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Bactericidal effect of electrolyzed neutral water on bacteria isolated from infected root canals

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Objective. The purposes of this study were to examine the time-related changes in pH, oxidation-reduction potential, and concentration of chlorine of electrolyzed neutral water and to evaluate the bactericidal effect of electrolyzed neutral water against bacteria from infected root canals.
Study design. Various properties of electrolyzed neutral water—pH value, oxidation-reduction potential, and concentration of chlorine—were measured at different times after storage of the water in the open state, the closed state, or the closed-and-dark state. The bactericidal effect of the various electrolyzed neutral water samples was then tested against 17 strains of bacteria, including 15 strains isolated from infected canals, as well as against 1 strain of fungus. Each bacterial or fungal suspension was mixed with electrolyzed neutral water, and the 2 substances were reacted together for 1 minute. After incubation for 1 to 7 days, the bactericidal effect of the electrolyzed neutral water was determined.
Results. The pH value and oxidation-reduction potential of electrolyzed neutral water remained almost unchanged when the water was stored in a dark, closed container. However, the concentration of chlorine decreased from 18.4 ppm to 10.6 ppm. Electrolyzed neutral water showed a bactericidal or growth-inhibitory effect against the bacteria.
Conclusions. The results indicate that electrolyzed neutral water maintains a constant pH and oxidation-reduction potential when kept in a closed container without light and that it exhibits a bacteriostatic/bactericidal action against isolates obtained from infected root canals.
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A variety of bacteria are present in infected root canals.[1] For that reason, mechanical enlargement and chemical disinfection of the root canal system are performed. Various agents are used to irrigate and disinfect root canals.

Sodium hypochlorite, ethylene diaminetetraacetic acid, and the like have been used as irrigants. These solutions have bactericidal effects.[2,3] In addition, Yamada et al[4] found the smear layer to be removed by irrigation with both solutions in alternation.

Recently, electrolyzed water has been used in Japan to disinfect produce for the sake of preventing food poisoning.[5,6] Electrolyzed water has a strong and immediate bactericidal effect[2,7]; data suggest that electrolyzed water, combined with with tap water and salt, is safe for human consumption. In addition, electrolyzed water does not produce allergic responses in humans.[8]

The active factors responsible for the bactericidal effect of electrolyzed water are chlorine-related substances, such as chlorine (Cl_2), hypochlorous acid (HClO), and hypochlorous acidic ion (ClO^-).[9] The bactericidal effect of the chlorine-related substances is stronger with nondissociated HClO than with dissociated ClO^-. In electrolyzed neutral water (ENW), Cl_2, having the strongest bactericidal effect, dissolves poorly, whereas HClO dissolves easily.

In medical facilities, electrolyzed water has been used as a disinfectant for hands and as a cleaning agent for surfaces such as floors and beds for the prevention of opportunistic infection.[5] Recently, electrolyzed water has been used in dentistry for disinfection of dental instruments,[10] root canal irrigation,[11] irrigation of the gingival pocket,[12] and gargles.[13]

The purpose of this study was to examine time-related changes in pH, oxidation-reduction potential

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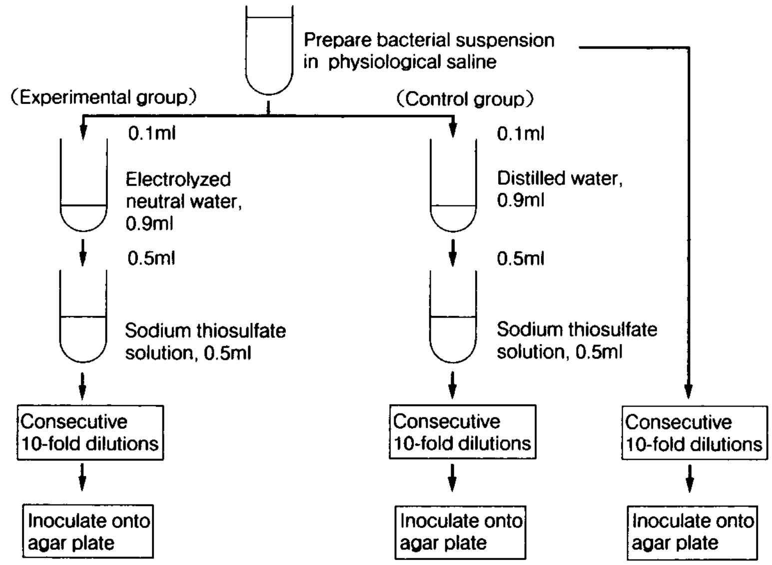

Fig 1. Flow diagram shows experiment designed to determine bactericidal effect of freshly prepared ENW. Sodium thiosulfate solution was used to stop reaction; reaction time was 1 minute. All operations were performed in anaerobic glove box.

