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Reduction in the Adherence of *Pseudomonas aeruginosa* to Human Buccal Epithelial Cells with Neuraminidase Inhibition

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Abstract

The aim of this study was to evaluate the reduction in the adherence of 33 strains of *Pseudomonas aeruginosa* isolated from humans and different animals to human buccal epithelial cells with neuraminidase inhibition. Buccal epithelial cells were incubated with strains of *Pseudomonas aeruginosa* in the presence or absence of the neuraminidase inhibitors, 2,3-dehydro-2-deoxy-N-acetyl-neuraminic acid (DANA) or N-acetyl-neuraminic acid (NANA). Incubation of cells with bacteria in the presence of either DANA or NANA reduced bacterial adherence significantly by 35.24 ± 23.90%, and 68.00 ± 22.51%, respectively. We suggest that the *in vivo* effects of such interventions should be explored as potential mechanisms reducing *Pseudomonas aeruginosa* in the binding to buccal cells.

Key words: adherence, *P. aruginosa*, bacterial infection

Adherence to cell surfaces, though to be the initiating step in many bacterial infections, has been shown in the majority of studies on *Pseudomonas aeruginosa* (Saiman *et al*., 1992; Davies *et al*., 1999). It has been demonstrated that adherence of *Pseudomonas aeruginosa* to buccal epithelial cells (BECs) is related to the pathogenesis of *Pseudomonas aeruginosa* – induced lung infection (Woods *et al*., 1980a). Several *in vitro* and *in vivo* studies have found poor adherence of *Pseudomonas aeruginosa* to functional and intact BECs (Woods *et al*., 1980b). Bacterial binding can be substantially increased by modification of the epithelial surface by acid (Ramphal and Pyle, 1983), trypsin (Woods *et al*., 1981; Wolska *et al*., 2003), influenza infection, trauma (Ramphal *et al*., 1980) or the effect of the accumulated *Pseudomonas aeruginosa* exoproducts in culture supernatants (Saiman *et al*., 1990). The ability of *Pseudomonas aeruginosa* to adhere to upper respiratory cells is associated with surface structures known as pili (Woods *et al*., 1980b). Pili adhere to cell surfaces *via* the Gal Nac β1-4 Gal moiety of certain asialylated glycolipids including asialoGM1 receptors (Saiman and Prince, 1993). The *Pseudomonas aeruginosa* exoproducts, particularly a neuraminidase, may increase the availability of such receptors by cleaving terminal sialic acid residues from cell surface gangliosides. Increased *Pseudomonas aeruginosa* adherence has been reported after exposure of cultured epithelial cells to bacterial exoproducts or purified neuraminidase (Cacalano *et al*., 1992; Saiman *et al*., 1992). Thus, there may be a potential role for neuraminidase inhibitors, which have been shown to be of benefit in limiting the pathogenicity of *Pseudomonas aeruginosa*.

Two groups of synthetic inhibitors of neuraminidase have been described. One diverse group consists of high molecular weigh substances like Congo red and trypan blue and low molecular weigh compounds, such as derivatives of oxamic acid, substituted β-aryl-α-mercaptoacrylic acids and other more complicated heterocyclic substances such as benzimidazoles. These substances, belonging to different chemical classes show varying *in vitro* inhibitory activity against neuraminidases but are nonspecific enzyme inhibitors. In contrast to these nonspecific inhibitors a second group of neuraminidase inhibitors has been described, which consists of analogues and derivatives of neuraminic acid with a high degree of specificity for neuraminidases. Among this second group, small N-glycosides and small S-glycosides of N-acetylneuraminic acid have been shown to be very effective neuraminidase inhibitors. Another synthetic neuraminic acid derivative, 2-deoxy-2,3-dehydro-N-acetylneuraminic acid which differs from naturally occurring N-acetylneuraminic

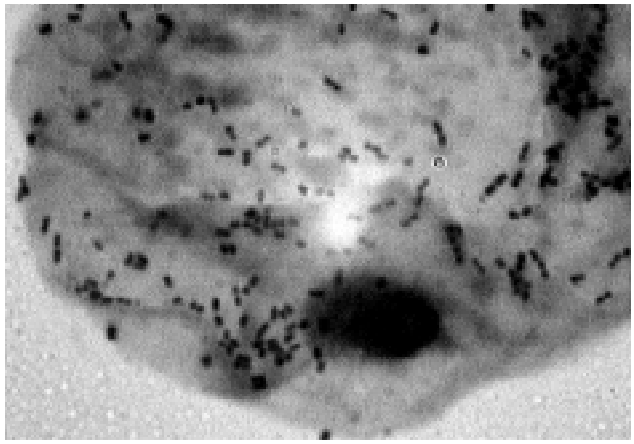

Fig. 1. Adherence of *Pseudomonas aeruginosa* to buccal epithelial cells

acid by one double bond between carbon atom 2 and carbon atom 3 has been shown to inhibit bacterial, viral and mammalian neuraminidases (Khorlin *et al*., 1970; Meindl *et al*., 1974).

