Effectiveness of Electrolyzed Oxidized Water Irrigation in a Burn-Wound Infection Model

Hajime Nakae, MD, PhD, and Hideo Inaba, MD, PhD

Objective: **The purpose of the study was to determine whether electrolyzed oxidized water (EOW) functions as a bactericide in burn injury with *Pseudomonas aeruginosa* infection in a rat burn-wound model.**

Methods: **Anesthetized Sprague-Dawley rats (n = 31) were subjected to third-degree burns to 30% of total body surface area. Two days after injury, all rats were infected with *P. aeruginosa* using 1 mL of a suspension containing 1 × 10^8 colony-forming units. Rats were assigned to one of three groups: no irrigation (group I), irrigation with physiologic saline (group II), or irrigation with EOW (group III). Blood culture, endotoxin levels, and survival rates were determined.**

Results: **Survival rate was significantly higher in group III than in groups I or II ($p < 0.0001$). Serum endotoxin levels on day 3 after infection in group III were significantly lower than the levels in group I ($p < 0.01$) and group II ($p < 0.01$). There were significant differences between the three groups in the culture of *P. aeruginosa* ($p < 0.05$).**

Conclusion: **Irrigation and disinfection with EOW may become useful in preventing burn-wound sepsis.**

Key Words: **Electrolyzed oxidized water, Burn-wound infection, *Pseudomonas aeruginosa* infection.**

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Major burn injury can lead easily to sepsis caused by wound infection, even when acute burn shock is under good control. We have reported that numerous chemical mediators, such as cytokines, type II phospholipase A2, platelet-activating factor, eicosanoids, polymorphonuclear leukocyte elastase, complement, endothelin-1, thrombomodulin, nitric oxide, and adhesion molecules, are involved in biologic responses to burn injury and that levels of these mediators are not very high in the acute phase of burn injury. However, levels increase rapidly because of complications that occur in the infection phase, and they are markers of the severity of illness.[1–7] The larger the wound, the greater the chance of burn-wound infection. The risk of *Pseudomonas aeruginosa* infection is particularly high on days 5 to 7 after burn injury. Thus, burn-wound infection must be prevented.

Electrolyzed oxidized water (EOW) is produced with an anode current by electrolyzing salt-containing water through a diaphragm.[8] EOW, which has a high positive oxidation-reduction potential (ORP) and high concentrations of dissolved chloride and oxygen, functions as a bactericide and is used clinically for the treatment of various types of infection and for cleaning and disinfecting of hemodialysis equipment.[9] Using a rat model, we studied whether or not EOW shows a bactericidal function in burn injury with *P. aeruginosa* infection.

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From the Department of Emergency and Critical Care Medicine, Akita University School of Medicine, 1–1-1 Hondo, Akita 010-8543, Japan.

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Address for reprints: Hajime Nakae, MD, Department of Emergency and Critical Care Medicine, Akita University School of Medicine, 1–1-1 Hondo, Akita 010-8543, Japan. Fax: 018-884-6450.

MATERIALS AND METHODS

The protocols for animal experimentation described in this paper were approved by the Animal Research Committee of Akita University School of Medicine. All animal experiments were kept to the Guidelines for Animal Experimentation of the University (approval number 98–5).

Animals

Thirty-one male Sprague-Dawley rats (aged 8 weeks; 230–260 g; Nippon SLC Ltd., Sendai, Japan) were used in the experiment. All rats were housed in individual cages in a temperature-controlled room with alternating 12-hour light-dark cycles, and the animals were acclimated for a minimum of 3 days before the study. All rats were provided with standard laboratory chow ad libitum.

Burn Injury

Experimental burn injury was induced in all rats according to the method of Walker et al.[10] and Yalcin et al.[11] In summary, rats were anesthetized by intraperitoneal injection of pentobarbital (40 mg/kg). Hair on the back of each rat was removed with an electric clipper, and dorsal skin surfaces were exposed to 95°C water for 10 seconds through a template designed to produce a third-degree burn that covered approximately 30% of the total body surface area. Immediately after the burn injury, 5 mL of physiologic saline was administered intraperitoneally to each rat for fluid resuscitation.

EOW

The equipment we used to produce EOW was an Oxilyzer (Miura Denshi Co., Ltd., Akita, Japan). The EOW generated was acidic (pH $\leq$ 2.7) and had an effective chlorine concentration of 0.287 to 1.148 mEq/L, and a dissolved oxygen concentration of 6.25×10^{-3} to 2.5 mEq/L, with a redox potential of at least +1,000 mV. The temperature of the EOW delivered to the wound surface was 21°C.

