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Effects of phosphate supplementation on *Pseudomonas aeruginosa* invasive behavior in burn wound infections: A simple approach to a big problem

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ABSTRACT

This study was designed to investigate the effect of inorganic phosphate supplementation on invasive behavior of *Pseudomonas aeruginosa* in burn wound infections. An emulsion-based lotion containing sodium dihydrogen phosphate was formulated and then 50 female Sprague-Dawley rats with burn wounds were used to assess the effect of phosphate supplementation on swarming motility of *P. aeruginosa*. On the second day after burn, four groups of rats were inoculated with *P. aeruginosa* and one group was left as negative control. The treatment was started on day 3 and the animals were followed up for 4 weeks. Significant improvement in wound healing was observed in the phosphate-receiving group after the 4-week follow-up, compared to the negative control, positive control, and silver sulfadiazine-receiving groups. Histopathological assessment of the tissue samples also indicated the healing process in phosphate-enriched lotion receiving group. The results showed that inorganic phosphate supplementation results in alteration of the virulence behavior of *P. aeruginosa* and improvement in the wound healing process. In conclusion, phosphate supplementation would be a rational strategy in the eradication of *P. aeruginosa* wound infection.

1. Introduction

Skin burn is one of the most common forms of trauma. Although critical care of burn-related trauma has improved considerably during the last decades, there are still many reports confirming the mortality rate in patients with burns over more than 40% of the total body surface area [1,2]. Bacterial superinfection of the burn wounds, especially with *Pseudomonas aeruginosa*, is a common complication in patients with burn trauma [1]. A number of protocols are available for local and systemic treatment to minimize superinfection. Nevertheless, severe burn wounds are infected at relatively high rates with various environmental and nosocomial bacteria. *P. aeruginosa* accounts for approximately half of all severe burn infections [2]. Biofilm formation by these bacteria

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provides survival advantages and resistance to host immune responses or antibiotics [3,4].

P. aeruginosa is a Gram-negative opportunistic pathogenic bacterium which is normally found in water, soil, and vegetation [5]. Bacterial colonization and the subsequent infection in patients occur in several conditions such as trauma, surgery, or insertion of various devices such as urinary catheter or electrode. The bacterium can also colonize through disruption of the normal flora balance during the administration of broad-spectrum antibiotics or immune system dysfunction [6]. *P. aeruginosa* causes life-threatening infections in various tissues and organs and despite the availability of various antibiotics during the last decades, the eradication of these infections is still difficult [7]. Wound superinfection due to bacterial contamination is still considered as a common complication in patients with burns, potentially leading to considerable morbidity and mortality [8–10]. Based on the literature, sepsis accounts for 50–60% of mortality in patients with burns despite improvements in antimicrobial therapies [11,12]. The antibiotic treatment of *P. aeruginosa* infections may actually cause drug resistance and survival of selected pathogenic variants [13,14].

Studies conducted in some experimental models confirm that phosphate depletion induces phenotypic changes in *P. aeruginosa* [15]. It has been proposed that phosphate depletion in host organs induces three global virulence pathways in *P. aeruginosa*. They include phosphate signaling (phosB), the MvfR-PQS pathway of quorum sensing, and the pyoverdin-mediated iron acquisition system [15]. In addition, some reports indicate injury-induced phosphate depletion [16]. The present study aims to determine whether inorganic phosphate supplementation prevents the activation of lethal phenotype in *P. aeruginosa* in a rat model.

2. Materials and methods

2.1. Materials

Ketamine, xylazine, vaseline, cetostearyl alcohol, sodium dihydrogen phosphate, glycerin, and liquid paraffin were purchased from Merck (Germany). Methyl hydroxybenzoate and propyl hydroxybenzoate were procured from Sigma (USA) and cetomacrogol 1000 was from Darou Pakhsh, Iran. Silver sulfadiazine ointment was purchased from the domestic market in Shiraz, Iran. All other chemicals and reagents were of analytical grade and were used without any modification.

2.2. Methods

2.2.1. Lotion formulation

Sodium dihydrogen phosphate, glycerin, methyl hydroxybenzoate and propyl hydroxybenzoate were mixed at appropriate concentrations with distilled water and heated up to 75 °C in a beaker. Vaseline, liquid paraffin, cetostearyl alcohol and cetomacrogol 1000 were heated up to 75 °C in a separate beaker. Then, the beakers were removed from the water bath, and the aqueous phase was added to the oil phase followed by mixing at 500 rpm for 10 min using a drive mixer. Once the lotion was cooled, it was packed in an appropriate jar and kept in refrigerator for use in animal study. Sodium dihydrogen phosphate concentration was 80 mM in phosphate-enriched formulation.

2.2.2. Particle size and Zeta potential determination

Particle size and Zeta potential of the base emulsion and phosphate-enriched emulsion were determined using Microtrac instrument (Germany). In this case, 1 g of emulsion was diluted in 100 ml of distilled water and 1 ml of the diluted samples was used to measure the Zeta potential and particle size. The mean volume based size of the emulsified phase was 977 nm in base formulation and 684 nm in phosphate-enriched formulation, respectively. The mean Zeta potential of the emulsified oil was 97.6 ± 1.2 mV for base formulation and 70.2 ± 0.4 mV for phosphate-enriched formulation, respectively.

2.2.3. Ethic statement

Animal studies were conducted according to the approved protocols of Shiraz University of Medical Sciences (Shiraz, Iran) for animal handling. The ethical code of the animal study was ec_p_91_4490 and was approved on 1/6/2013 by the Ethics Committee of Shiraz University of Medical Sciences.

2.2.4. Animal study

A total of 50 female Sprague Dawley rats (230 ± 50 g), aged between 8 and 10 weeks, from the Laboratory Animal Center of Shiraz University of Medical Sciences, Shiraz, Iran were randomly divided into five different groups. All the rats were anesthetized using ketamine–xylazine cocktail (100 mg/kg ketamine and 10 mg/kg xylazine). Then, the back of the anesthetized rats was shaved with scissors and the predetermined sites were burnt for the same duration using a hot square plate of 1-cm^2 surface area, which had been heated on top of the flame.

P. aeruginosa was isolated from the infected burn wound of the patient in Ghotbedin Burn Hospital, Shiraz, Iran. Preliminary identification of isolated bacteria was performed by colony morphology, odor, zone of hemolysis and oxidase, methyl red, Voges–Proskauer, and citrate tests and finally the isolated strain PTCC number (1811) was specified by Iranian Research Organization for Science and Technology (IROST). This strain was resistant to gentamicin, tetracycline, tobramycin, ceftazidime, ceftriaxone, ciprofloxacin, carbenicillin, imipenem, and intermediate to piperacillin–tazobactam. *P. aeruginosa* was cultured and incubated for 24 h. It was then suspended in normal saline solution and the microbial concentration was adjusted to 1.2×10^9 CFU/ml (4 Macfarlane).

P. aeruginosa suspension of 1.2×10^9 CFU/ml was applied to the inflamed areas of four rat groups and one group was assigned as the negative control group (group 2). Each rat was kept in isolated separate cage to prevent the transmission of infection between animals. At 24 h after inoculation and confirmation of the infection in the injured areas, treatment was started, according to the following group classification:

Phosphate-receiving group: Includes 10 infected rats. Phosphate-enriched lotion formulation was applied on the surface of the wounds one time a day.

Negative control group: Includes 10 rats that were burnt. *P. aeruginosa* suspension was not applied, neither did they receive any treatment.