J. Basic Microbiol. **38** (1998) 5–6, 383–388

(Institute of Preventive and Clinical Medicine, Department of General Microbiology, Bratislava, 83301 Slovakia)

Serum sensitivity and cell surface hydrophobicity of *Klebsiella pneumoniae* treated with gentamicin, tobramycin and amikacin

ANNA HOŠTACKÁ

(Received 20 January 1998/Accepted 14 May 1998)

A suppression of bacterial growth of *Klebsiella pneumoniae* after 30 min treatment with gentamicin, tobramycin and amikacin at suprainhibitory concentrations was found (postantibiotic effect, PAE). The antibiotics at a concentration of 2 × MIC induced PAEs in the range of 0.5–1.7 h, PAEs manifested by aminoglycosides at a concentration of 4 × MIC varied between 0.8–3.3 h. Susceptibility of exposed bacteria to serum bactericidal activity was efficiently enhanced compared with controls (without antibiotics). A survival of treated bacteria was between 12.7–36.6% (2 × MIC) or 5.0–8.3% (4 × MIC). The percentage of viable nontreated bacteria moved between 70.2–83.0% at these conditions. Surface hydrophobicity of bacteria exposed to aminoglycosides was only slightly increased. The results indicate that exposure of *K. pneumoniae* to a suprainhibitory concentrations of aminoglycosides *in vitro* enhanced the susceptibility of this strain to human serum bactericidal activity. Whether this phenomenon occurs *in vivo*, remains to be determined.

Klebsiella pneumoniae as one of widely recognized opportunistic pathogen has been encountered in outbreaks of hospital-acquired infection, including mainly urinary tract infections, pneumonia, septicaemia (BODEY *et al.* 1989, STAMM 1991, MALONEY and WILLIAMS 1995). The treatment of many of these infections becomes increasingly complicated because resistant strains have emerged (DAVIES 1994, PITT 1996). Values of minimal inhibitory concentration of antimicrobial drugs alone are poor indicators of antimicrobial activities because they are determined in a static situation in which the antibiotic concentration remains the same over the whole incubation period (ELKHAÏLI *et al.* 1997). The pharmacodynamics of antibiotics may be evaluated in several ways, including the postantibiotic effect (PAE) (suppression of bacterial growth after a limited exposure of microorganisms to the antimicrobials). This parameter may represent an important value leading to the optimal selection of drug dosage interval (CRAIG and VOGELMAN 1987). Antimicrobial agents inducing long PAEs can be administered with longer dosing intervals than before without losing efficacy and with lower frequency of unfavourable reactions. PAE is often accompanied with changes in some bacterial activities (GUAN *et al.* 1992, GOTTFREDSSON *et al.* 1993, GARCIA *et al.* 1995, HOŠTACKÁ and KARELOVÁ 1997).

Postantibiotic effects, the serum sensitivity changes and the cell surface hydrophobicity alterations induced by supra-inhibitory concentrations of gentamicin, tobramycin and amikacin in a clinical isolate of *K. pneumoniae* were studied.

Materials and methods

Bacterial strain: *Klebsiella pneumoniae* was isolated from a patient suffering from respiratory infection.

Antibiotics: Gentamicin (Gentamicin sulfate, Lek, Ljubljana), tobramycin (Tobramycin sulfate, Biogal, Hungary) and amikacin commercially manufactured as amikin (Amikacin sulfate, Bristol – Myers SQIBB, Co., USA) were used in the study.

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

Minimal inhibitory concentration (MIC): The macrodilution broth method using serial twofold dilutions of antibiotic was applied. MUELLER-HINTON broth supplemented with 25 mg of $CaCl_2$ and 12.5 mg of $MgSO_4$ per litre was used in all experiments. The MIC was defined as the lowest concentration of antibiotic allowing no visible growth of bacteria after incubation at 37 °C for 24 h. MIC values were 0.78 mg/l for gentamicin and tobramycin and 6.25 mg/l for amikacin.