(ORP), and chlorine concentration of ENW, which is one type of electrolyzed water. Also investigated was the bactericidal effect of this water against bacteria isolated from infected root canals.

MATERIAL AND METHODS

Changes in ENW properties with storage time

Ameni Clean (National Co/Matsushita Seico Co, Osaka) was used to produce ENW by mixing tap water with a solution of a specific electrolyte. The ENW was immediately placed into polypropylene containers, and each sample was stored for 3 weeks at room temperature (approximately 20°C) under 1 of 3 different conditions: (1) the open state, (2) the closed state, and (3) the closed-and-dark state.

The pH, ORP (as indicated by pH Meter HM-60S, TOA Electronics Ltd, Tokyo), and concentration of chlorine (as indicated by DO Meter UC-101, Central Kagaku Co, Tokyo) of the ENW under each of the 3 storage conditions were measured at various times during the storage period.

Bacterial species

The bacteria used were as follows: methicillin-resistant *Staphylococcus epidermidis* (MRSE), isolated from a human nasal cavity; *Bacillus subtilis*, obtained from the Department of Microbiology of Aichi-Gakuin University School of Dentistry; and 15 strains isolated and identified from infected root canals[14] at our department. The facultative anaerobes comprised 5 strains: *Staphylococcus aureus, Streptococcus sanguis, Lactobacillus acidophilus,* MRSE, and *B subtilis*. The 12 obligative anaerobes were *S intermedia, Peptococcus niger, Peptostreptococcus anaerobius, Veillonella parvula, L rogosae, Actinomyces israelii, Eubacterium lentum, Bifidobacterium bifidum, Propionibacterium acnes, Porphyromonas endodontalis, Prevotella melaninogenica,* and *Fusobacterium nucleatum*. *Candida albicans*, a fungus, was also obtained and examined.

These 18 strains were subcultured anaerobically at 37°C in anaerobic bacterial culture medium broth (ABCM Broth, Eiken Co, Tokyo) with 0.2% agar.

Bactericidal effects of ENW

An outline of the experiment is shown in Fig 1. All bacteria were grown anaerobically at 37°C in an anaerobic glove box on ABCM agar plates prepared with or without 5% defibrinated sheep blood. The blood was added to promote the growth of *P endodontalis* and *P melaninogenica*. Bacterial suspensions were made at 10^5 to 10^7 colony-forming units per mL in physiological saline solution. A 0.1-mL volume of the suspension and 0.9 mL of freshly prepared ENW were mixed and reacted together for 1 minute at 37°C. Then 0.5 mL (volume) of the mixure was added to an equal volume of 0.6% sodium thiosulfate solution to stop the reaction in the mixture.[15,16] Thereafter, 0.1 mL of the fluid was inoculated onto the agar plates, and the plates were incubated at 37°C for 1 to 7 days anaerobically. The number of

Table I. Time-dependent change in pH

Time	pH Open*	Closed†	Closed-and-dark‡
Immediate	6.10	NT	NT
1 h	6.19	6.11	6.05
3 h	6.20	6.11	6.05
6 h	6.20	6.11	6.04
1 d	6.24	6.26	6.05
2 d	6.25	6.10	6.03
4 d	6.34	6.06	6.05
7 d	6.29	6.07	5.90
14 d	6.21	5.88	5.92
21 d	6.30	5.57	6.04

NT, Not tested.
*No cover on container used for ENW storage.
†Cover on container used for ENW storage.
‡Container used for ENW storage covered and kept in dark.

Table II. Time-dependent change in oxidation-reduction potential

Time	Potential (mV) Open	Closed	Closed-and-dark
Immediate	880.7	NT	NT
1 h	842.4	857.4	859.5
3 h	879.9	870.7	866.6
6 h	870.3	856.5	874.5
1 d	861.1	853.9	869.6
2 d	845.6	851.3	873.1
4 d	817.0	833.1	856.1
7 d	793.3	843.5	845.5
14 d	750.4	843.3	856.4
21 d	650.4	800.1	826.9

See legend to Table I for explanations of storage states.
NT, Not tested.

