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NEW INSTRUMENTS AND TECHNIQUES

STRONGLY ACIDIC ELECTROLYZED WATER: VALUABLE DISINFECTANT OF ENDOSCOPES

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Background: Glutaraldehyde (GA) is currently considered to be the best disinfectant for endoscope disinfection. However, GA poses high risks for medical staff involved in the process and also to the environment. Strongly acidic electrolyzed water (SAEW) has been recently re-evaluated for its potent bactericidal effect and environmental safety.
Methods: Through the aspiration channel of the scopes, upper GI endoscopes and colonoscopes were experimentally contaminated with *Pseudomonas aeruginosa*, *Mycobacterium avium* and hepatitis B surface antigen positive blood. Four disinfection methods were tested: manual washing only, soaking in 3% GA for 5 and 10 min, and a 10-s soak in SAEW with 50 or 100 mL of aspiration.
Results: Direct plating culture was positive for *Pseudomononas* contamination after manual washing only (1/5) and after a 5-min soak in 3% GA. Complete disinfection, confirmed by enrichment culture and polymerase chain reaction (PCR) of *Pseudomonas* and hepatitis B surface antigen positive blood on the contaminated upper GI endoscope was obtained after a 10-min soak in GA and after using SAEW (0/5). *Mycobacterum avium* are rather resistant against SAEW as determined by broth culture and PCR (1/5).
Conclusion: Strongly acidic electrolyzed water is a valuable disinfectant for endoscopes.

Key words: disinfection, glutaraldehyde, manual washing, strongly acidic electrolyzed water, super-oxidized water.

INTRODUCTION

Endoscopy has become an indispensable tool for diagnosing and treating digestive diseases. Accordingly, one endoscope is used more than once during the course of a day. Since 1974, transendoscopic infections have been reported[1] and several guidelines for endoscope cleaning and disinfection have been recommended.[2,3] The standard method of disinfection is a 20-min soak in glutaraldehyde (GA) in an automatic endoscope reprocessor.[4] However, in addition to its high cost, GA has various adverse effects for medical staff and the environment.[5]

Strongly acidic electrolyzed water (SAEW) is produced by using water and salt under electrolysis with membrane separation. It contains HClO, generating hydroxy radicals that have a rapid and potent bactericidal effect.[6,7] Additionally, the low pH (pH 2.7) and high oxidation–reduction potential (1100 mV) of SAEW are toxic to microorganisms[8] (Table 1). This water is quite easily neutralized with organic solutions and thus is safe for humans and the environment. Strongly acidic electrolyzed water was made from Super oxseed α1000 (Shionogi Pharmacolog. Inc.; Fig. 1) and also from ESW-45 (Olympus Optical Comp.; Fig. 2). These machines continuously produce 750–1000 mL/min of SAEW from tap water and salt.

We introduced SAEW as an endoscope disinfectant in 1994 and proved its efficacy and safety.[9] In this paper, we discuss the effect of SAEW compared with the use of GA by using experimentally contaminated endoscopes.

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MATERIALS AND METHODS

Twelve endoscopes, which had been routinely used at the Kanto Medical Center, Nippon Telegraph and Telephone East Corporation, Tokyo, were used in this experiment: half were upper GI endoscopes (Olympus video endoscope XQ200, XQ230) and the other half were colonoscopes (Olympus video endoscope CF200, CF230). Before the experiment, each endoscope was cleaned using an automatic reprocessor (Olympus OW30) with a 30-min soak in GA and alcoholic flush. *Pseudomonas aeruginosa* (ATCC 27853), *Mycobacterium avium* (ATCC 25291) and hepatitis B surface antigen (HbsAg) positive, e antigen positive blood were used as markers of contamination. Each bacterial suspension was prepared from precultures in sterile distilled water and adjusted to 10^6 CFU/mL. Then 10 mL of this suspension was aspirated from the tip of the endoscope through a suction channel; 20 mL was used for the colonoscope.

Fig. 1. Strongly acidic electrolyzed water generator used for this experiment: α1000 320 (w)×460 (d)×570 (h); produces 1000 mL/min of SAEW; storage tank is necessary.