Postantibiotic effects (PAE): PAE was determined as previously described (HOŠTACKÁ and KARELOVÁ 1997). Bacterial suspension was exposed to 2 × or 4 × MIC of the antibiotic for 30 min. Control cultures (without antibiotic) were left untreated. Then the bacterial suspension was diluted to eliminate the antibiotic. Three different dilutions of the control culture were made (10^{-4}, 10^{-5}, 10^{-6}) to obtain a control with an inoculum as close as possible to the inoculum of the treated culture. The samples were then incubated (37 °C) and regrowth of bacteria was followed for 24 h. After 24 h, the treated as well as control bacterial suspensions were centrifuged and bacterial pellets were used for serum sensitivity and cell surface hydrophobicity assays. The PAE was defined as the difference between the time required for the exposed 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) (ODENHOLT-TORNQVIST and BENGTSSON 1994).

Serum bactericidal assay: The assay was a slight modification of a method previously described (MILER *et al.* 1979, SIEGFRIED *et al.* 1992). The bacterial suspensions (treated with antibiotic as well as control ones) were incubated with 10% serum for 180 min. Then the samples were withdrawn and spread on agar plates to determine the viable count. Susceptibility of bacteria to serum bacteriocidal activity was expressed as the percentage of bacteria surviving after incubation with serum in relation to the original count of bacterial enumerated at 0 min in the controls. The values in Tables 2 and 3 show means from four measurements ± standard deviation (SD).

Cell surface hydrophobicity: The method of ROSENBERG *et al.* (1980) was applied. The exposed as well as control cultures (adjusted to an OD_{470} of 1.0) were vortexed with xylene for 60 s and then incubated for 30 min. Following phase separation, the optical density of the lower aqueous phase was measured at 470 nm. The results were expressed as a percentage decrease in the optical density of the aqueous phase compared with the optical density of the initial cell suspension.

Results

Postantibiotic effects of the tested antibiotics against *K. pneumoniae* are given in Table 1. Gentamicin at a concentration of 2 × MIC induced a PAE of 5.1 h, PAEs obtained with the same concentration of tobramycin was 0.55 h and of amikacin 1.7 h. The antibiotics at a concentration of 4 × MIC showed longer PAEs compared with 2 × MIC. A PAE of 1.5 h was manifested by gentamicin, of 0.8 h by tobramycin and of 3.3 h by amikacin. Delay of regrowth of treated bacteria was accompanied with significant changes in the sensitivity of *K. pneumoniae* to the human serum bactericidal activity. The susceptibility of exposed bacteria was increased after treatment with all aminoglycosides at both concentrations tested. The survival of bacteria exposed to 2 × MIC of antibiotics was 35.7% (gentamicin), 36,6% (tobramycin) and 12.7% (amikacin) (Table 2). More effective was a higher concentration of antibiotic. In this case, the survival of bacteria was 5.0% (gentamicin), 21.5% (tobramycin) and 8.3% (amikacin). The viability of nontreated bacteria was between 70.2% and 83.0% at these conditions. Further experiments showed that suprainhibitory concen-

Table 1
PAEs of the antibiotics against *K. pneumoniae*

Antibiotic	Concentration (mg/l)	PAE* (h)
Gentamicin	2 × MIC	0.5
	4 × MIC	1.5
Tobramycin	2 × MIC	0.5
	4 × MIC	0.8
Amikacin	2 × MIC	1.7
	4 × MIC	3.3

* PAE, the difference in the time required for exposed and unexposed cultures to grow to a chosen point (A_{25}) on the absorbance curve

Table 2
Serum sensitivity (mean ± SD) in exposed (2 × or 4 × MIC) and control cultures of *K. pneumoniae*

Antibiotic	Concentration (mg/l)	Serum sensitivity*
Gentamicin	0	70.9 ± 5.3
	2 × MIC	35.7 ± 5.7
	4 × MIC	5.0 ± 1.5
Tobramycin	0	83.0 ± 11.2
	2 × MIC	36.6 ± 1.2
	4 × MIC	21.5 ± 1.0
Amikacin	0	70.2 ± 6.7
	2 × MIC	12.7 ± 2.8
	4 × MIC	8.3 ± 1.2

