Folia Microbiol. 42 (6), 565–568 (1997)

Hydrophobicity and Outer Membrane Proteins of *Shigella dysenteriae* Type 1 after Treatment with Subinhibitory Concentrations of Aminoglycosides

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Received May 28, 1997

ABSTRACT. Hydrophobicity and profiles of outer membrane proteins of *Shigella dysenteriae* type 1 after treatment with subinhibitory concentrations (½ or ¼ of the MIC) of aminoglycosides were studied. The antimicrobial activity of the antibiotics tested was 3.12 mg/L (amikacin, tobramycin) and 6.25 mg/L (gentamicin). The hydrophobicity of the cell surface of *S. dysenteriae* type 1 was decreased after exposure to all aminoglycosides at a concentration of ½ of the MICs; ¼ of the MICs of the antibiotics did not affect bacterial aggregation in the presence of ammonium sulfate. SDS-polyacrylamide gel electrophoresis showed that the profiles of outer membrane proteins of the strain treated with aminoglycosides at both subinhibitory concentrations were not changed as compared to the control.

Recently, multiresistant bacteria including *Shigella* spp. have begun to be frequently isolated (Voogd *et al.* 1992; Brito-Alayon *et al.* 1994). Such microorganisms became a serious clinical problem. Shigellosis is a gastrointestinal infection which may cause diarrhea and dysentery and is still the cause of a great deal of morbidity and mortality, mainly in developing countries. The primary virulence determinant of shigellae is the invasive capacity of the organisms.

Antimicrobial substances affect bacteria not only at concentrations that visually inhibit their growth. Literature data suggest that antimicrobials at subinhibitory concentrations (sub-MICs), which are generally present during the interval between two therapeutic doses, can modify the different bacterial properties including the physico-chemical characteristics of the cell surface (Stessman and Stessman 1980; Geers and Baker 1987; Adinolfi *et al.* 1988; Grimwood *et al.* 1989; Braga *et al.* 1994; Hoštacká and Majtán 1993).

The purpose of the present paper was to study the hydrophobicity of the cell surface and the profiles of outer membrane proteins of *Shigella dysenteriae* type 1 after treatment with subinhibitory concentrations of aminoglycosides.

MATERIAL AND METHODS

Bacterial strain. Shigella dysenteriae type 1 was isolated from a patient suffering from dysentery (*International Centre for Diarrhœal Disease Research*, Dhaka, Bangladesh).

Antibiotics. Amikacin commercially manufactured as selemycin (Amikacin sulphate, *Medochemie Ltd.*, Limassol, Cyprus), gentamicin (Gentamicin sulphate, *ICN Biochemicals*) and tobramycin (Tobramycin sulphate, *Biogal*, Hungary) were used.

MIC determination was made by the macrodilution broth method in culture medium consisting of 3 % casein hydrolysate (*Merck*, Darmstadt, Germany), 0.3 % yeast extract (*Difco*, Detroit, USA), 0.3 % glucose and 1 mmol/L $CaCl_2$, pH 7.4. The lowest dilution of the antibiotic allowing no visible growth after 1 d at 37 °C was considered as MIC.

Effect of sub-MICs of antibiotics on S. dysenteriae *type 1 and isolation of outer membrane proteins (OMPs).* Bacterial suspensions exposed to ½ or ¼ of the MIC of the antibiotic tested as well as a control culture (without antibiotic) were incubated for 1 d at 37 °C. After centrifugation of all bacterial suspensions, bacterial pellets were used for isolation of OMPs and determination of hydrophobicity. Bacterial pellets were resuspended in distilled water and stirred for 1 h at room temperature (Oaks *et al.* 1986). The supernatant obtained after centrifugation of stirred suspension (16 000 **g**, 20 min) was again centrifuged (100 000 **g**, 2 h). The OMPs present in the supernatant were concentrated against polyethyleneglycol (PEG 6000) and lyophilized.

