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Comparison of octenidine dihydrochloride (Octenisept®), polihexanide (Prontosan®) and povidon iodine (Betadine®) for topical antibacterial effects in Pseudomonas aeruginosa-contaminated, full-skin thickness burn wounds in rats

Research Article

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Abstract: *Pseudomonas aeruginosa* is one of the most frequently isolated organisms from infected burn wounds and a significant cause of nosocomial infection and septic mortality among burn patients. In this animal study, three antiseptic agents which were Octenidine dihydrochloride (Octenisept®, Schülke & Mayr, Norderstedt, Germany), polyhexanide (Prontosan®, B. Braun, Melsungen AG, Germany) and povidon iodine (Betadine, Purdue Pharma L.P, Stamford, USA) were compared to assess the antiseptic effect of their applications on experimental burn wounds in rats contaiminated with *P. aeruginosa*. All treatment modalities were effective against *P. aeruginosa* because there were significant differences between treatment groups and control groups. The mean eschar concentrations were not different between polyhexanide and povidon iodine groups, but there were significant differences between the octenidine dihydrochloride group and the other treatment groups, indicating that the Octenidine dihydrochloride significantly eliminated *P. aeruginosa* more effectively in the tissues compared to the to other agents. All treatment modalities were sufficient to prevent the *P. aeruginosa* invasion into the muscle and to cause systemic infection. In conclusion, Octenidine dihydrochloride is the most effective antiseptic agent in the treatment of the *P. aeruginosa*-contaminated burn wounds; Octenidine dihydrochloride can be considered as a treatment choice because of its peculiar ability of limit the frequency of replacing wound dressings.

Keywords: *Pseudomonas aeruginosa* • *Burn wounds* • *Antiseptic*

1. Introduction

Thermal destruction of the skin barrier and concomitant depression of local and systemic host cellular and humoral immune responses cause infectious complications in burn patients [1]. Prevention of burn wound infection is dependant on optimal wound care including the use of antiseptic agents and prompt wound closure [2,3].

Pseudomonas aeruginosa is one of the most frequently cultured organisms from infected burn wounds. It also remains as a significant cause of nosocomial infection and septic mortality in burn patients [4-7].

There is no report in the literature that compares the activities of commonly used antiseptic agents which are Octenidine dihydrochloride (Octenisept®, Schülke & Mayr, Norderstedt, Germany), polyhexanide (Prontosan®,

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B. Braun, Melsungen AG, Germany) and povidon iodine (Betadine, Purdue Pharma L.P, Stamford, USA) against *Pseudomonas aeruginosa* on a burn wound model. We present the comparison of these agents in treating a full-skin thickness burn in rat wound seeded 24 hour earlier with a standard strain of P. aeruginosa.

2. Materials and Methods

The study beganafter the experimental protocol had been approved by the Ethical Committee of the Haydarpasa Training Hospital. Male Sprague- Dawley rats (n = 32) weighing 250g to 300 g were used and housed under standard conditions at ambient room temperatures and given laboratory chow and water ad libitum throughout the study. The back skins of the rats were shaved after intraperitoneal anesthetic application (ketamine hydrochloride 80 mg/kg body weight). After the shaving a full-skin thickness scald burn at back skin on approximately 15% of each rat's body surface was made by placing them in boiling water [8]. Fluid rescucitation with an intraperitoneal injection of 2 ml of lactated Ringer's solution was made on all rats. Each burn wound was seeded with 0.5 ml of broth containing 10^8 colony forming units of *P. aeruginosa* (ATCC 27853; American Type Culture Collection, Rockville, MD) by swabbing ten minutes after the burn. The rats were placed in separate sterilized cages and allowed to recover. The animals were then assigned at random to four groups 24 hours after the trauma..

Group 1 was assigned as the control group, and no topical agent was applied, group 2 was assigned as the Octenidine dihydrochloride group, group 3 was assigned as the polihexanide group, and group 4 was assigned as povidone- iodine group. Treatment began at 24 hours after burn injury. Octenidine dihydrochloride was sprayed over the burn areas daily, polihexanide and povidone- iodine were applied over them using sterile gauze and all burn areas left exposed. All the animals were anesthetized and killed on 7^{th} day after burn injury.

All cultures were obtained using aseptic techniques. After thoracotomy was performed, blood cultures from the left ventricle and lung biopsies were obtained. Full-skin thickness 9-mm punch biopsies were obtained from the center of the burn eschar. After removal of eschar and underlying fascia, a separate biopsy of paravertebral muscle deep to the burn eschar was obtained. Separate quantitative cultures of eschar and muscle were performed using a standard method [9].