This study examined whether the adherence of *Pseudomonas aeruginosa* strains to human buccal epithelial cells can be reduced *in vitro* by 2,3-dehydro-2-deoxy-N-acetyl-neuraminic acid (DANA) and N-acetyl-neuraminic acid (α-sialic acid) (NANA), broadspectrum neuraminidase inhibitors. 33 of *Pseudomonas aeruginosa* strains were isolated from humans (12 strains), flowers – *Zantedeschia aethiopica* (1), community sewage (1) and different animals: deer (1), chicken (3), dog (1), fox (1), minks (2), cattle (2), swine (2), chinchillas (1), fish (5), cat (1). Adherence of *Pseudomonas aeruginosa* to buccal epithelial cells was assayed by method of W o o d s *et al*. (1980b) and D a v i s *et al*. (1999). Buccal epithelial cells were collected from healthy people, nonsmoking volunteers by vigorous swabbing of the buccal mucosa with a sterile, cotton-tipped swab. The cells were suspended in phosphate-buffered saline (PBS), pH 7.4 and washed three times by centrifugation (10 min at 150×g) to remove any unattached bacteria. To investigate reduction in the adherence of *Pseudomonas aeruginosa* strains to buccal epithelial cells, cells were incubated with DANA (Sigma) or NANA (Sigma). These inhibitors were used at a final concentration of 50 μM. The DANA (or NANA) was added to cell samples (5×10^4/ml) at the same time as application of 200 μl of bacterial suspension (5×10^6/ml) and incubated for 3h at 37°C in a shaking water bath. Control samples were incubated with PBS alone. After incubation the bacterium – buccal cells – inhibitor mixture was washed again three times with PBS. Smears were made, air dried, fixed in methanol, and stained with Giemsa staining solution. The number of bacteria adhering to buccal epithelial cells was counted under a light microscope. In each experiment, the first 30 well-defined of epithelial cells were observed. Three independed trials were used to obtain the mean number of bacteria adhering to cells in each experiment.

The results were statistically worked out by means of 1-factor variance analysis counted with the method of least squares.

The effects of neuraminidase inhibitors on bacterial adherence to buccal epithelial cells are presented in Figs 2–4. Incubation of cells with the bacterium and DANA resulted in a significant reduction in adherence compared with the control (the bacterium-buccal cells) (Fig. 2 and Fig. 4). The mean number of bacteria adhering to cells after application DANA amounted 4.85 ± 3.13 and to cells in the absence of DANA – 17.00 ± 10.44. We suggested that the samples demonstrating high values of adherence (*e.g.* 43.8; 34.8; 32.2; 28.5) also demonstrated great degree of inhibition in the presence of DANA (4.1; 6.9; 4.6; 5.9). There

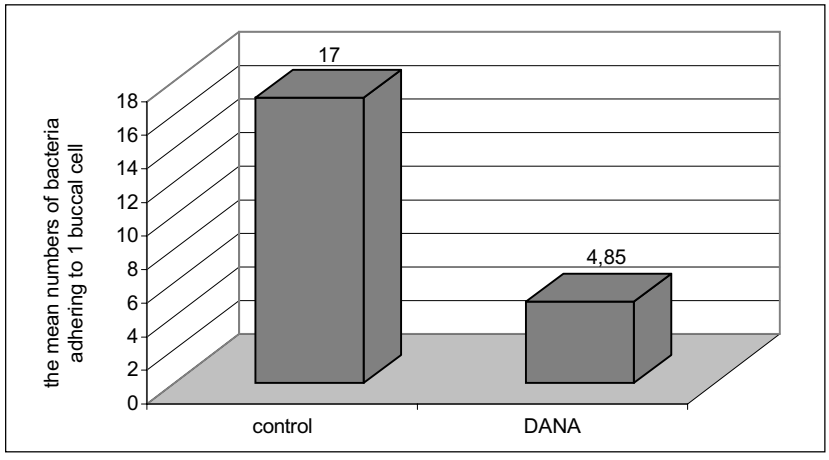

Fig. 2. Effect of 2,3-dehydro-2-deoxy-N-acetyl-neuraminic acid on the adhesion of *Pseudomonas aeruginosa* strains to human buccal epithelial cells

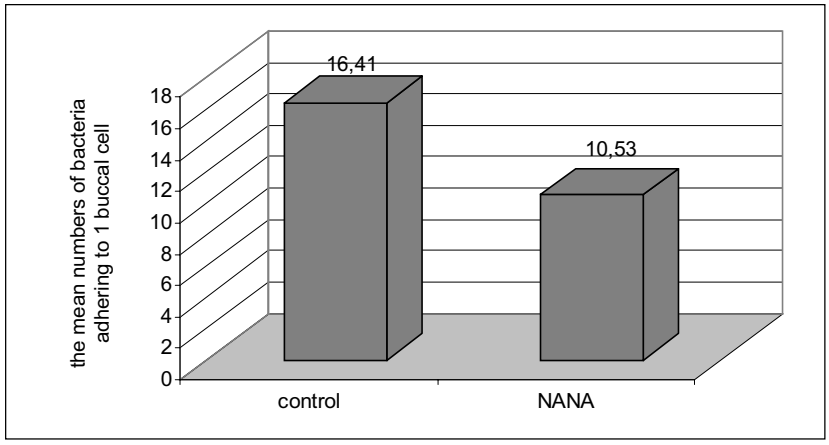

Fig. 3. Effect of alfa-N-acetyl-neuraminic acid on the adhesion of *Pseudomonas aeruginosa* strains to human buccal epithelial cells