Experiment

Two days after injury, all rats were infected with *P. aeruginosa* (ATCC 27853) using 1 mL of a suspension containing 1×10^8 colony-forming units. The suspension fluid was dripped onto the wound and rubbed into the skin by fingertip covered with a sterile glove.[11] Rats were assigned to the following groups: no irrigation (n = 9) (group I); irrigation with 20 mL of physiologic saline for approximately 20 seconds once a day (group II), starting 8 hours after the application of *P. aeruginosa* (n = 11); or irrigation with 20 mL EOW for approximately 20 seconds once a day, starting 8 hours after the application of *P. aeruginosa* (n = 11) (group III). The wounds were kept open after irrigation. On day 5 after injury, blood samples were taken from the subclavian vein of each rat to obtain blood cultures and to determine levels of endotoxin. On day 7 after injury, the burn-wound cultures were analyzed for *P. aeruginosa*. Mortality was recorded daily until postburn day 23. All rats that survived beyond day 23 were killed by an overdose of pentobarbital.

Endotoxin Assay

Blood samples were collected in endotoxin-free heparinized blood-specimen tubes. They were centrifuged immediately at 3,000 rpm for 40 seconds, and platelet-rich plasma was obtained and stored at −80°C until assay. Endotoxin levels were measured by Chromogenic Substrate Assay (Seikagaku Corp., Tokyo, Japan). Normal values are below 10.0 pg/mL.

Statistical Analysis

Data are expressed as mean ± SD. Mortality was analyzed by Kaplan-Meier plots and compared using the log-rank test. A post-hoc Fisher's paired least significant difference test was used to compare endotoxin levels. The Kruskal-Wallis rank test was used for group comparisons. A *p* value of less than 0.05 was considered significant.

RESULTS

The mortality rate was 100% in group I, 90.9% in group II, and 9.1% in group III. The survival rate for group III was significantly higher than for groups I or II ($p < 0.0001$) (Fig. 1). *P. aeruginosa* was detected by blood culture on day 3 after infection in 5 of 9 rats in group I, 4 of 11 in group II, and 2 of 11 in group III; there were no significant differences in the detection of *P. aeruginosa* between the three groups ($p = 0.3654$). The values of serum endotoxin on day 3 after infection in group III (7.4 ± 3.4 pg/mL) were significantly lower than values in group I (18.9 ± 5.7 pg/mL) ($p = 0.0017$) or group II (18.3 ± 10.7 pg/mL) ($p = 0.0017$), although there was no significant difference between groups I and II ($p = 0.8516$) (Fig. 2). *P. aeruginosa* was detected in burn-wound cultures on day 5 after infection in 2 of 3 rats in group I, 3 of 5 in group II, and 0 of 11 in group III; these differences were significant ($p = 0.0118$) (Table 1).

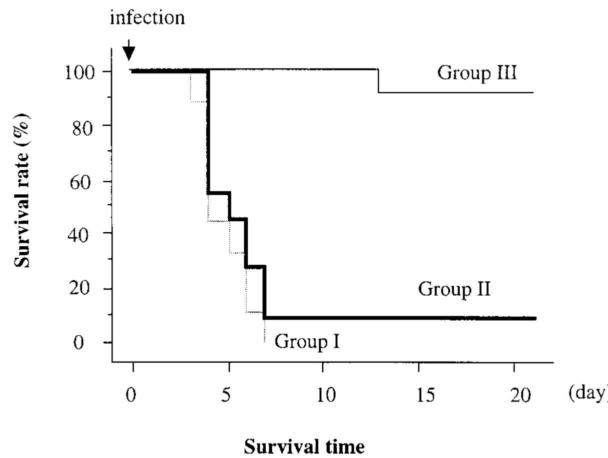

Fig. 1. *The survival curve of the three groups determined by Kaplan-Meier methods. The survival rate in group III (EOW) (n = 10) (10 of 11) was significantly higher than the survival rate in group I (control) (n = 1) (1 of 11) or II (physiologic saline) (0 of 9) (p < 0.0001).*

DISCUSSION

The method for producing electrolyzed water is shown in Figure 3. The source is tap water (Na^+: 0.49 mEq/L; Cl^-: 0.24 mEq/L) with added salt as an electrolysis-promoting agent. Positive and negative electrodes separated by an ion-exchange membrane are contained in an exchange. When voltage is applied, water is electrolyzed to field-oxidized water at the positive electrode and reduced water at the negative electrode according to the following chemical formulas:

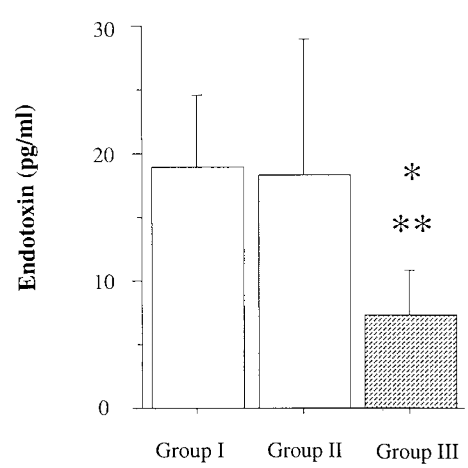

Fig. 2. *Serum endotoxin levels on day 3 after infection. Values shown are mean ± SD. *p < 0.01 for comparison between group III (EOW) and group I (control). **p < 0.01 for comparison between group III and group II (physiologic saline).*

Table 1 Burn-Wound Culture Findings on Day 5 According to Groups

	Number of Positive Cultures (%)
Group I	2/3 (66.7)
Group II	3/5 (60.0)
Group III	0/11[a] (0)

Group I, control; group II, physiologic saline; group III, electrolyzed oxidized water.
[a] $p < 0.05$ (Kruskal-Wallis rank test).