* Percentage of viable bacteria after incubation with human serum

Table 3
Hydrophobicity (mean ± SD) in exposed (2 × or 4 × MIC) and control cultures of *K. pneumoniae*

Antibiotic	Concentration (mg/l)	Hydrophobicity*	
Gentamicin	0	58.2 ± 4.2	(100)**
	2 × MIC	63.7 ± 1.6	(109.4)
	4 × MIC	67.6 ± 1.0	(116.2)
Tobramycin	0	55.2 ± 3.5	(100)
	2 × MIC	66.0 ± 2.6	(119.6)
	4 × MIC	56.5 ± 1.2	(102.4)
Amikacin	0	58.1 ± 3.3	(100)
	2 × MIC	70.2 ± 2.1	120.9)
	4 × MIC	60.1 ± 1.0	(103.6)

* Percentage decrease in absorbance of the lower aqueous phase compared with that of the original suspension
** Percentage hydrophobicity in parentheses

trations of aminoglyosides did not alter the surface hydrophobicity of *K. pneumoniae* as significantly as they did serum sensitivity of *K. pneumoniae* (Table 3). The aminoglycosides at 2 × MIC increased surface hydrophobicity to 109% (gentamicin), to 119.6% (tobramycin) and to 120.9% (amikacin) of the control values. Surface hydrophobicity of bacteria exposed to antibiotics at 4 × MIC was enhanced to 116.2% (gentamicin), to 102.4% (tobramycin) and to 103.6% (amikacin) compared with controls.

Discussion

The emergence of multiresistant microbes, including *Klebsiella* spp., mainly in the immunocompromised host, has made the treatment of these infections difficult (GRUNEBERG 1990, DAVIES 1994). Aminoglycosides represent an important class of antibiotics in the treatment of severe bacterial infections despite their toxicity and the introduction of newer and safer antimicrobials. The postantibiotic effect being one of the pharmacodynamic parameters can provide a more precise and suitable basis for the antibiotic use with prolonged dosing intervals and with no loss of efficacy (CRAIG and VOGELMAN 1987). Results of this study showed a delay of regrowth of *K. pneumoniae* after a short time treatment with the tested aminoglycosides. Longer PAEs were induced by a higher concentration of antibiotics. The most effective was amikacin. Similar results were published also by some other authors. MAGNUSSON *et al.* (1955) observed postantibiotic effect of gentamicin against *K. pneumoniae* in the range between 0.5–0.8 h in dependence on temperature. Significant postantibiotic effect was reported also with amikacin, netilmicin, isepamicin and tobramycin for *K. pneumoniae* strains (DE MONTCLOS *et al.* 1994, DEN HOLLANDER *et al.* 1996). To the contrary, no clear PAE of ciprofloxacin was found by HOWARD *et al.* (1993) for *K. pneumoniae.*

