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Effects of azithromycin in *Pseudomonas aeruginosa* burn wound infection

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ABSTRACT

Background: Cutaneous thermal injuries (i.e., burns) remain a common form of debilitating trauma, and outcomes are often worsened by wound infection with environmental bacteria, chiefly *Pseudomonas aeruginosa*.

Materials and methods: We tested the effects of early administration of a single dose of azithromycin, with or without subsequent antipseudomonal antibiotics, in a mouse model of standardized thermal injury infected with *P aeruginosa via* both wound site and systemic infection. We also tested the antimicrobial effects of these antibiotics alone or combined in comparative biofilm and planktonic cultures *in vitro*.

Results: In our model, early azithromycin administration significantly reduced wound and systemic infection without altering wound site or circulating neutrophil activity. The antimicrobial effect of azithromycin was additive with ciprofloxacin but significantly reduced the antimicrobial effect of tobramycin. This pattern was reproduced in biofilm cultures and not observed in planktonic cultures of *P aeruginosa*.

Conclusion: These data suggest that early administration of azithromycin following burn-related trauma and infection may reduce *P aeruginosa* infection and potential interactions with other antibiotics should be considered when designing future studies.

1. Introduction

Cutaneous thermal injury (i.e., skin burn) is one of the most common and debilitating forms of trauma. Each year there are over 1.3 million fires in the United States, and approximately 45,000 of these involve human injury severe enough to require hospitalization [1]. Burns continue to be a common cause of combat-related trauma and often the majority of those injured are civilian rather than military personnel [2]. Though burn trauma critical care and outcomes have improved, there are still greater than 3000 deaths annually [1]. After reaching the hospital, outcomes are often worsened by an acquired

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state of immunosuppression complicated by opportunistic infection. In patients with >40% of total body surface area burn, 75% of deaths are now secondary to infectious complication (e.g., pneumonia, sepsis) or inhalation injury rather than shock [3–6]. Infection is an even greater cause of death from burn trauma in military personnel than in the general population [7]. This disparity may be related to challenges with rapid access to advanced medical care.

Despite aggressive local and systemic treatment to minimize infection, severe burn wounds continue to become infected with environmental and nosocomial pathogens at relatively high rates. Among these, *Pseudomonas aeruginosa* is paramount, accounting for over half of all severe burn infections [8]. This gram-negative bacteria is well adapted to the environment, utilizing biofilm colony growth, which provides a tremendous survival advantage for the pathogen and effectively prevents eradication by the host immune system or antimicrobial drug treatment. A recent review of burn trauma patients who acquired secondary infection with *P aeruginosa* reported that mortality was approximately four-fold greater than in those without *P aeruginosa*, with an average of 23 ventilator-assisted days in *P aeruginosa*–infected patients [9]. Historically, mortality in burn patients with *P aeruginosa* bacteremia has been as high as 77% over a 25-y period [10]. In light of such high incidence of pulmonary infection and morbidity in severe burn-related trauma, interventions capable of limiting systemic spread to the lung may be useful adjuncts to current therapy.

Excessive neutrophil accumulation, combined with impaired clearance of the dead and dying leukocytes, has been shown to worsen tissue damage at injured sites. Recent studies also find that neutrophil products can accelerate *P aeruginosa* biofilm formation, a key feature of infected burn wounds [11–13]. As neutrophils undergo necrosis, long strands of DNA and F-actin are released into the inflammatory milieu and polymerize through covalent attraction. *P aeruginosa* can exploit the neutrophil-rich environment by utilizing these polymers as scaffolding, significantly enhancing early biofilm development [11–13]. Therefore, early and excessive neutrophil recruitment to the site of injury may by a therapeutic target when trying to minimize wound infection.

The pathologic confluence of altered immune function, neutrophilic inflammation, and biofilm-enhanced *P aeruginosa* infection present in thermal injury is also central to airway diseases such as cystic fibrosis (CF) and diffuse panbronchiolitis. In these chronic pulmonary conditions, macrolide therapy can effectively reduce neutrophilic inflammation and improve long-term outcomes [14–17]. The mechanism by which this occurs is multifactorial and not completely understood, as numerous antimicrobial and antiinflammatory or immunomodulatory properties have been reported for azithromycin therapy [16,18–21]. Given the apparent efficacy of macrolide therapy in CF and other diseases, we hypothesized that azithromycin would reduce *P aeruginosa* infection and systemic spread when administered early in a model of cutaneous burn with *P aeruginosa* wound inoculation. Our data support this hypothesis. We also sought to test the impact of early azithromycin administration on more conventional antipseudomonal antibiotics, including ciprofloxacin and tobramycin. Our data indicate that this macrolide may inhibit the antimicrobial effect of tobramycin against *P aeruginosa*, particularly in a biofilm growth state.

2. Materials and methods

2.1. Animals

Eight-week-old sex-matched C57BL/6J mice ranging in weight from 17–25 g were obtained from Jackson Laboratories (Bar Harbor, ME). Animal care and use were in accordance with the Institutional Animal Care and Use Committee and with the permission of National Jewish Health. Animals were housed in microisolator cages within a clean, pathogen-free animal facility and fed irradiated chow to minimize the risk of bacterial contamination. All animals undergoing thermal injury were anesthetized with a single intraperitoneal (i.p.) injection of 0.1 mL 0.1% xylazine – 1% ketamine solution. Three days before injury, hair was removed over the dorsum using an electric shaver and depilation cream (Surgi-Cream; Church & Dwight Co, Inc, Princeton, NJ) to expose the skin surface as described [22].

2.2. Thermal injuries

A 10% total body surface area, partial-thickness, third-degree burn was made on the exposed skin of anesthetized mice as previously described [22–24]. Our modified technique employed a uniform thermal injury by exposing the depilated area for 5 s to a round brass probe (diameter: 28 mm) heated to thermal equilibration with boiling tap water [23]. Sterile saline (2 mL i.p.) was administered to support fluid balance during recovery. Mice were then placed under a warming light and supervised until full recovery. Control mice were shaved but no thermal injury was performed.

2.3. Infectious challenge

A derivative of *P aeruginosa* strain PAO1 was obtained from the Pseudomonas Genetic Stock Center (East Carolina University, Greenville, NC). Bacteria were grown overnight in 2% heat-inactivated platelet-poor pooled human plasma RPMI liquid media at 37°C with shaking and adjusted to an optical density at 600 nm (OD_{600}) of 0.30 (corresponding to 5×10^8 colony-forming units [cfu]/mL) before dilution. Viable bacterial counts were performed by serial dilutions and plating on solid *P aeruginosa* selective media to determine the exact stock titer on the day of each experiment. Before the bacterial challenge, the depilated skin surface of all the anesthetized mice was abraded with an 18G needle to promote infection after bacterial inoculation. Control mice without thermal injury received the same abrasion injury. Two hours following thermal injury, *P aeruginosa* suspension (100 μL) containing 1×10^6 cfu in presterilized saline was placed on the wound site and remained in place while the mice recovered from anesthesia. Body weights were recorded at the time of injury and daily thereafter.