Positive electrode:

$$H_2O \rightarrow 1/2O_2 + 2H^+ + 2e^-$$
$$2Cl^- \rightarrow Cl_2 + 2e^-$$
$$Cl_2 + H_2O \rightarrow H^+ + Cl^- + HClO$$

Negative electrode:

$$2H_2O + 2e^- \rightarrow H_2 + 2OH^-$$

Thus, water at the positive electrode becomes EOW, which has a low pH and a high ORP and is rich in dissolved chloride, dissolved oxygen, and hydroxy radical. Water at the negative electrode becomes an electrolyzed basic aqueous solution, which has a high pH and a low ORP and is rich in alkaline minerals, and in which organ compounds can be reduced.[8,12] There is no explosion hazard, because the device produces a very small amount of hydrogen gas.

The environment required by mitochondria in all microorganisms must be within the range from 2 to 12 in pH and from −400 to 850 mV in ORP. However, EOW requires a pH below 2.7, dissolved oxygen above 20 ppm, hypochlorite ion above 30 ppm, and ORP above 1,100 mV.[8] The fact that some EOW specifications fall outside the range required by mitochondria in microorganisms is one reason for its germicidal effects. Moreover, the solution contains a high concentration of hypochlorous acid, a well-known and potent germicidal agent in which bactericidal action increases markedly in acid solution. The hydroxy radical also has a germicidal effect, and the electrical energy of the solution itself destroys microorganisms including viruses, fungi, mycobacteria, spirochetes, and bacteria. Almost all germs will perish within 10 seconds in such a solution. Shimizu et al. [13] reported that bactericidal effects of EOW differ from those of hypochlorous acid only because substances contained in EOW, such as Cl^-, ClO_2, H_2O_2, OH^+ (hydroxyl radical), etc., seem to support synergistically such activity by balancing in a competitive state in acidic conditions. It is important to note that the solution loses both its electrolytic potential and its germicidal action when it comes into contact with multicellular organic matter, and it reverts to ordinary water with no added function.[8,14,15] EOW does not provide a long-lasting antibacterial effect after irrigation. Therefore, infected tissues should be irrigated with a sufficient amount of solution to obtain the necessary effect. We applied 20 mL of EOW slowly for approximately 20 seconds during irrigation.

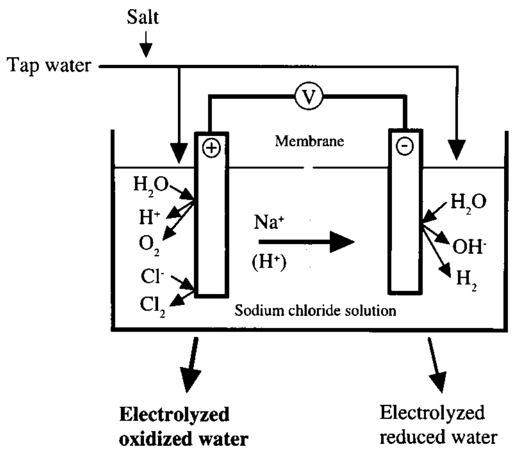

Fig. 3. *Production of electrolyzed water.*

Irrigation using povidone-iodine solution, a conventional treatment for infected burn wounds, sometimes causes adverse effects. These effects include thyroid dysfunction, liver dysfunction, restrictive pericarditis, electrolyte derangement, metabolic acidosis, skin erosions, and damage to underlying healthy tissues, which impedes granulation and sometimes results in the failure of the treatment.[16,17]

We usually use physiologic saline to irrigate abdominal cavity, thoracic cavity, or dirty injured sites. Therefore, we used physiologic saline for group II. In this study, the survival rate in the EOW irrigation group was significantly higher, serum endotoxin levels in the EOW irrigation group were significantly lower, and *P. aeruginosa* levels in burn wounds were significantly lower than in the other groups. These data suggest that EOW irrigation may be effective bactericidally.

The prevention of sepsis is critical to a good prognosis for patients with severe burn injury. It is important to control infection at the wound site. Irrigation and disinfection with EOW may prevent burn-wound sepsis in humans. Additional studies need to be done to confirm that EOW irrigation does not cause tissue damage and that it promotes tissue growth in a partial-thickness injury model.

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