Inhibitory effects of hypertonic saline on P. aeruginosa motility

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Abstract

Salt transport defects in CF lungs predispose to overwhelming and fatal respiratory infection caused by Pseudomonas aeruginosa. Motility of this organism is central to pathogenesis in a number of settings. Incubation of numerous strains of P. aeruginosa with hypertonic saline caused a concentration-dependent decrease in bacterial motility. Reduction of P. aeruginosa virulence through this effect may contribute to clinical efficacy of hypertonic saline aerosols in CF patients.

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Sir,

Chronic pulmonary infection with Pseudomonas aeruginosa is a hallmark of cystic fibrosis (CF). P. aeruginosa motility and swimming are likely to represent important factors in CF pulmonary disease progression and severity. P. aeruginosa lacking flagella are significantly less virulent in rodent models of pneumonia, including defective motility, immuno-stimulation, and adaptation. [1,2] Flagella-deficient P. aeruginosa are also less damaging in murine models of chronic corneal and burn infections. [3–5] Bacterial chemotaxis may promote migration of P. aeruginosa towards more favorable sites of infection, improved nutrient availability, and away from host defense and clearance: all potential mechanisms pertinent to the CF lung.

Numerous clinical reports [6–8] including two recent randomized, placebo-controlled trials from two independent centers [9,10] indicate beneficial effects of hypertonic saline (HS, 7% NaCl) aerosols in CF lung disease. This intervention was shown to restore airway surface liquid (ASL) depth and mucociliary clearance. [10] However, the results of the studies presented a paradox: a 48 week treatment with 4 milliliters (ml) hypertonic saline aerosolized twice daily to CF subjects led to a dramatic decrease in acute pulmonary exacerbations of CF (and diminished need for antibiotics), but had minimal effect on pulmonary function (FEV1 improved only 68 ml and was statistically significant only when analyzed as a composite endpoint). [9] A shorter (two week, higher dose intensity) trial of 5 ml HS 4 times daily led to a larger increase in pulmonary function (113 ml). However, when amiloride was co-administered with HS (intended to stabilize a presumed increase in ASL depth by blocking NaCl reabsorption through ENaC), the benefits of HS on both pulmonary function and mucociliary clearance were essentially lost. [10].

Even modest increases in extracellular NaCl (400–500 mOsm/L) have been shown to arrest swimming and impair the flIC regulatory element in E. coli [11,12]. Similarly, 500 millimolar NaCl in an environmental isolate of P. aeruginosa abolished motility and diminished flagellar gene synthesis. [13] Daily aerosols of up to 20 ml HS (2178 mOsm/L) as tested in recent clinical trials are likely to increase the salt of the pulmonary secretions by 2–3 fold over weeks to months of daily aerosolization.

As one test of possible effects of high salt solutions on P. aeruginosa motility, swim plates at increasing NaCl concentrations were established with 0.4% agar in LB media (Fig. 1).
As a control, *P. aeruginosa* strains lacking flagella were non-motile at all time points and all salt conditions. The common laboratory strain PA01 (www.pseudomonas.com), the CF clinical isolate FRD1, and several other strains were also studied. An isogenic FRD fliC::Gm mutant was constructed via allelic exchange and sucrose counterselection.[14] An algT(U)::Tn501 was previously derived by transposon mutagenesis[15] and confirmed by Southern blot. All these strains, as well as patient derived *P. aeruginosa* (isolated and typed by the clinical microbiology laboratory at Children’s Hospital of Alabama and judged non-mucoid (NM) by conventional phenotyping) exhibited a strong dose and time dependence of motility on ambient salt concentration (Panel B). Concentrations of saline ≥2% or <0.9% inhibited *P. aeruginosa* motility. Panel C: The growth of *P. aeruginosa* (PA01) is also impaired by exposure to high salt concentration. Growth curves for 7%, 2%, and 0.9% NaCl liquid cultures are shown. By 24 hours (Panel C, lower), growth is nearly ablated in all strains in 7% salt. However, 2% salt appears normal despite pronounced effects on motility.

The observation that high salt impairs *P. aeruginosa* motility and inhibits growth suggests that the clinical benefit attributable to HS aerosols might be due in part to these effects. Although Elkins et al. showed no effects on *P. aeruginosa* colony counts during long term HS administration, *P. aeruginosa* motility was not evaluated and our data suggest that lower NaCl may have significant effects on motility. Normal motility is crucial to *P. aeruginosa* pathogenesis in rodent models of pneumonia[1,2], as well as other models of infection.[3–5] Clinical studies to monitor salt accumulation in the CF mucus itself (i.e. the site of *P. aeruginosa* infection; rather than within ASL as examined previously) and tests of flagellar structure and function (known to be inhibited by high salt in *E. coli*[11,12]), should be considered as part of future clinical trials of the HS intervention in CF individuals. Confirmation of increased salt in CF mucus following HS administration could validate the potential for HS to confer these beneficial effects.[16].

Our findings do not connote effects on Pseudomonas motility as the only mechanism by which HS ameliorates CF lung disease. Rather, our data implicate impairment of bacterial motility as a potential contributor to the HS intervention. Elkins et al. did not describe a subgroup analysis regarding uninfected CF individuals. However, a 1.5 ml deposition (approximately 30%) of a 5 ml dose of HS[9] represents an estimate of lower

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**Fig. 1.** *P. aeruginosa* spreading is blocked by hypertonic saline. Panel A: Depicts a standard swim motility plate in which agar (0.4% in LB media) has been inoculated either with motility competent (PA01) or incompetent (fliC mutant) strain. The fliC mutant was devoid of swim behavior at all salt concentrations and time points studied. (not shown). Panel B: Time dependent swimming from the inoculation site at varying salt concentrations was studied at 24, 48, and 72 hours. PA01, FRD1 (mucoid) strain lacking fliC, FRD1 (algT(U)), or FRD1 parent, and two non-mucoid (NM) clinical strains are depicted. Motility was measured by the area of spread under each condition. Concentrations of saline ≥2% or <0.9% inhibited *P. aeruginosa* motility. Panel C: The growth of *P. aeruginosa* (PA01) is also impaired by exposure to high salt concentration. Growth curves for 7%, 2%, and 0.9% NaCl liquid cultures are shown. By 24 hours (Panel C, lower), growth is nearly ablated in all strains in 7% salt. However, 2% salt appears normal despite pronounced effects on motility.
airway delivery over a 30-minute interval in vivo [17]. While airway epithelia can adjust to a fluid load of this magnitude, much less is known regarding clearance rates for NaCl deposited within purulent secretions in CF lungs (i.e. where mucus plaques represent a predominant site of bacterial infection), or the mechanisms by which added salt is managed within CF secretions, themselves. The present findings indicate the importance of additional endpoints addressing motility and other microbial virulence factors following HS aerosolization.

The mechanism by which increased salt or osmolarity disrupt motility function is likely to be multifactorial. Inorganic polyphosphate (polyP), a regulator of the bacterial networks responding to environmental stress, also controls motility of numerous bacterial pathogens [18,19], although the chronic effects of salt administration on polyP and motility have not been examined previously. [20] Because flagellar activity appears to be necessary for biofilm formation in *P. aeruginosa* [21], it is possible that the effects of salt described here could also influence a functional step in the biofilm adaptation and quorum sensing response. Clinical interventions specifically targeting both chemotaxis and flagellar machinery have not been studied and should be considered as a potential therapeutic target for inhibiting *P. aeruginosa* in CF and other lung diseases.

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