Effect of Electrolyzed Water on Wound Healing

Artificial Organs 2000; 24:984-987

*Naoki Yahagi, *Masashi Kono, *Masaki Kitahara,
*Akito Ohmura, †Osao Sumita,
‡Toshimasa Hashimoto, ‡Katsuaki Hori,
‡Chen Ning-Juan, §Paul Woodson, ¶Shoji Kubota,
**Arata Murakami, and **Shinichi Takamoto,
*Department of Anesthesiology, Teikyo University
Mizonokuchi Hospital; †Coherent Technology Inc.;
‡Faculty of Engineering, Tokyo Institute of
Technology; §Basic Sciences Department, Kenmore
Bastyr University; ¶Water Design Laboratories, and
**Department of Thoracic Surgery, University of
Tokyo, Tokyo, Japan

Abstract: Electrolyzed water accelerated the healing of full-thickness cutaneous wounds in rats, but only anode chamber water (acid pH or neutralized) was effective. Hypochlorous acid (HOCl), also produced by electrolysis, was ineffective, suggesting that these types of electrolyzed water enhance wound healing by a mechanism unrelated to the well-known antibacterial action of HOCl. One possibility is that reactive oxygen species, shown to be electron spin resonance spectra present in anode chamber water, might trigger early wound healing through fibroblast migration and proliferation.

Key Words: Wound healing— Electrolyzed water—Anode water—Cathode water—Hypochlorous acid.

Electrolyzed water, in a variety of forms and made by a variety of different processes, is widely used in

Japan as a topical disinfectant (1,2). We describe here our findings that some types of electrolyzed water appear to accelerate the healing of full-thickness cutaneous wounds in rats.

Materials and methods

Six different types of water were made and tested. The first, ultrapure water (resistivity $\simeq 18\ M\Omega \cdot cm$), was produced by a sequence of treatments applied to city tap water: charcoal filtration, reverse osmosis, and ion exchange. One group of experimental animals was treated with this ultrapure, nonelectrolyzed water. Four types of electrolyzed water were made from this ultrapure water. In each case, the water was electrolyzed (voltage gradient 13 V and apparent current density 200 mA/cm^2) while being pumped (500 ml/min) through a 3 chamber device (Coherent Technology, Tokyo, Japan) (3). One chamber contained a platinum-plated titanium anode. Usually, the middle chamber was filled with a saturated sodium chloride solution made with the ultrapure water. The third chamber contained the cathode, also made of platinum-coated titanium. Proprietary ion-exchange membranes separated the chambers. The following 3 types of electrolyzed water were made using the Coherent Technology device in this configuration (all measurements made at 25°C). The first, acid pH water, Ac(+), was taken from the anode chamber [pH 2.50–2.63, oxidation-reduction potential (ORP) 1104–1191 mV, concentration of residual chlorine (determined by the o-toluidine method) 80 to 100 ppm]. Neutral pH (7.40) anode chamber water, N(+), was made by adding NaOH (1N) to the electrolyzed water taken from the anode chamber [pH 7.4, ORP 749–784 mV, concentration of residual chlorine almost the same as in the Ac(+) solution]. Alkaline pH water, Al(−), was taken from the cathode chamber (pH 10.65–10.85, ORP 212–297 mV, residual chlorine only a few ppm). Replacing the platinum cathode in the Coherent Technology device by one made of carbon and replacing the center-chamber solution by 5 *M* citrate (organic acid; citric acid) in ultrapure water allowed us to make a fourth type of electrolyzed water. This acidic water [Ac(−)] was taken from the cathode chamber (pH 3.86–3.87, ORP 212–297 mV, no residual chlorine). Finally, hypochlorous acid (HOCl) solution was made by electrolyzing 0.45% NaCl in a single-chamber device (Omuko Co. Ltd., Tokyo, Japan) (Voltage gradient, apparent current density, and pump rate were the same as for the Coherent Technology device.). The pH and ORP of this solution were 7.45 and 780 mV, respectively. When stored in PET bottles, the pH and ORP of all 6 types of water remained stable for at least a month.

Forty-two Wistar rats (8 weeks old) were randomly assigned to 6 experimental groups, housed in individual metabolic cages at 25°C, and fed rodent chow and water ad libitum. Under pentobarbital anesthesia, the back was shaved and two 1.0 cm square, full-thickness cutaneous wounds were made, one behind the other and 1.5 cm apart, on the back of each animal. In each rat, 1 wound (selected randomly) was treated twice a day for 7 days with 1 of the 6 types of water described previously; the other wound was left untreated. The rat was observed carefully until the water was absorbed by the wound to ensure that no spillage occurred. The first treatment was administered immediately after surgery. All wounds were allowed to heal without dressings. For 17 days after surgery, wound areas were measured daily by planimetry, using digital video camera images displayed via a personal computer.

Acid and neutralized anode waters were studied by electron spin resonance (ESR; JEOL-JES-RE2X; Nihon Denshi, Tokyo, Japan) with the addition of a spin trapping agent [5,5-dimethyl-1-pyrroline-N-oxide (DMPO, Sigma, St. Louis, MO, U.S.A.)] using a flat cell of 1 mm width (4). This was carried out 24 hr after the preparation of the electrolyzed water. The duration of the incubation of the electrolyzed water with DMPO was 2 min. The magnetic field was of 335 ± 5 mT. Signal strength was compared with the signal derived from Mn^{2+} (as a control).

All protocols were approved by the Animal Research Review Committee of Teikyo University Medical School.

For each group, the results are presented as mean wound area ± SD. The comparison between the areas of water-treated and nontreated wounds was performed using a paired Student's *t* test. The comparisons among the experimental groups were made using a one-way analysis of variance. When statistical significant was detected, Dunnett's test was used to determine which values differed significantly from those obtained using the nonelectrolyzed ultrapure water. Values of $p < 0.05$ were considered significant.

Results

As shown in Table 1, both types of anode chamber water [acidic and neutral: Ac(+) and N(+), respectively] accelerated wound healing (when compared to the effect of nonelectrolyzed ultrapure water) ($p < 0.05$). This acceleration of wound healing was evident from as early as postoperative Day (POD) 1 in Ac(+) or POD 2 in N(+). The alkaline water from the cathode chamber [Al(−)] showed a slight, but