Table 1. Bactericidal effects of SAEW compared with 0.1% NaOCl

Microorganism	SAEW	0.1% NaOCl*
Staphylococcus aureus	<5 s	<5 s
Staphylococcus epidermis	<5 s	<5 s
Escherichia coli	<5 s	<5 s
Serratia marcescens	<5 s	<5 s
Bacillus cereus	<5 s	<5 s
Mycobacterium sp.	1–3 min	5–30 min
Fungi	5–30 s	5–15 s
Herpes simplex	<5 s	<5 s
Influenza virus	<5 s	<5 s

SAEW, strongly acidic electrolyzed water; NaOCl, standard disinfectant of chloride. * Data for NaOCl courtesy of Dr Iwasawa.[6]

At our hospital, routine manual washing is performed by nursing staff using an aseptic brush under tap water. After manual washing, a control and three conditions of disinfectant were studied: 5-min soak in 3% GA, 10-min soak in 3% GA, and 10-s soak in SAEW followed by 50 mL (upper GI endoscope) and 100 mL (colonoscope) suction of SAEW. An appropriate broth was then injected through the biopsy orifice and collected from the endoscope tip.[10] Each broth (50 μL) was spread on NAC agar (Eiken Chemical Co. Ltd, Tokyo, Japan) and Mycobacteria 7H11 agar (Difco Laboratories, Detroit, MI, USA), for detection of *P. aeruginosa* and *M. avium.* The former was incubated for 48 h, and the latter was incubated for 3 weeks at 35°C. Afterwards, viable cell counts were determined by conventional plating methods. For the enrichment culture for *P. aeruginosa*, 100 μL of 10 mL was collected from the endoscope tip and inoculated

Fig. 2. Strongly acidic electrolyzed water generator used in this experiment: overview of ESW-45 with storage tank; generator: 290 (w)×510 (d)×510 (d); 750 mL/min of SAEW from tap water; tank stores 100 L max. of SAEW.

in Heart infusion broth (Difco) containing nalidixic acid (15 μg/mL; Sigma Chemical Co. MO USA). The broths were incubated at 35°C for up to 5 days. The polymerase chain reaction (PCR) technique for the detection of *Pseudomonas* has been described elsewhere.[11] The MGIT 960 system was used for the cultivation of *M. avium*, and the AMPLICOR Mycobacterium Avium system (Roche Diagnostic Systems, Inc, NJ, USA) was used for *M. avium* PCR.[12]

The HBsAg positive, e antigen positive blood was ob tained from one volunteer. The blood (200 mL) was diluted with 800 mL of saline and 50 mL was aspirated from the tip of the endoscope through a suction channel. After contamination, four disinfection methods were studied: routine manual washing (control), manual washing plus a 5- and 10-min soak in 3% GA, and manual washing plus a 10-s soak in SAEW with SAEW suction. Then, 10 mL of saline was collected in the same manner and checked for hepatitis B virus (HBV) DNA as reported elsewhere.[13]

Manual washing comprised four steps following the guidelines of the Japan Gastroenterological Endoscopy Technicians Society. First, tap water was aspirated through the tip of the endoscope. Second, soap and tap water used.

Table 2. Viable cells of *Pseudomonas aeruginosa* and *Mycobacterium avium* after treatment with strongly acidic electrolyzed water (SAEW) and 3% glutaraldehyde (GA)

Test strain	Disinfectant	Incubation time (min)					
		0.5	1	2	3	5	10
Pseudomonas aeruginosa							
	SAEW	$<2.0\times10^1$	$<2.0\times10^1$	$<2.0\times10^1$	$<2.0\times10^1$	$<2.0\times10^1$	$<2.0\times10^1$
	3% GA	$<2.0\times10^1$	$<2.0\times10^1$	$<2.0\times10^1$	$<2.0\times10^1$	$<2.0\times10^1$	$<2.0\times10^1$
Mycobacterium avium							
	SAEW	$>10^4$	$>10^4$	$>10^4$	1.0×10^3	2.0×10^2	$<2.0\times10^1$
	3% GA	$>10^4$	$>10^4$	2.4×10^3	3.2×10^2	$<2.0\times10^1$	$<2.0\times10^1$

Table 3. Effects of disinfection of upper GI endoscopes contaminated with *Pseudomonas aeruginosa* and *Mycobacterium avium*

Test strain	Disinfectant	Positive number		
		Bacterial plate count (CFU/mL)	After cultivation	PCR product
Pseudomonas aeruginosa				
	Control (before)	0/5	0/5	0/5
	Manual washing	1/5 (4.0×10^2)	5/5	5/5
	3% GA (5 min)	1/5 (6.0×10^1)	1/5	1/5
	3% GA (10 min)	0/5	0/5	0/5
	SAEW	0/5	0/5	0/5
Mycobacterium avium				
	Control (before)	0/5	0/5	0/5
	Manual washing	0/5	3/5	3/5
	3% GA (5 min)	0/5	1/5	1/5
	3% GA (10 min)	0/5	0/5	0/5
	SAEW	0/5	0/5	0/5

3% GA (5 min), 5-min soak in glutaraldehyde, 3% GA (10 min), 10-min soak in 3% glutaraldehyde, SAEW, strongly acidic electrolyzed water.

Third, each channel was brushed. Finally, further aspiration using tap water was performed.

Glutaraldehyde was prepared by using 3% GA (Sterihide) manufactured by Maruishi Pharmacological Inc. Japan. Just before use, the pH and chloride concentration were checked (pH 2.7; residual chloride ion concentration 50 ppm; oxidation–reduction potential 1100 mV).