Table III. Time-dependent change in chlorine concentration

Time	Chlorine concentration (ppm) Open	Closed	Closed-and-dark
Immediate	17.4	NT	NT
1 h	17.5	18.8	18.4
3 h	18.9	18.3	18.5
6 h	18.6	18.1	18.0
1 d	17.4	18.2	18.7
2 d	16.4	16.2	18.2
4 d	14.4	16.1	16.4
7 d	11.1	13.9	17.7
14 d	9.0	12.1	14.1
21 d	6.2	8.8	10.6

See legend to Table I for explanations of storage states.
NT, Not tested.

colonies that appeared were then counted. As a control, sterilized distilled water was used instead of ENW.

Bactericidal effects of stored ENW

Freshly prepared ENW was kept for 2 or 7 days in the closed state at room temperature before being mixed with the bacterial suspensions and tested as described. All bacterial strains except for *B subtilis* and *C albicans* were examined. The latter were excluded because the freshly prepared ENW showed low bactericidal effect against these strains; stored ENW would thus be ineffective against them.

RESULTS

Changes in ENW properties with storage time

The time-dependent changes in pH value are shown in Table I. With the container in the closed state, the pH value of the water stored for 21 days decreased slightly, from 6.10 to 5.57. However, the water under the other 2 storage conditions (open and closed-and-dark) remained constant throughout the experimental period.

The changes in ORP value are shown in Table II. With the container in the open state at room temperature, the ORP value of the water stored for 21 days decreased from 880 mV to 650 mV. The samples maintained under the other 2 storage conditions remained at more than 800 mV even after 21 days.

The effect of storage time and condition on the concentration of chlorine is shown in Table III. At room temperature, the concentration of chlorine showed the least change in the closed-and-dark state.

Bactericidal effects of fresh ENW

The bactericidal effects of freshly prepared ENW against the 18 strains tested are shown in Table IV. ENW was bactericidal against 12 strains: *S aureus*, MRSE, *S sanguis*, *P niger*, *Ps anaerobius*, *L acidophilus*, *L rogosae*, *A israelii*, *E lentum*, *B bifidum*, *P endodontalis*, and *F nucleatum*. In addition, the ENW reduced the bacterial numbers of the other 6 strains. However, ENW showed little effectiveness against *B subtilis*.

With distilled water used as a control, the bacterial number is theoretically reduced to 1/20 of that in the physiological saline solution, as indicated in Fig 1. Practically, as shown in Table IV, the bacterial number for distilled water showed a value nearly 1/20 of that for the physiological saline solution. Therefore, distilled water did not inhibit the growth of bacteria during the short time of exposure.

Bactericidal effects of stored ENW

Overall, there was decreased bactericidal activity after ENW storage. The ENW that was produced 2 days before testing showed a bactericidal effect against 10 strains: *S aureus, MRSE, S sanguis, P niger, L acidophilus, A naeslundii, E lentum, B bifidum, P*

Table IV. Bactericidal effect of freshly prepared electrolyzed neutral water

	Concentration*		
Strain	*Nontreated†*	*Control‡*	*Experiment (ENW)*
Staphylococcus aureus	4.8×10^6	1.7×10^5	ND
MRSE	4.6×10^6	3.1×10^4	ND
Streptococcus sanguis	2.3×10^6	1.2×10^5	ND
Streptococcus intermedia§	5.9×10^5	1.8×10^6	<10
Peptococcus niger§	7.2×10^6	3.1×10^5	ND
Peptostreptococcus anaerobius§	5.5×10^6	2.3×10^5	ND
Veillonella parvula§	1.1×10^7	9.2×10^5	<10
Lactobacillus acidophilus	3.2×10^7	1.8×10^6	ND
Lactobacillus rogosae§	1.2×10^7	4.9×10^5	ND
Actinomyces israelii§	3.3×10^5	1.4×10^4	ND
Eubacterium lentum§	5.8×10^5	2.0×10^4	ND
Bifidobacterium bifidum§	4.0×10^7	2.2×10^6	ND
Propionibacterium acnes§	1.1×10^7	8.2×10^5	<10
Porphyromonas endodontalis§	1.1×10^5	3.0×10^4	ND
Prevotella melanimogenica§	9.8×10^7	5.6×10^6	<10
Fusobacterium nucleatum§	4.4×10^5	1.6×10^4	ND
Bacillus subtilis	9.1×10^7	3.9×10^6	7.9×10^5
Candida albicans (fungus)	3.9×10^6	1.4×10^5	4.1×10