Relationships between postantibiotic effects and modification of some bacterial properties were documented. Alterations in the production of some virulence factors, in macromolecular biosynthetis, in ultrastructure of bacterial cells, in the profile of outer membrane proteins, were observed (GUAN *et al.* 1992, GOTTFREDSSON *et al.* 1993, MAJTÁN and HOŠTACKÁ 1996, HOŠTACKÁ and KARELOVÁ 1997). Serum bactericidal activity is one of the most important host defense mechanisms against bacterial infections (TAYLOR 1983). Resistance to this bactericidal activity may be a significant virulence factor of bacteria. Increased serum sensitivity of *K. pneumoniae* after treatment with gentamicin, tobramycin and amikacin at both suprainhibitory concentrations was found in our experiments. That means, exposed bacteria manifested lowered resistance to serum bactericidal activity *in vitro.* The most significant increase in the susceptibility of bacteria was seen after treatment with gentamicin. Different results concerning the modification of serum sensitivity of bacteria treated with antibiotics at subinhibitory concentrations (sub-MICs) were published. Cefepime, ceftazidime and imipenem at sub-MICs, but not amikacin or ciprofloxacin rendered a serum resistant *Pseudomonas aeruginosa* more sensitive to the bactericidal action of serum (DARVEAU and CUNNINGHAM 1990). Similarly ADINOLFI and BONVENTRE (1988) observed that Escherichia coli strain treated with sub-MICs of imipenem and exposed to serum was reduced in viability compared with control, while serum susceptibility of *Staphylococcus aureus* was not changed. Also some aminoglycosides as well as quinolones at sub-MICs enhanced the sensitivity of *K. pneumoniae* strains to the serum bactericidal activity (HOŠTACKÁ 1997a, b). TATEDA *et al.* (1994) suggested that enhanced serum sensitivity of *P. aeruginosa* caused by erythromycin was associated with changes in bacterial surface components, such as outer membrane proteins and lipopolysaccharide.

Cell surface hydrophobicity represents the initial nonspecific step to the adhesion in the pathogenesis of infections. Hydrophobic cells were more virulent in mice and manifested a higher degree of adherence to cells (SELTMANN *et al.* 1986, QADRI *et al.* 1988, HAZEN *et al.* 1991). In this study, suprainhibitory concentrations of the aminoglycosides tested did not

affect the surface hydrophobicity of *K. pneumoniae* as effectively as they did the serum sensitivity. The cell surface hydrophobicity was only slightly increased. Effects of antibiotics, but mainly those at sub-MICs concentrations, on surface hydrophobicity of bacteria are known, but these results are not unambiquous. Some studies showed an increased bacterial surface hydrophobicity (KADURUGAMUWA *et al*. 1985, NOMURA and NAGAYAMA, 1995), on the other hand, a decreased surface hydrophobicity of bacteria treated with sub-MICs of antibiotics was also reported (TATEDA *et al.* 1993, BRAGA *et al*. 1995, HOŠTACKÁ 1977a, b). Results obtained by KADURUGAMUWA *et al.* (1985) indicated that increased surface hydrophobicity of *K. pneumoniae* after sub-MICs of antibiotics was connected with reduced capsule formation, or with reduction of the thickness of the capsular layer (NOMURA and NAGAYAMA 1995).

In conclusion, gentamicin, tobramycin and amikacin at suprainhibitory concentrations induced postantibiotic effects against *K. pneumoniae.* Delay of bacterial regrowth was accompanied with increased sensitivity of bacteria to serum bactericidal activity. It is possible that, *in vivo*, the enhanced serum sensitivity of treated bacteria would contribute to the decrease of bacterial infection in the host.

Acknowledgements

The author would like to express her appreciation to ANNA KORENAČKOVÁ for expert technical assistance.

References

ADINOLFI, L. E. and BONVENTRE, P. F., 1988. Enhanced phagocytosis, killing and serum sensitivity of *Escherichia coli* and *Staphylococcus aureus* treated with sub-MICs of imipenem. Antimicrob. Agents Chemother., **32**, 1012–1018.

BODEY, G. P., ELTING, L. S., RODRÍGUES, S. and HERNÁNDES, M., 1989. *Klebsiella bacteremia* – a 10-year review in a cancer institution. Cancer, **64**, 2368–2376.

BRAGA, P. C., DAL SASSO, M., MACI, S., REGGIO, S. and PIATTI, S., 1995. Influence of subinhibitory concentrations of brodimoprim and trimethoprim on the adhesiveness, hydrophobicity, hemagglutination and motility of *Escherichia coli.* Chemotherapy, **41**, 50–58.

CRAIG, W. A. and VOGELMAN, B., 1987. The postantibiotic effect. Ann. Intern. Med., **106**, 900–902.

DARVEAU, R. P. and CUNNINGHAM, M. D., 1990. Influence of subinhibitory concentrations of cephalosporins on the serum sensitivity of *Pseudomonas aeruginosa.* J. infent. Dis., **162**, 914–921.