RESULTS

Bactericidal activity of 3% GA and SAEW (Table 2)

The bacterial suspensions (100 µL, 10^8 CFU/mL) of the *P. aeruginosa* and *M. avium* strains tested were prepared and mixed with a 10-mL volume of 3% GA and SAEW. The mixture was allowed to stand at a room temperature for 10 min. Viable cell counts in the mixture were determined. Both GA and SAEW had complete bactericidal effects against *P. aeruginosa* strain tested within 30 s. Glutaraldehyde had a more rapid (5 min) bactericidal effect than SAEW (10 min) against *M. avium*.

Effects of disinfection on *Pseudomonas aeruginosa and Mycobacterium avium* contamination of upper GI endoscopes (Table 3)

Two colonies (4.0×10^1 CFU/mL) of *P. aeruginosa* were grown on NAC agar incubated after manual washing alone from 1 out of 5 upper GI endoscopes. From the remaining 4 endoscopes, *P. aeruginosa* was detected by enrichment culture and PCR. After a 5-min soak in 3% GA group, 1 out of 5 endoscopes tested positive; after a 10-min soak in 3% GA, *P. aeruginosa* strains were not detected by direct plating culture or broth culture. After a 10-s soak in SAEW, residual bacteria was not evident. The PCR technique for *P. aeruginosa* gave the same result.

After manual washing, *M. avium* strains were not detected on Mycobacteria 7H11 agar after incubation, but 3 out of 5 upper GI endoscopes tested positive by enrichment culture and PCR. After a 5-min soak in 3% GA, one endoscope tested positive for *M. avium* as detected by enrichment culture and PCR. After a 10-min soak in 3% GA, the endoscopes were all negative for *M. avium*. After a 10-s soak in SAEW, *M. avium* could not be detected on Mycobacteria 7H11 agar after incubation, but one endoscope tested positive for *M. avium* after enrichment culture and PCR.

Effect of disinfection on *Pseudomonas aeruginosa* and *Mycobacterium avium* contamination of colonoscopes (Table 4)

Colonoscopes were contaminated in the same manner as the upper GI endoscopes. However, as positive cultures were obtained for upper GI endoscopes after a 5-min soak in 3%

Table 4. Effects of disinfection of colonoscopes contaminated with *Pseudomonas aeruginosa* and *Mycobacterium avium*

Test strain	Disinfectant	Positive number		
		Bacterial plate count (CFU/mL)	After cultivation	PCR product
Pseudomonas aeruginosa				
	Control (before)	0/5	0/5	0/5
	Manual washing	0/5	5/5	5/5
	3% GA (10 min)	0/5	0/5	0/5
	SAEW	0/5	1/5	1/5
Mycobacterium avium				
	Control (before)	0/5	0/5	0/5
	Manual washing	0/5	3/5	3/5
	3% GA (10 min)	0/5	0/5	0/5
	SAEW	0/5	0/5	0/5

3% GA (10 min), 10-min soak in 3% glutaraldehyde; SAEW, strongly acidic electrolyzed water.

Table 5. Disinfection of hepatitis B viral contamination of upper GI endoscopes (HbsAg positive, e antigen positive blood; HBV DNA: 3300 pg/dL)

Endoscope no.	Before	Manual washing	3% GA (5 min)	3% GA (10 min)	SAEW
1	–	–	–	–	–
2	–	–	–	–	–
3	–	–	–	–	–
4	–	–	–	–	–
5	–	–	–	–	–

–, negative.

GA, experiments were limited to a 10-min soak in 3% GA and SAEW. After manual washing, *P. aeruginosa* tested negative by direct plating culture. However, all colonoscopes tested positive for *P. aeruginosa* by enrichment culture and PCR. After a 10-min soak in 3% GA, no colonoscopes tested positive for bacteria. After a 10-s soak in SAEW followed by suction with 100 mL of SAEW, test strains were not detected on NAC agar, but 1 out of 5 tested positive when determined by enrichment culture and PCR.

Colonoscopes were similarly contaminated with *M. avium*. After manual washing, all samples contaminated with *M. avium* tested negative on agar plates. However, 3 of the 5 colonoscopes tested positive for *M. avium* by using MGIT960 and PCR. Complete disinfection was obtained with a 10-min soak in 3% GA. The same effect was observed after a 10-s soak in SAEW followed by suction of 100 mL of SAEW.

Effects of disinfection on HBV contamination of upper GI endoscopes (Table 5)

The HBV positive (HBsAg positive, e antigen positive) blood was obtained from one unit of donated blood. After 50 mL of aspiration, manual washing was performed as above. No HBV virus was detected after manual washing, a 5- or 10-min soak in GA, or SAEW.