ND, Not detected.
*Colony-forming units per mL, as determined on agar plates.
†Bacterial concentration in stock preparations suspended in physiological saline solution at beginning of assay.
‡Distilled water.
§Obligative anaerobe.

acnes, and *F nucleatum*. For the other six strains, the number of bacteria was reduced. With the ENW produced 7 days before being mixed with the bacteria, the number of strains killed decreased to 8; this compared with 12 strains killed with freshly mixed ENW. However, there still was a reduction in the bacterial number for the other 8 strains (data not presented).

DISCUSSION

There are several reports on the bactericidal effect of superoxidized water (SOW), which is one type of electrolyzed water.[2,7,15,17] However, few reports on ENW have appeared until now.[18] SOW, which is strongly acidic, is produced by electrolysis of tap water containing a small quantity of NaCl. The SOW obtained from the anode site is characterized by a pH of less than 2.7, an ORP of 1000 mV or more, and a concentration of chlorine ranging from 20 ppm to 50 ppm.[19] ENW is produced by mixing tap water and a solution obtained by electrolyzing an electrolyte containing NaCl. This water is characterized by a pH in the range of 5.5 to 7.0, an ORP of 600 to 800 mV, and a concentration of chlorine of roughly 20 ppm.[19]

SOW and ENW have both been used as disinfectants for hands and as cleaning agents for floors and beds in Japan. The active factors responsible for the bactericidal effect in both types of water are chlorine-related substances, such as chlorine (Cl_2), hypochlorous acid (HClO), and hypochlorous acidic ion ($C10^-$).[9] The bactericidal effect of the chlorine-related substances is stronger with nondissociated HClO than with dissociated $C10^-$. Cl_2, having the strongest bactericidal effect, dissolves poorly in ENW, whereas HCIO dissolves easily in ENW. According to a comparison of the values indicated previously, there are great differences in pH and ORP between SOW and ENW. An environment of neutral pH and an ORP below approximately 900 mV is conducive to the growth of microorganisms. These facts suggest that chlorine-related substances, especially HCIO, may be the main bactericidal factor in ENW.

In the present study, the pH value of the ENW was approximately 6.0 under all storage states, and the ORP value was approximately 800 mV, except in the open state, during the entire experimental period. The concentration of chlorine was 6.3 ppm at 21 days with storage in the open state. ENW was more stable with time than SOW, as reported by Sakai et al[15] and Hiratsuka et al.[17] The slight time-dependent change in ENW may be related to the high concentration of dissolved HClO or to the low volume of volatilized Cl_2.

The bactericidal effect of freshly prepared ENW was shown in 12 of the 18 strains tested. 6 strains were not killed: *S intermedia*, *V parvula*, *P acnes*, *P melaninogenica*, *B subtilis*, and *C albicans* (fungus). Of these, the last 2 are also resistant to SOW.[17] Hirano and Ueda[18] reported that ENW did not have any bactericidal effect against *B subtilis* and 2 kinds of molds. Shiba et al[12] reported that SOW exerted a stronger bactericidal effect against obligative anaerobes than against facultative anaerobes. Our finding of a bactericidal effect against *S intermedia, V parvula, P acnes,* and *P melaninogenica* is similar to that of Shiba et al.[22] The bactericidal effect of ENW produced 7 days before testing was shown in 8 of the 16 strains examined. Hiratsuka et al[17] reported that SOW produced 7 days before the test was bactericidal for 2 of 12 strains. It is possible that the factor responsible for the bactericidal effect of ENW is more stable than the corresponding factor in SOW. This may be related to the fact that dissolved chlorine does not decrease as much over time in ENW as in SOW, as shown by Sakai et al[15] and Hiratsuka et al.[17] In the light of our results, ENW may have potential as an intracanal irrigant, though in vitro conditions and in vivo conditions may be not the same and results thus may differ.

Sodium hypochlorite has been routinely used in the root canal as an intracanal irrigant. However, it is highly irritating as well as cytotoxic.[20] Deleterious effects of ENW have not yet been reported. It would be worthwhile to conduct further studies, including studies on cytotoxicity and on the clinical application of ENW for root canal treatment as an irrigant or intracanal medicament.

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