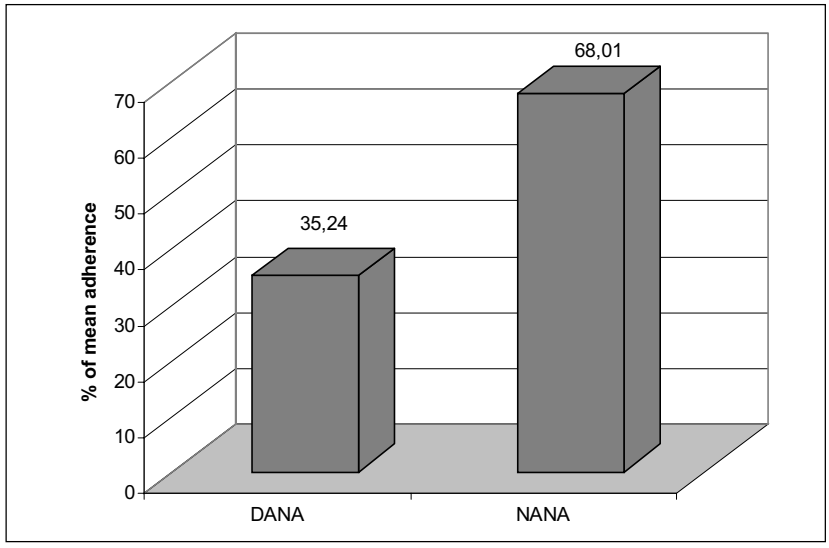

Fig. 4. Reduction in bacterial adherence (%) after exposure of buccal epithelial cells and bacteria to DANA and NANA

was again a wide range of inhibition (1.14–96.04% decrease). 27 of samples demonstrated over 50% decrease of adherence. This result is in agreement with the work of Davis *et al*. (1999) who observed an inhibition of adhesion of piliated, nonmucoid *Pseudomonas aeruginosa* to fresh, noncultured CF respiratory cells in the presence of 2,3-dehydro-2-deoxy-N-acetyl-neuraminic acid (DANA) by 34%. In the same study they used anti-asialoGM1 (anti-aGM1), which significantly reduced bacterial adherence by 51%. Other workers also identified DANA as a potent inhibitor of neuraminidase (Meindl *et al*., 1974). The substitution of the C-4 hydroxyl group of DANA with a guanidino group resulted in a 10,000-fold increase in the potency of DANA (Holzer *et al*., 1993). α-sialic acid (NANA) has been designed as a weak neuraminidase inhibitor (Varghese *et al*., 1992). In our study there was no significant effect of NANA on bacterial adherence to buccal epithelial cells (Fig. 3 and Fig. 4). The mean reduction in the bacterial adherence compared with control was 32%. Only 6 of the strains demonstrated over 50% of inhibition in the adherence. We indicated also that N-acetylneuraminic acid enhanced the adhesion of 1 of *Pseudomonas aeruginosa* strain isolated from humans. McEachran and Irvin (1985) found that N-acetylneuraminic acid enhanced the adhesion of the bacterium to untrypsinized BECs and inhibited the adhesion to trypsinized BECs. α-sialic acid was an efficient inhibitor of adhesion of *Pseudomonas aeruginosa* to fibronectin (Rebiere-Huet *et al*., 2004).The results of White *et al*. (1995) showed that the inhibition of neuraminidase influenza virus by a phosphonate analog of N-acetyl-neuraminic acid was approximately 100-fold better than NANA inhibition. The same authors indicated that the contributions of the inhibitor functional groups

are not equal for active site binding. The most important inhibitor functional group was the acidic carboxyl or phosphonoyl group that interacted with the arginine pocket in the active site of neuraminidases through charge-charge interactions. The new neuraminidase inhibitors, zanamivir and oseltamivir blocked influenza neuraminidase and prevented cleavage of sialic acid residues (McNicholl and McNicholl, 2001). Experimental studies demonstrated that viral neuraminidase exposes pneumococcal receptors on host cells by removing terminal sialic acids. The inhibition of viral neuraminidase activity reduced adherence and invasion of *Streptococcus pneumoniae* (Peltola and McCullers, 2004).

This study demonstrates that the increased binding of *Pseudomonas aeruginosa* to fresh buccal cells can be significantly reduced *in vitro* by inhibition of the bacterial exoproduct, neuraminidase. A significant reduction in binding was seen with the sialic analogue DANA, a highly-specific neuraminidase inhibitor. It is interesting to note that the greatest degree of inhibition by DANA was seen on those samples with the highest levels of adherence of control, suggesting that this high control may have been as a result of neuraminidase-induced exposure of increased asialo receptors.

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