DAVIES, J., 1994. Inactivation of antibiotics and dissemination of resistance genes. Science, **264**, 375–382.

DE MONCLOS, M., GUINET, M. and CHAPUIS, C., 1994. Post antibiotic effect of three aminoglycosides against *Klebsiella pneumoniae.* Path. Biol., **42**, 419–424.

DEN HOLLANDER, J. G., MOUTON, J. W., BAKKER-WOUDENBERG, A. J. M., VLEGGAAR, F. P., VAN GOOR, M. P. J. and VERBRUGH, H. A., 1996. Enzymatic method for inactivation of aminoglycosides during measurement of postantibiotic effect. Antimicrob. Agents Chemother., **40**, 488–490.

ELKHAÏLI, H., KAMILI, N., LINGER, L., LEVÊQUE, D., POMPEI, D., MONTEIL, H. and JEHL, F., 1997. *In vitro* time-kill curves of cefepime and cefpirome combined with amikacin, gentamicin or ciprofloxacin against *Klebsiella pneumoniae* producing extended-spectrum beta-lactamase. Chemotherapy, **43**, 245–253.

GARCIA, L. B., BENCHETRIT, L. C. and BARRUCAND, L., 1995. Penicillin post-antibiotic effects on the biology of group A streptococci. J. Antimicrob. Chemother., **36**, 475–482.

GOTTFREDSSON, M., ERLENDSDÓTTIR, H., KOLKA, R., GUDMUNDSSON, A. and GUDMUNDSSON, S., 1993. Ultrastructural alterations of bacteria during the postantibiotic effect. Chemotherapy, **39**, 153–162.

GRUNEBERG, R. N., 1990. Changes in the antibiotic sensitivities of urinary pathogens 1971–1989. J. Antimicrob. Chemother., **26**, 3–11.

GUAN, L., BLUMENTHAL, R. M. and BURNHAM, J. C., 1992. Analysis of macromolecular biosynthesis to define the quinolone-induced postantibiotic effect in *Escherichia coli.* Antimicrob. Agents Chemother., **36**, 2118–2124.

HAZEN, K. C., BRAWNER, D. L., RIESSELMAN, M. H., JUTILA, M. A. and CUTLER, J. E., 1991. Differential adherence of hydrophobic and hydrophilic *Candida albicans* yeast cells to mouse tissues. Infect. Immun., **59**, 907–912.

HOŠTACKÁ, A., 1997a. Alterations in serum sensitivity and hydrophobicity of *Klebsiella pneumoniae* after the treatment with subinhibitory concentrations of aminoglycosides. Zbl. Bakt., in press.

HOŠTACKÁ, A., 1997b. Hydrophobicity and serum sensitivity of *Klebsiella pneumoniae* treated with sub-MICs of quinolones. Microbios, **91**, 137–146.

HOŠTACKÁ, A. and KARELOVÁ, E., 1997. Outer membrane proteins and elastase of *Pseudomonas aeruginosa* after the postantibiotic effect induced by amikacin. Chemotherapy, **43**, 118–122.

HOWARD, B. M. A., PINNEY, R. J. and SMITH, J. T., 1993. Contribution of post-antibiotic lag and repair-recovery to the post-antibiotic effects of ciprofloxacin on *Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus* and *Streptococcus pyogenes*. Chemotherapy, **39**, 22–31.

KADURUGAMUWA, J. L., ANWAR, H., BROWN, M. R. W. and ZAK, O., 1985. Effect of subinhibitory concentrations of cephalosporins on surface properties and siderophore production in iron-depleted *Klebsiella pneumoniae*. Antimicrob. Agents Chemother., **27**, 220–223.

MAGNUSSON, V., JONSDOTTIR, TH., GUDMUNDSDOTTIR, H., ERLENDSDOTTIR, H. and GUDMUNDSSON, S., 1995. The *in vitro* effect of temperature on MICs, bactericidal rates and postantibiotic effects in *Staphylococcus aureus*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*. J. Antimicrob. Chemother., **35**, 339–343.