One milliliter of intracardiac blood samples drawn in aseptic conditions were inoculated into blood culture bottles (Bactec Peds Plus™, USA). Tissue samples were weighed and transferred to the laboratory wrapped in sterile aluminium foil. The weight of each tissue sample was noted upon arrival at the microbiology laboratory. Tissue biopsies were then grinded completely in sterile dishes and homogenized after adding 1 ml of Brain Heart Infusion Broth (BHIB). The homogenates were diluted in sterile saline for concentrations of 10^{-1} to 10^{-4}. For culturing, 0.1 ml from each dilution were inoculated onto sheep blood agar and Eosin Methylene Blue (EMB) agar and incubated at 37°C for 24 hours. Isolated bacteria were identified via conventional biochemical tests and BBL Crystal E/NF ID Kit™ (Becton-Dickinson, USA). Thirty to 300 colonies grown on solid media were counted for quantitative evaluation. Colony counts per gram of tissue were then calculated for each sample. Growth ≥ 10^5 CFU/gr were accepted as infection criteria. For calculation; the formula N X D X V 10 /W = CFU/gr tissue was used [N: number of colonies on the plate (CFU); D: dilution factor (10^{-1} -10^{-4}); V: volume of broth used for diluting the tissues (1 ml); constant factor for 0.1 ml standart inoculation (10); W: weight of the sample (gr)].

The median, mean, and standard deviation of counts for each treatment group were determined. For statistical analysis, the package program SPSS (Statistical package for Social Sciences for Windows 10.0, SPSS Institute, Chicago, IL) was used. Kruskal–Wallis and Mann–Whitney U test were used to compare medians of groups. Probability levels less then 0.05 were considered significant.

3. Results

No rats died throughout the study. The frequency of recovery of the seeded organisms from each culture site is detailed in Table 1. The swabbed organism was recovered from the eschar of all groups. A comparison of quantitative cultures performed on burn eschar and muscle is shown in Table 2.

Statistically the results were significantly different between treatment and control groups (control – Octenidine dihydrochloride [$p < .001$], control - polihexanide [$p < .001$], and control - PI [$p < .05$]). The mean eschar concentrations were not significantly different in the polihexanide and PI ($P > .05$), but significant differences were found between Octenidine dihydrochloride and the other treatment groups (octenidine dihydrochloride -polihexanide, $p < .05$; octenidine- PI, $P < .001$).

Table 1. Frequency of recovery of seeded organisms from each culture site.

	Number of rats	Eschar	Muscle	Lung	Blood
Control	8	8	6	5	7
Octenidine	8	-	-	-	-
Polihexanide	8	3	1	1	-
Povidon- iodine	8	2	2	-	1

Table 2. Comparison of quantitative cultures performed on burn eschar and muscle.

	Number of rats	**Eschar**		**Muscle**	
		Mean±SD	**Median**	**Mean±SD**	**Median**
Control	8	$7.1\pm2.4x\ 10^6$	$8.2X10^6$	$8.3\pm3.9x\ 10^5$	$7.8X10^5$
Octenidine	8	Not recovered	Not recovered	Not recovered	Not recovered
Polihexanide	8	$4.7\pm1.2x\ 10^6$	$5.0X10^6$	$3.1\pm1.9x\ 10^6$	$3.3X10^6$
Povidon- iodine	8	$4.6\pm1.9x\ 10^6$	$5.1X10^6$	$2.9\pm1.1x\ 10^6$	$3.0X10^6$

4. Discussion

In this study, three topical antiseptic solutions; Octenidine dihidrochloride, Polihexanide and Povidone- iodine against were compared *P. aeruginosa* in burn wounds. To our knowledge, there has been no report comparing the activities of these antiseptics against *P. aeruginosa* on a in-vivo model. This is the first study in this field which presents the efficacies of three different antiseptic agents for the treatment of experimental burn wounds in contaminated with *P. aeruginosa.*

The in-vivo model is better than in vitro test which does not prove clinical potency. The use of antiseptic agents play an important role in preventing invasive burn wound infection. An efficient antiseptic agent used for burn injury should provide antimicrobial cover to minimize the growth of the pathogenic bacteria and prevent colonization. Different antimicrobial agents were used against *P. aeruginosa* and their influence was discussed in the literature [10-13].

