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Comparison of the Microbicidal Activities of Superoxidized and Ozonated Water in the Disinfection of Endoscopes

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The microbicidal activities of superoxidized water (electrolysed strong acid water [ESAW] or electrolysed weak acid water [EWAW]), ozonated water, 0.05% chlorhexidine and 2% glutaraldehyde were tested against seven strains of clinical micro-organism isolates. Following incubation of bacterial suspensions in ESAW and EWAW for 10 s, the number of micro-organisms was reduced below the detection limit. The microbicidal activities of ESAW and EWAW were similar to that of glutaraldehyde, and superior to ozonated water and 0.05% chlorhexidine. The microbicidal activities of ESAW, EWAW and ozonated water were markedly diminished in the presence of albumin. Microbial contamination of upper gastrointestinal endoscopes was detected after 90 endoscopic procedures, but treatment of the endoscope with ESAW, EWAW or ozonated water eradicated the microbes. These results indicate that ESAW and EWAW are effective disinfectants after mechanical cleaning of upper gastrointestinal endoscopes, and can, therefore, be used in the endoscopy unit.

KEY WORDS: DISINFECTANTS; ELECTROLYSED STRONG AND WEAK ACID WATER; OZONATED WATER; MICROBICIDAL ACTIVITY; ENDOSCOPY; CONTAMINATION

Introduction

Widespread use of endoscopic procedures in daily practice has raised concerns regarding the risk of transmission of infectious organisms via these procedures. In a recent review, many episodes of infection were found to have been transmitted by upper gastrointestinal endoscopy,[1,2] findings which emphasize the importance of disinfecting the endoscope after use. Glutaraldehyde has been recommended as a suitable disinfecting agent,[3,4] but it is toxic, an irritant, and sensitizing to the skin, eyes, and respiratory and gastrointestinal tracts.[5,6] Furthermore, Glutaraldehyde is only slowly effective

against mycobacteria and spores, with suppliers of the disinfectant advising a contact time varying from 20 min for high-level disinfection to 10 h for sterilization.[7]

Newer disinfectants, such as electrolysed strong acid water (ESAW), electrolysed weak acid water (EWAW), and ozonated water have a high oxidation-reduction potential.[8,9] These solutions can be prepared cheaply using salt and tap water, and lose their oxidative and acidic properties when exposed to the environment. They are therefore safe, and do not harm human tissues,[10,11] and their application in medicine is gradually increasing.[12 – 14]

Materials and methods

MICRO-ORGANISMS AND DISINFECTANTS

Seven strains of clinical micro-organism – *Helicobacter pylori*, methicillin-resistant *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Mycobacterium avium* and *Candida albicans* were randomly chosen from clinical isolates at Nagasaki University Hospital, and the microbicidal properties of ESAW, EWAW, ozonated water, 0.05% chlorhexidine (Zeneca-Pharma, Osaka, Japan) and 2% glutaraldehyde (Cidex®; Johnson and Johnson, Tokyo, Japan) were evaluated.

Electrolysed strong acid water of pH 2.3 – 2.7 was prepared by electrolysis of tap water, using a Super Oxseed Alpha 1000® (Janix Inc., Kanagawa, Japan). This had an oxidation-reduction potential of 1000 – 1100 mV and contained approximately 30 ppm of dissolved chlorine. EWAW was prepared using the NDX-70KMW® (OMCO, Saitama, Japan); its pH was 5.0 – 6.0, with a dissolved chlorine concentration of 50 – 80 ppm. Ozonated water at a concentration of 10 ppm was prepared using Gaiya Water® (Mizutomo, Tokyo, Japan). The concentrations selected represented those commonly used in solutions prepared for hand washing. All disinfectant solutions were mixed with sterilized distilled water at the time of use, and sterilized distilled water was used as a control.

ASSESSMENT OF MICROBICIDAL ACTIVITY

One millilitre of solution containing the test micro-organism (concentration: 10^7 cfu/ml), in saline, was added to 5 ml of the test disinfectant solution. Following incubation at room temperature for 10 s, 60 s or 300 s for *H. pylori*, MRSA, *E. coli*, *P. aeruginosa* and *C. albicans*, and 60 s, 300 s or 600 s for *B. subtilis* and *M. avium*, 0.1 ml of the mixture was transferred into tubes containing 0.9 ml of neutralizing agents. The neutralizing agents – confirmed through a series of preliminary experiments to have inactivating effects against 0.05% chlorhexidine, ESAW, EWAW and ozonated water (data not shown) – consisted of 10% Tween 80, 3% lecithin and 0.5% sodium thiosulphate.

Once neutralized, samples were cultured immediately on appropriate media, under specific conditions as follows: *H. pylori*, on *H. pylori* agar (Nissui Pharmaceutical Co., Tokyo, Japan) containing 10% horse serum at 37 °C in 5% O_2 and 15% CO_2 for 7 days; MRSA, *E. coli*, *P. aeruginosa* and *B. subtilis*, on triptic soy agar (Difco Laboratories, Detroit, MI, USA) at 37 °C for 24 h; *C. albicans*, on Sabouraud dextrose agar (Difco Laboratories) at 30 °C for 48 h; *M. avium*, on egg-based Ogawa medium (Nissui Pharmaceutical Co.) at 37 °C for up to 6 weeks. These procedures were performed in duplicate, and the bactericidal activity results expressed (according to Haley *et al.*[15]) as mean colony forming units (CFU) of recovered bacteria per 0.1 ml after indicated contact time with the disinfectant.