MAJTÁN, V. and HOŠTACKÂ, A., 1996. Influence of the postantibiotic effect and postantibiotic sub-MICs effect of netilmicin, tobramycin, ciprofloxacin and perfloxacin on alginate production by *Pseudomonas aeruginosa*. Folia Microbiol., **41**, 228–232.

MALONEY, S. B. and WILLIAMS, W. J., 1995. Epidemic nosocomial pneumonia in the intensive care unit. Clin. Chest Med., **16**, 209–223.

MILER, I., VONDRÁČEK, J. and HROMÁDKOVÁ, L., 1979. The bactericidal activity of sera of healthy neonates and of newborns with hyperbilirubinaemia to *Escherichia coli*. Folia Microbiol., **24**, 143–152.

NOMURA, S. and NAGAYAMA, A., 1995. Mechanism of enhancement of bactericidal activity of phagocytes against *Klebsiella pneumoniae* treated with subminimal inhibitory concentrations of cefodizime. Chemotherapy, **41**, 267–275.

ODENHOLT-TORNQVIST, I. and BENGTSSON, S., 1994. Postantibiotic effect, and postantibiotic effect of subinhibitory concentrations, of sparfloxacin on Gram-negative bacteria. Chemotherapy, **40**, 30–36.

PITT, T. L., 1996. Epidemiology of multiply resistant gram-negative bacteria in hospitals. Med. Microb. Lett., **5**, 284–291.

QUADRI, F., HOSSAIN, S. A., ČIŽNÁR, I., HAIDER, K., LJUNGH, A., WALDSTRÖM, T. and SACK, D., 1988. Congo red binding and salt aggregation as indicators of virulence in *Shigella* species. J. Clin. Microbiol., **26**, 1343–1348.

ROSENBERG, M., GUTNICK, D. and ROSENBERG, E., 1980. Adherence of bacteria to hydrocarbons: a simple method for measuring cell-surface hydrophobicity. FEMS Microbiol. Lett., **9**, 29–33.

SELTMANN, G., PAL, T. and TSCHÄPE, H., 1986. Surface hydrophobicity of plasmid-carrying virulent *Shigella flexneri* and their avirulent variants. J. Basic Microbiol., **26**, 283–287.

SIEGFRIED, L., PUZOVÁ, H., KMEŤOVÁ, M. and KERESTEŠOVÁ, A., 1992. Killing of α-haemolytic and non-haemolytic *Escherichia coli* strains in human serum and polymorphonuclear leucocytes. J. Med. Microb., **37**, 3–7.

STAMM, W. E., MC KEVITT, M., ROBERTS, P. L. and WHITE, N. J., 1991. Natural history of recurrent urinary tract infections in women. Rev. Infect. Dis., **13**, 77–84.

TATEDA, K., HIRAKATA, Y., FURUYA, N., OHNO, A. and YAMAGUCHI, K., 1993. Effects of sub-MICs of erythromycin and other macrolide antibiotics on serum sensitivity of *Pseudomonas aeruginosa*. Antimicrob. Agents Chemother., **37**, 675–680.

TATEDA, K., ISHII, Y., HIRAKATA, Y., MATSUMOTO, T., OHNO, A. and YAMAGUCHI, K., 1994. Profiles of outer membrane proteins and lipopolysaccharide of *Pseudomonas aeruginosa* grown in the presence of sub-MICs of macrolide antibiotics and their relation to enhanced serum sensitivity. J. Antimicrob. Chemother., **34**, 931–942.

TAYLOR, P. W., 1983. Bactericidal and bacteriolytic activity of serum against gram-negative bacteria. Microbiol. Rev., **47**, 46–83.

Mailing address: ANNA HOŠTACKÁ, Institute of Preventive and Clinical Medicine, Limbova 14, 83301 Bratislava, Slovak Republic