



Full length article

Synergy between tobramycin and trivalent chromium ion in electrochemical control of *Pseudomonas aeruginosa*



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ABSTRACT

We recently demonstrated that the effectiveness of tobramycin (Tob), an aminoglycoside, against antibiotic-tolerant persister cells of *Pseudomonas aeruginosa* can be enhanced by electrochemical factors generated from direct currents (DC). Supplementation of Ni(II), Cr(III) and Fe(II) during carbon-mediated DC treatment revealed that these metal cations promote killing of persister cells in the presence of tobramycin, which led to our hypothesis that specific interactions between Tob and some metal ions contribute to the synergistic killing of persister cells. In this study, the interactions between selected metal cations and Tob were investigated using ¹H-¹³C HSQC NMR. Increase in the concentration of Cr(III) (in the form of [CrCl₂(H₂O)₄]⁺) in solutions containing Tob was found to shift the HSQC NMR peaks of Tob to new positions, suggesting the formation of a Cr(III)-Tob complex. Crystal field effects and electrochemical properties of the complex were further studied using UV-visible spectroscopy and cyclic voltammetry, which led to the finding that the Cr(III)-Tob complex has increased affinity with negatively charged nucleic acids. These findings are helpful for understanding the mechanism of electrochemical control of bacterial cells and for developing more effective antimicrobial therapies based on aminoglycosides and electrochemical species released from various metallic biomaterials.

Statement of Significance

Medical device associated infections present a major challenge to healthcare and the quality of life of affected individuals. This problem is further exacerbated by the emergence of multidrug resistant pathogens. Thus, alternative methods for microbial control are urgently needed. Recently, we reported synergy between tobramycin and low-level electrochemical currents generated using stainless steel electrodes in killing bacterial persister cells, a dormant population with high-level intrinsic tolerance to antibiotics. In this article, we describe how electrically-induced interaction between aminoglycosides and certain metal cations enhance the potency of tobramycin in bacterial killing. The findings will help design new methods for controlling infections through electrochemical disruption of cellular function and associated drug resistance.

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1. Introduction

Bioelectric effects are a well-known phenomenon, by which the antimicrobial activities of antibiotics are enhanced by low-level electrochemical currents [1–4]. Although such activities are documented and can be achieved with a variety of metallic biomaterials [5–7], the underlying mechanism of synergy that enhances the

effectiveness of combined factors [e.g. DC and tobramycin (Tob), or Cr(III) and Tob] is still poorly understood, hindering the application of this approach in clinical treatment of chronic infections. It is also important to understand what materials can release ions/compounds that have synergy with antibiotics in bacterial killing, instead of lowering the activities of antibiotics. We are interested in stainless steel because it is a biocompatible material used in many implanted medical devices [8,9]. It has also been reported that stainless steel-based medical implants release biologically active metal ions [10–13], which have possible uses for controlling medical device-associated bacterial infections [14–16].

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Recently, we explored the potential of stainless steel as an electrode material for controlling bacterial persister cells (a dormant subpopulation that is highly tolerant to antibiotics [17,18]). We reported the synergy between 1.5 $\mu\text{g}/\text{mL}$ Tob and 70 $\mu\text{A}/\text{cm}^2$ direct current (DC) mediated with stainless steel 304 (SS304) electrodes in killing of *Pseudomonas aeruginosa* PAO1 persister cells, corresponding to a 5 logs reduction of cell viability vs. 2 logs in the absence of DC [17]. The electrochemical treatment generated low-concentrations of metal species from the SS304 electrodes (mainly composed of Fe, Cr, Ni, Mn), which contributed to such observed synergy [17]. More explicitly, supplementation of 0.1 μM Ni(II), 0.27 μM Cr(III) in the form of $[\text{CrCl}_2(\text{H}_2\text{O})_4]^+$, or 0.82 μM Fe(II) during DC treatment mediated with graphite electrodes (lacking metal elements) improved the antimicrobial activity of 1.5 $\mu\text{g}/\text{mL}$ Tob against *P. aeruginosa* persister cells by $84.5 \pm 1.2\%$, $38.1 \pm 5.7\%$, and $20 \pm 7.4\%$, respectively, compared to the treatment without these metal ions [17]. Interestingly, the synergistic effects between Tob and metal cations in persister killing occurred only in concurrent treatment, and were not observed in sequential treatment with cells exposed to DC first and subsequently to Tob [19]. These findings led to our hypothesis that some interactions between SS304-released metal cations and Tob may be important to the control of bacteria with low-level DC; and elucidating the underlying mechanism could guide the design of novel medical devices and therapeutics. In this study, we investigated the interaction between metal cations and Tob, and the associated effects on persister killing.

Both Tob and the structurally related kanamycin (Kan) are excellent antibiotics against a wide range of multidrug-resistant pathogens including *P. aeruginosa*, *Staphylococcus aureus* and some *Enterobacteriaceae* spp. [20–23]. These agents kill bacteria by binding to 16S ribosomal RNA and preventing the association of 30S and 50S subunits, which ultimately blocks translation [24,25]. Since Tob has five primary amine groups instead of four in Kan, the former has a higher cationic charge at physiological pH. This makes Tob a stronger binder toward negatively charged ribosomal RNA, which contributes to the higher bactericidal activities of Tob than Kan [25].