DISCUSSION

Strongly acidic electrolyzed water is quite easily produced using water and salt. Its main antibacterial effect is owing to hydroxy radicals generated from HClO produced by the electrolysis of water and salt. Hydroxy radicals are rapid and potent bactericidal agents, also used by polymorphonuclear leukocytes. Strongly acidic electrolyzed water contains a high level of HClO with low pH and a high electrical potential that is suitable for killing bacteria within a few seconds. This activated water is used widely for decubitus wounds, intraperitoneal lavage[14] and operative wound infection.[15] Although the antibacterial effect is rapid and potent, bacterial spores are the most resistant against SAEW. The second most resistant organisms are mycobacteria, the same as for GA. Additionally, viral DNA and RNA are easily damaged by SAEW and NaOCl.

Organic substances inactivate the bactericidal effects of SAEW. Selkon *et al.* reported the protein concentration and bactericidal effect of superoxidized water (Sterilox).[16] Sterilox is a type of electrolyzed water with weak acidity (pH 6–7). In their research, Selkon *et al.* demonstrated that the antibacterial effect of electrolyzed water was equivalent to GA for various bacteria and that this effect was blocked by 1% horse serum. Therefore, they concluded that manual washing was important to reduce the organic loading before contact with Sterilox or SAEW. The bactericidal effect of SAEW is rapid and strong compared with Sterilox because SAEW contains a greater amount of hydroxy radicals than Sterilox. Additionally, the high chloride concentration of Sterilox can cause a chemical change on the endoscope coating.

Another disadvantage is that SAEW is corrosive to metals after long-term exposure. However, the contact time between the endoscope and SAEW is relatively short

(within 15 s). With the cooperation of Olympus, we examined the endoscope after disinfection 1000 times using SAEW. The deterioration of metals was within the normal range and no apparent corrosive changes were detected after cleaning using SAEW.

A 10-min soak in GA completely eliminated *P. aeruginosa, M. avium* and HBsAg positive blood contamination. The Working Party of the World Congress of Gastroenterology (Sydney) recommends for digestive endoscopes a 5–10-min soak in 2% GA.[17] Our study showed that a 5-min soak was incomplete and thus supports the 10-min cleaning method, which is quite applicable to the clinical setting, especially office endoscopy. However, GA must be filled through each channel by syringe until all air bubbles are dissipated. Medical staff are at some risk when performing this procedure as it may increase their exposure to GA by direct contact or through the formation of GA gas during aspiration.

Strongly acidic electrolyzed water showed equivalent bactericidal effects to GA with respect to the cleaning of upper GI endoscopes, except for *M. avium*. *Mycobacterium* species are rather resistant to SAEW, however, as this bacteria is widely distributed throughout our environment it may not be critical to eradicate it from the digestive endoscope. Enrichment culture and PCR tests for *P. aeruginosa* were positive for one colonoscope cleaned using SAEW. Direct plating culture negative, broth culture and PCR positive means detection of bacteria under the 10^1 level; 10^5 elimination of bacteria is adequate criteria for disinfection.

The negative PCR result for HBV was quite impressive because even manual washing alone can destroy HBV DNA activity. DNA is very fragile under various conditions and it is reasonable that HBV DNA can be inactivated with either GA or SAEW. Harada *et al.* reported on the anti-HIV effect of SAEW and peracetic acid. Both have been proved to inactivate these pathogens within 30 s.[18] Manual washing involves using tap water and brushing. The tap water contains chlorine and therefore small doses of active ions may alter viral DNA activity irreversibly. However, manual washing alone is not recommended because of the lack of standardization in the brushing technique and diligence of medical staff .

The clinical benefits of SAEW are now widely confirmed in many endoscopy centers in Japan. Abe *et al.* cultured *Helicobacter pylori* using direct plating culture and PCR from 25 continuous routine upper GI endoscopes with manual washing plus SAEW cleaning. Direct plating culture and PCR detected no *H. pylori.*[19] Furthermore, Kaise *et al.* studied the efficacy of endoscope disinfection in 154 cases of patient-to-patient disinfection. *Helicobacter pylori* were completely destroyed after SAEW washing.[20]

The gold standard for disinfection of endoscopes is a 20-min soak in 2% GA with using an endoscope reprocessor. However, for routine endoscopy, this standard is almost impossible to achieve because it is time consuming and is also hazardous to the medical staff performing endoscopy. In fact, some endoscopy centers may skirt strict guidelines for disinfection because endoscope cleaning using GA can be so toxic.[21] On the other hand, there are many advantages in using SAEW as it has rapid action against microorganisms and there is little chance of developing resistance to SAEW. In addition, the cost of using SAEW is much less expensive (5.3 yen/L) compared with GA (1200 yen/L). Thus, we conclude that SAEW is a suitable disinfectant for cleaning endoscopes against bacteria and viruses and will be widely used in the near future.

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