SDS-polyacrylamide gel electrophoresis (SDS-PAGE). The method of Laemmli (1970) was used. The OMPs were denatured by boiling at 100 °C for 5 min with 2 % SDS–5 % 2-mercaptoethanol

and their profile was determined by electrophoresis in 4 % stacking and 12 % separating gel. SDS-PAGE was carried out at a constant current (40 mA) using a Bio-Rad apparatus (*Biorad*, Richmond, USA). The gels were stained with Coomassie brilliant blue.

Salting-out test (SAT). The relative hydrophobicity of the bacterial cell surface was determined by the method of Lindhal *et al.* (1981). Bacterial pellets of control and treated cultures (*cca* 10^9 cells) were suspended in a series of dilutions of $(NH_4)_2SO_4$ ranging from 0.25 to 2.5 mol/L. The resulting mixture was then gently shaken on a glass for 2 min. The lowest concentration of ammonium sulfate at which bacterial aggregation was visible was determined.

RESULTS AND DISCUSSION

Aminoglycosides still represent an important class of antibiotics in the treatment of severe bacterial infections despite their toxicity and the continued introduction of newer and safer antimicrobials with a broad antibacterial spectrum. The mode of action of these antibiotics is pleiotropic. The pleiotropic actions of aminoglycosides include inhibition of ribosomal function, membrane damage and suppression of the initiation of DNA replication (Bryan and Kawan 1963; Walker and Beveridge 1988; Kadurugamuwa *et al.* 1993; Matsunaga *et al.* 1986). Our results showed that the MICs of antibiotics for *S. dysenteriae* type 1 were 3.12 mg/L (amikacin and tobramycin) and 6.25 mg/L (gentamicin) (Table I). Previous papers documented that many antibiotics at sub-MICs changed different bacterial characteristics. In the majority of these cases the antimicrobial agents suppressed the activity of some bacterial toxic enzymes (Warren *et al.* 1985; Grimwood *et al.* 1989; Hoštacká and Majtán 1993) and other virulence factors (Shibl 1987; Morris and Brown 1988; Nichterlein *et al.* 1996) and affected bacterial adherence (Breines and Burnham 1994; Ravizzola *et al.* 1994; Braga *et al.* 1995; Vranes 1996).

Table I. MICs of some aminoglycosides and hydrophobicity of *S. dysenteriae* type 1

Antibiotic	MIC mg/L	SAT[a]		
		Control	½ MIC	¼ MIC
Amikacin	3.12	1	1.5	1
Gentamicin	6.25	1	1.5	1
Tobramycin	3.12	1	1.5	1

[a]The lowest molar concentration of $(NH_4)_2SO_4$ causing visible bacterial aggregation.

It is known that bacterial adhesion, as a phenomenon contributing to the development of infection, is associated with hydrophobicity (Qadri *et al.* 1988). Bacterial adhesion increases with increasing bacterial hydrophobicity and is lowered with decreasing hydrophobicity (Hermansson *et al.* 1982; Loosdrecht *et al.* 1987). Our results showed that the hydrophobicity of the cell surface of *S. dysenteriae* type 1 strain studied by the salting-out test was decreased after the exposure to all aminoglycosides tested, albeit only at concentrations equal to ½ of the MICs (Table I). Visible cell aggregation in bacterial suspensions after treatment with concentrations equal to ¼ of the MICs occurred at the same molar concentration of ammonium sulfate (1 mol/L) as in controls. Similar results were published by Braga *et al.* (1995) and Braga and Reggio (1995), who found that brodimoprim at certain subinhibitory concentrations effectively decreased the surface hydrophobicity of *E. coli* and *S. aureus*.

By contrast, sub-MIC levels of some antimicrobial agents did not significantly change the hydrophobicity of bacterial cells (Cai *et al.* 1996; Hoštacká and Karelová 1997*b*). The OMP patterns of the control strain determined by SDS-PAGE revealed a considerable number of protein bands (Fig. 1). Five prominent bands showed apparent molar mass ranging from 62 to 35 kDa (62, 58, 43, 38 and 35 kDa). The mobility of three bands (62, 43 and 38 kDa) corresponded to the mobility of the major outer membrane protein antigens (Ipa proteins). These proteins are encoded by a large plasmid which is responsible for the invasiveness of *Shigella* spp. The analysis also showed that *S. dysenteriae* type 1 strain treated with aminoglycosides at both subinhibitory concentrations exhibited the same protein profile as the control.