The chemical composition and ionic strength of the environment have strong impact on the affinity of an aminoglycoside with its cellular target, and therefore its effectiveness as an antimicrobial. For example, the presence of divalent cations such as Mg^{2+} or Ca^{2+} in Mueller–Hinton agar has been shown to reduce the susceptibility of *P. aeruginosa* cells to Tob and other aminoglycosides [26–28]. It was believed that the increased ionic strength of the medium altered the electrostatic binding of Tob to target RNA and thus reduced the bactericidal activity of Tob. In contrast, the chelation of Tob by iron chelators such as deferoxamine and deferasirox can enhance the bioactivity of the antibiotic against *P. aeruginosa* biofilm in the treatment of a cystic fibrosis model involving human bronchial epithelial cells [29]. These earlier findings and our recent report [17] of the electrochemical control of bacterial persister cells (ECCP) indicate the possibility that interactions between metal cations and aminoglycosides may modulate the efficacy of Tob in bacterial killing. In this study, we investigated the electrochemical properties of Tob in the presence of a group of first row transition metal ions to elucidate the possible roles of these factors in killing *P. aeruginosa* cells.

2. Materials and methods

2.1. Bacterial strain and growth medium

The wild-type *P. aeruginosa* PAO1 (henceforth PAO1) was cultured in Lysogeny Broth (LB) containing 10 g/L tryptone, 5 g/L yeast

extract, and 10 g/L NaCl [30]. Each overnight culture was incubated at 37 °C with shaking at 200 rpm.

2.2. Chemicals and reagents

Metal salts purchased from Acros Organics (Thermo Fisher Scientific, Waltham, MA, USA) include Nickel (II) chloride (NiCl_2), referred to as Ni(II); chromium (II) chloride (CrCl_2), referred to as Cr(II) (note-this compound is rapidly oxidized to Cr(III) in water that is exposed to air); chromium (VI) oxide (CrO_3), referred to as Cr(VI); and chromium (III) chloride hexahydrate ($[\text{CrCl}_2(\text{H}_2\text{O})_4]\text{Cl}\cdot 2\text{H}_2\text{O}$), containing the complex cation $\text{trans}[\text{CrCl}_2(\text{H}_2\text{O})_4]^+$, referred to as Cr(III). MgCl_2 was purchased from Amresco (Solon, OH, USA). Anhydrous iron (III) chloride (FeCl_3), referred to as Fe(III), and manganese (II) chloride tetrahydrate ($\text{MnCl}_2\cdot 4\text{H}_2\text{O}$), referred to as Mn(II), were purchased from Fisher scientific (Waltham, MA, USA). Iron (II) chloride tetrahydrate ($\text{FeCl}_2\cdot 4\text{H}_2\text{O}$), ammonium iron (II) sulfate hexahydrate ($(\text{NH}_4)_2\text{Fe}(\text{SO}_4)_2\cdot 6\text{H}_2\text{O}$) and Tris-HCl buffer were purchased from Sigma-Aldrich (St. Louis, MO, USA). Tobramycin was purchased from TCI America (Portland, OR, USA). Stock solutions of these chemicals were prepared with Milli-Q water.

2.3. Electrochemical treatments

An electrochemical cell (Fig. 1) as previously described [17] was constructed using working and counter electrodes made with TGON 805 (Laird Technologies, Schaumburg, IL, USA), a carbon based conductive material. An Ag/AgCl electrode was used as the reference electrode. Solutions containing 9 mM Tob and 10 μM Fe(II), Fe(III), Cr(II), Cr(III), Cr(VI), Mn(II), or Ni(II) were prepared with Milli-Q water. Three milliliters of each mixture were treated with 70 $\mu\text{A}/\text{cm}^2$ DC for 1 h using TGON 805 electrodes to generate electrochemical products and screen for complexes formed between Tob and the metal cations. After DC treatment, the mixtures and untreated control samples (DC-free samples) were stored on ice till NMR analysis.

2.4. Nuclear magnetic resonance (NMR) spectroscopy

Sensitivity-enhanced ^1H - ^{13}C HSQC-NMR experiments were performed on DC-free or DC-treated mixtures of ions and

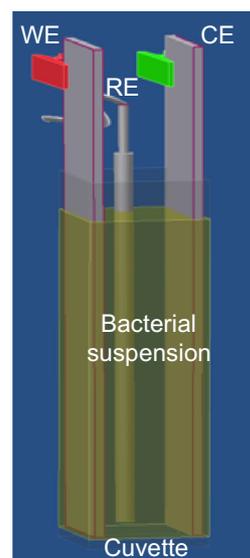


Fig. 1. Electrochemical cell including a working electrode (WE) and counter electrode (CE) and an Ag/AgCl reference electrode (RE).

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