ASSESSMENT OF MICROBICIDAL ACTIVITY IN THE PRESENCE OF ALBUMIN

One millilitre of solution containing the test micro-organism, in saline, was added to 0.1 ml of 0.5 mg/ml albumin (from bovine serum) and 4.9 ml of the test disinfectant solution (final albumin concentration was 0.01 mg/ml).[7,14] Following incubation at room temperature (for 10 s, 60 s or 300 s for *H. pylori*, MRSA, *E. coli*, *P. aeruginosa* and *C. albicans*), 0.1 ml of the mixture was transferred into tubes each containing 0.9 ml of neutralizing agents. Solutions were cultured on the appropriate media and under the specific conditions described in the previous section.

ASSESSMENT OF CONTAMINATION

Clinical contamination of Olympus GIF-XQ 200® gastrointestinal endoscopes (Olympus, Tokyo, Japan) was examined after 90 upper-gastrointestinal endoscopic procedures. Saline was aspirated through the suction channel of the endoscope before and after disinfection with ESAW, EWAW or ozonated water, and the endoscopic washings collected. Five millilitres of each sample were placed in a sterile suction trap attached directly to the endoscope. Samples were cultured on appropriate media and under the conditions previously described.

Results

MICROBICIDAL ACTIVITY

Electrolysed strong acid water, EWAW and 2% glutaraldehyde killed *H. pylori*, MRSA, *E. coli*, *P. aeruginosa* and *C. albicans* within 10 s of contact (Table 1), whereas 600 s of contact was required by all three agents to kill *B. subtilis*, and *M. avium* was killed by ESAW and EWAW after 300 s and by glutaraldehyde after 60 s (Table 2). Ozonated water did not kill *H. pylori*, *B. subtilis* or *M. avium*, and 0.05% chlorhexidine did not kill MRSA, *B. subtilis* or *M. avium*, even after 300 s of contact (Table 1 and Table 2).

MICROBICIDAL ACTIVITY IN THE PRESENCE OF ALBUMIN

In the presence of albumin, glutaraldehyde killed all test micro-organisms within 10 s of contact, but ESAW and EWAW only killed *H. pylori* (even after 300 s of contact). Ozonated water did not kill any micro-organisms and chlorhexidine did not kill MRSA and only killed *C. albicans* after 300 s of contact (Table 3).

CONTAMINATION OF UPPER GASTROINTESTINAL ENDOSCOPY

After endoscopic examination of 90 patients, the micro-organisms most frequently detected on endoscopes were α-streptococci (70/90), γ-streptococci (41/90) and assorted Neisseria species (53/90) (Table 4). Other micro-organisms present included *H. pylori* (5/90), *Enterobacter cloacae* (2/90), *Klebsiella oxytoca* (2/90), *Serratia marcescens* (1/90) and *C. albicans* (1/90). No micro-organisms were detected after disinfection with ESAW, EWAW and ozonated water (Table 4).

Discussion

Helicobacter pylori is an important aetiologic agent in acute gastritis, chronic active gastritis and peptic ulcer, and gastrointestinal endoscopic cross-infection with *H. pylori* has been reported.[1,2,16] Complete decontamination of the endoscope can prevent this risk, but an optimal disinfectant is required. Several reports have recommended the use of glutaraldehyde for the disinfection of gastrointestinal endoscopes, based on its broad-spectrum disinfective activity against bacteria and viruses. Glutaraldehyde is, however, an irritant, and some individuals may develop acute allergic reactions.[3–6]

TABLE 1:
Microbicidal effects of various disinfectants *in vitro* against clinical isolates after 10 s, 60 s and 300 s of contact. Data represent the number of micro-organisms remaining in samples after contact with the disinfectant

	Electrolysed strong acid water			Electrolysed weak acid water			Ozonated water			Chlorhexidine			Glutaraldehyde			Control (distilled water)
Contact times (s)	10	60	300	10	60	300	10	60	300	10	60	300	10	60	300	10
Helicobacter pylori	0	0	0	0	0	0	> 500	108	88	0	0	0	0	0	0	> 500
Methicillin resistant *Staphylococcus aureus*	0	0	0	0	0	0	0	0	0	> 500	> 500	> 500	0	0	0	> 500
Escherichia coli	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	> 500
Pseudomonas aeruginosa	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	> 500
Candida albicans	0	0	0	0	0	0	0	0	0	> 500	> 500	0	0	0	0	> 500

TABLE 2:
Microbicidal effects of various disinfectants *in vitro* against *Bacillus subtilis* and *Mycobacterium avium* after 60 s, 300 s and 600 s of contact. Data represent the number of micro-organisms remaining in samples after contact with the disinfectant