N,N'-(1,10 decanediyldi-1[4H]-pyridinyl-4-ylidene) bis-(1-octanamine) dihydrochloride (= octenidine dihydrochloride) is a chemical substance with two cation active centers in its molecule not interacting with each other due to the fact that they are separated by a long aliphatic hydrocarbon chain. Octenidine dihydrochloride had no sensitising potential. In a battery of in vivo studies on local tolerance no irritant effects on skin, the vagina, or the eyes were observed [14]. Although several in vitro studies have suggested that antiseptic agents are cytotoxic to fibroblasts and to other cell cultures, in vivo studies with octenidine and octenidine-containing preparations have failed to demonstrate an adverse effect to wound healing [15]. As a cation-active substance octenidine dihydrochloride binds readily to the negatively charged bacterial cell envelope, consequentlly disrupting the vital functions of the cell membrane and killing the cell. Octenidine dihydrochloride exhibits a broad spectrum of antimicrobial efficacy against gram-positive and gram-negative bacteria and fungi. Sedlock and Bailey evaluated the determining capacity of Octenidine dihydrochloride on skin estimated its potential as a skin antiseptic by examining its antimicrobial activity against *P. aeruginosa* and common nosocomial pathogens. They showed that Octenidine dihydrochloride was effective against to *P. aeruginosa* [16]. In our experimental study, the results showed that Octenidine dihydrochloride was the most potent anti pseudomonal antiseptic agent in burn wound. It prevented the colonization of the *P. aeruginosa* in all of the tissues, including eschar, which the other agents could not achieve.

The antiseptic polyhexanide was introduced in the 1980's in Europe [17]. Polyhexamide, containing the polymeric biguanide polihexanide, possesses microbicidal activity against a broad spectrum of bacteria. Polyhexanide is the first known antiseptic which has a specific action against negatively charged cell layers of prokaryotic cells and is less affecting eukaryotic neutral lipid membranes [18,19]. It was shown that polyhexanide was efficient eradication of various pathogens in a chronic wound [20]. Daeschlein et al. demostrated that polihexanide proved clinically and histologically superior to povidone-iodine for the treatment of second-degree burns and it does not inhibit the re-epithelialization process [21]. According to the results of our study polihexanide prevented the penetration and systemic spreading of *P. aeruginosa* but could not remove it from the eschar as octenidine did.

Povidone-iodine (Betadine®), a complex of iodine, is a commonly used antimicrobial agent. It consists of the bactericidal component, with polyvinylpyrolidone

(povidone) and a synthetic polymer. The most common commercial form is a 10% solution in water yielding 1% available iodine [22]. Its antimicrobial action is due to iodination and oxidation of the membranes and cytoplasm of infective agents by free iodine of molecules [23]. Povidone-iodine solution is commonly used worldwide because of its potent germicidal activity, relatively low irritancy, toxicity and low cost. Povidone-iodine has been demonstrated to be effective at killing a broad range of the pathogens generally associated with wound infection [24]. But it is not always effective at killing common bacteria. Anderson discussed two reports of povidone-iodine solution contaminated with *Pseudomonas spp*. The contamination apparently occurred during production of the povidone-iodine solution. The bacteria remained viable for several weeks and were eventually involved in patient infections [25]. Although povidone- iodine prevented the penetration and systemic spreading *P. aeruginosa*, it could not remove *P. aeruginosa* from the eschar similar to the results that discussed before.

In this experimental study, the results showed that Octenidine dihydrochloride was the most potent anti pseudomonal agent. It prevented the colonization of the *P. aeruginosa* in all of the tissues, including eschar, which the other agents could not achieve. Polihexanide and povidone- iodine also prevented the penetration and systemic spreading *P. aeruginosa*, but they could not remove *P. aeruginosa* from the eschar.

We believe that this data will be useful clinically to select the efficient antiseptic agent used for burn wound contaminated with *P. aeruginosa* to minimize the growth of the bacteria and prevent colonization.

In conclusion, our results provide a basis for preclinical investigations of three antiseptic agents which were Octenidine dihydrochloride, Polyhexanide and Povidon Iodine were compared to assess the antiseptic effect of their applications on experimental burn wound model in contaminated with *P. aeruginosa* in rats. In the present study, animal data suggest that all antiseptic agents which are used this study are effective against *P. aeruginosa* because there are significant differences between treatment groups and control groups. Octenidine dihydrochloride is the most effective agent in the treatment of *P. aeruginosa* contaminated burn wound and can be considered as treatment choice.

We believe that this data will be useful clinically to select the efficient antiseptic agent used for burn wound contaminated with *P. aeruginosa* to minimize the growth of the bacteria and prevent colonization.

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