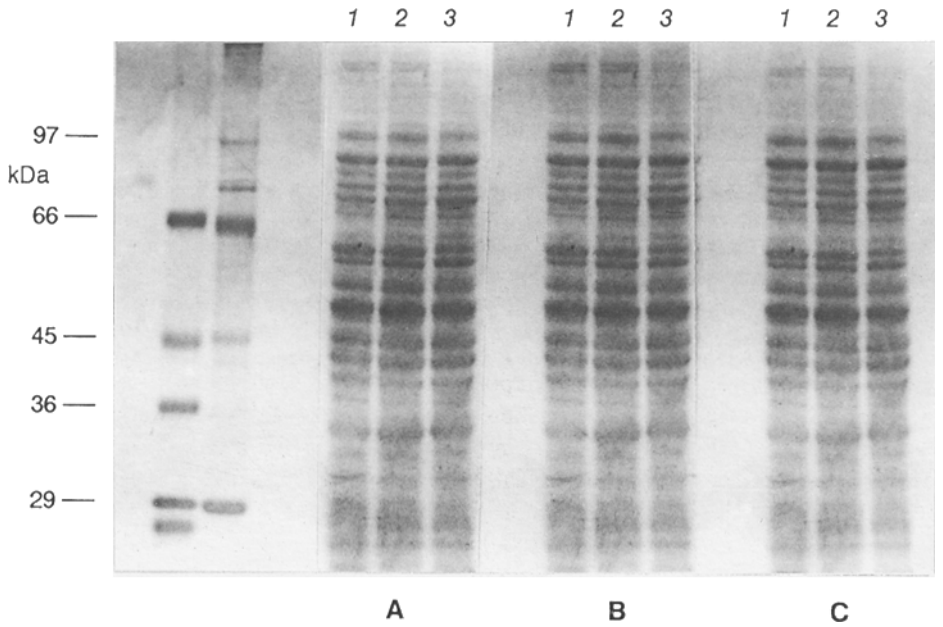

Fig. 1. SDS-polyacrylamide gel electrophoresis of outer membrane proteins of *S. dysenteriae* type 1 after treatment with subinhibitory concentrations of aminoglycosides; **A** — gentamicin, **B** — amikacin, **C** — tobramycin; control (without antibiotics) (*lanes 1*), at ¼ MICs (*lanes 2*) and ½ MICs (*lanes 3*). The migration positions of standard proteins (low and high molar mass mixtures) are shown on the left (kDa).

Several authors published results on the interaction between antibiotics (at different concentrations) and some bacterial components. Xiong *et al.* (1996) did not observe any alterations in outer and inner membrane proteins during the adaptive–resistance interval induced by aminoglycosides. Also our previous study showed that the profiles of OMPs of *S. dysenteriae* type 1 were not changed after treatment with quinolones at concentrations equal to ¼ or ⅛ of the MICs (Hoštacká and Karelová 1997*b*). Similarly, Karlowsky *et al.* (1996) found no changes in outer membrane proteins or lipopolysaccharide profiles when control, adaptively resistant and postadaptively resistant cells of *Pseudomonas aeruginosa* were campared. On the other hand, Kadurugamuwa *et al.* (1993) gave morphological evidence suggesting that the initial binding of gentamicin disrupts the packing order of the lipopolysaccharide of the outer membrane of bacterial cells and forms holes in the cell envelope. Also it was shown that norfloxacin (2× or 4× MIC) caused alterations in the outer membrane proteins of *P. aeruginosa* (Hoštacká and Karelová 1997*a*).

In conclusion, our results showed that aminoglycoside antibiotics at ½ MIC levels reduced the surface hydrophobicity of *S. dysenteriae* type 1 strain, but they did not affect the profile of outer membrane proteins of this strain.

The authors wish to thank Mrs. A. Korenačková and Mrs. D. Opoldusová for skilful technical assistance.

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