	Electrolysed strong acid water			Electrolysed weak acid water			Ozonated water			Chlorhexidine			Glutaraldehyde		
Contact times (s)	60	300	600	60	300	600	60	300	600	60	300	600	60	300	600
Bacillus subtilis	> 500	3	0	> 500	51	0	> 500	> 500	> 500	> 500	81	52	> 500	> 500	0
Mycobacterium avium	> 500	0	0	> 500	0	0	> 500	> 500	> 500	> 500	> 500	> 500	0	0	0

TABLE 3:
Microbicidal effects of various disinfectants *in vitro* against clinical isolates in the presence of albumin, after 10 s, 60 s and 300 s of contact. Data represent the number of micro-organisms remaining in samples after contact with the disinfectant

	Electrolysed strong acid water			Electrolysed weak acid water			Ozonated water			Chlorhexidine			Glutaraldehyde		
Contact times (s)	10	60	300	10	60	300	10	60	300	10	60	300	10	60	300
Helicobacter pylori	0	0	0	0	0	0	> 500	> 500	> 500	0	0	0	0	0	0
Methicillin resistant *Staphylococcus aureus*	> 500	> 500	> 500	> 500	> 500	> 500	> 500	> 500	> 500	> 500	> 500	> 500	0	0	0
Escherichia coli	> 500	> 500	> 500	> 500	> 500	> 500	> 500	> 500	> 500	0	0	0	0	0	0
Pseudomonas aeruginosa	> 500	> 500	> 500	> 500	> 500	> 500	> 500	> 500	> 500	0	0	0	0	0	0
Candida albicans	> 500	> 500	> 500	> 500	> 500	> 500	> 500	> 500	> 500	> 500	> 500	0	0	0	0

TABLE 4:
Microbial contamination of upper gastrointestinal endoscopes before and after disinfection. Data represent the number of procedures in which the micro-organism was found/the total number of procedures

Isolated micro-organisms	Before disinfection	After disinfection
Electrolysed strong acid water		
α-streptococci	22/30	0/30
γ-streptococci	15/30	0/30
Neisseria species	16/30	0/30
Helicobacter pylori	1/30	0/30
Serratia marcescens	1/30	0/30
Candida albicans	1/30	0/30
Electrolysed weak acid water		
α-streptococci	24/30	0/30
γ-streptococci	13/30	0/30
Neisseria species	19/30	0/30
Helicobacter pylori	2/30	0/30
Enterobacter cloacae	1/30	0/30
Klebsiella oxytoca	1/30	0/30
Ozonated water		
α-streptococci	24/30	0/30
γ-streptococci	13/30	0/30
Neisseria species	18/30	0/30
Helicobacter pylori	2/30	0/30
Enterobacter cloacae	1/30	0/30
Klebsiella oxytoca	1/30	0/30

Electrolysing water using salt and tap water could provide a useful low-cost disinfectant.[17] These acids have a strong level of ionization,[7,8,10,11] but in the case of water, no new hydrogen ions are generated; these are produced only by electrolysis of the saline solution. The full-strength solution is therefore not corrosive to skin and mucosa. The mechanism of action is postulated to be oxidation of the cell membranes, inactivation of enzymes and denaturation of the nucleic acids of pathogens.[18]

Electrolysed water is divided into two types – ESAW and EWAW – depending on the pH value: 2.3 – 2.7 for ESAW and 5.0 – 6.0 for EWAW. Some reports have described the usefulness of ESAW for endoscope disinfection, but there have been no reports of the use of EWAW. This study, however, shows the effectiveness of both agents for this purpose. ESAW is corrosive to metals,[8,12] so is probably not ideal for long-term use as a

disinfectant for endoscopes. EWAW however, is a weaker acid and contains higher concentrations of chlorine, making it more suitable.

This study tested seven strains of micro-organisms, all of which are important clinical pathogens. ESAW, EWAW and glutaraldehyde had immediate microbicidal effects (superior to those of ozonated water and 0.05% chlorhexidine) on five of these micro-organisms, but two strains (*B. subtilis* and *M. avium*) were highly resistant to the disinfectants, even after longer contact periods (600 s).

Ozonated water exhibits microbicidal activities against bacteria and fungi, and is used to disinfect food and waste water.[19,20] In our study, however, it showed no microbicidal activity for *H. pylori*, *B. subtilis* and *M. avium* within 5 min of contact, indicating it is not a suitable disinfectant for upper-gastrointestinal endoscopes.

Previous studies have indicated that the bactericidal effects of electrolysed water and ozonated water decrease in the presence of organic matter,[7,14] and our study supports this. Addition of albumin to bacterial solutions containing 10^7 cfu/ml reduced the bactericidal activity of the test disinfectants, suggesting that the bactericidal activities of ESAW and EWAW may be reduced in the presence of organic substances, and that simple disinfection may be insufficient to completely eradicate bacteria. For total eradication, the item to be disinfected should be rinsed or immersed in ESAW and EWAW for 5 – 15 min.

In conclusion, we have demonstrated that ESAW and EWAW have powerful micro-bicidal activities and can be used safely, after mechanical cleaning, for the disinfection of upper-gastrointestinal endoscopes. Further studies are necessary to explore the suitability of these disinfectants in a clinical environment.

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