactericidal activity was expressed as percentage of bacteria surviving after incubation with serum in relation to the original count of bacteria enumerated at 0 min in the controls. The values in Table 2 show means from four measurements ±SD (standard deviation).

4.2.4. Cell surface hydrophobicity

The slight modification of method described by Rosenberg et al. [36] was used. The treated as well as control cultures (without antibiotic) adjusted to an OD_{400} of 1.0 were vortexed with xylene and incubated for 30 min. After phase separation, the optical density of the lower aqueous phase was measured at 400 nm. Hydrophobicity was determined as a percentage decrease in the optical density of the lower aqueous phase compared with the optical density of the initial cell suspension.

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