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Journal of Virological Methods 85 (2000) 163–174

Journal of Virological Methods

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Disinfection potential of electrolyzed solutions containing sodium chloride at low concentrations

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Received 5 July 1999; received in revised form 1 November 1999; accepted 2 November 1999

Abstract

Electrolyzed products of sodium chloride solution were examined for their disinfection potential against hepatitis B virus (HBV) and human immunodeficiency virus (HIV) in vitro. Electrolysis of 0.05% NaCl in tap water was carried out for 45 min at room temperature using a 3 A electric current in separate wells installed with positive and negative electrodes. The electrolyzed products were obtained from the positive well. The oxidation reduction potential (ORP), pH and free chlorine content of the product were 1053 mV, pH 2.34 and 4.20 ppm, respectively. The products modified the antigenicity of the surface protein of HBV as well as the infectivity of HIV in time- and concentration-dependent manner. Although the inactivating potential was decreased by the addition of contaminating protein, recycling of the product or continuous addition of fresh product may restore the complete disinfection against bloodborne pathogens. © 2000 Published by Elsevier Science B.V. All rights reserved.

Keywords: Disinfection; Pathogenic viruses; Electrolyzed strong acid water; HBV; HIV

1. Introduction

Aldehydes have been used for the disinfection of endoscopes in a clinical setting (Jeng et al., 1987; Hanson, 1990; Rutala et al., 1991; Reynolds et al., 1992; Working Party of the British Society of Gastroenterology Endoscopy Committee,

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PII: S0166-0934(99)00165-2

1998). Although glutaraldehyde has been thoroughly evaluated as a suitable chemical for the disinfection of bloodborne pathogenic viruses (Russell, 1994; Meester et al., 1995) such as hepatitis B virus (HBV) (Bond et al., 1983; Kobayashi et al., 1984) and human immunodeficiency virus (HIV) (Hanson, 1990), insufficient rinsing of the chemical may cause bloody diarrhea and abdominal cramps (Durante et al., 1992). The chemical was also found to exhibit cytotoxic and genotoxic potential in cultured human cells (Sun et al., 1990; St.-Clair et al., 1991).

Chlorination is another method of disinfection which has been shown to be effective against bloodborne pathogens such as HIV (Katner et al., 1988; Bloomfield et al., 1990; Shapshak et al., 1994; Rutala and Weber, 1997). Sodium hypochlorite has been shown to be effective for the disinfection of HBV both in vitro (Bond et al., 1977; Sehulster et al., 1981; Nath et al., 1982) and in vivo (Bond et al., 1983; Kobayashi et al., 1984). Electrolyzed products in a sodium chloride solution contain free chlorine and have been shown to be effective for disinfection. In the 1990s, electrolyzed solutions of sodium chloride, containing high levels of free chlorine, have been investigated from the viewpoint of feasibility of clinical application in Japan. At present, two types of electrolyzed solutions are available, namely, electrolyzed strong acid water and electrolyzed weak acid water. Electrolyzed strong acid water is prepared from sodium chloride solution after it is electrolyzed with positive and negative electrodes in wells separated by a cationic membrane. This solution is obtained using high concentrations of salt in the well of the positive electrode (Kumon, 1997). Electrolyzed strong acid water is effective against many human pathogens (Iwasawa et al., 1993; Abe et al., 1994; Iwasawa and Nakamura, 1996) including *Bacillus cereus* (Iwasawa and Nakamura, 1995) and *Mycobacterium tuberculosis* (Iwasawa and Nakamura, 1993). However, the high oxidation-reduction potential (ORP), high concentrations of sodium chloride and low pH of electrolyzed strong acid water, also results in oxidation of metallic instruments and facilities. To prevent such damage, electrolyzed weak acid water, obtained by electrolysis of solutions containing high concentrations of sodium chloride in a single well without a cationic membrane, has been applied for disinfection. The electrolyzed weak acid water has been shown to be effective against various bacteria (Yoh et al., 1994; Wu et al., 1996) and bloodborne viruses (Kakimoto et al., 1997). Iwasawa and Nakamura (1996) showed that electrolyzed weak acid water is suitable for stable disinfection. Both types of electrolyzed water, however, corrode instruments and facilities of a hospital owing to the salt contained at high concentrations. Recently, to minimize the corrosive effect, electrolyzed strong acid water was prepared by electrolysis of a solution containing low concentrations of sodium chloride, and was confirmed to be effective as a disinfectant (Iwasawa and Nakamura, 1996). This solution is suitable for application in the clinical setting. Although the solution of water with low sodium chloride is well established as a bactericidal (referred to as the solution) disinfectant (Iwasawa and Nakamura, 1996), its disinfection potential against bloodborne viruses is not well studied. In this study, the effect of electrolyzed strong acid water with low concentration of sodium chloride on the antigenicity of the HBV surface antigen and the infectivity of HIV in vitro is examined.

2. Materials and methods

2.1. Electrolyzed strong acid water

Electrolyzed strong acid water containing sodium chloride at low concentrations was prepared in an electrolyzing apparatus (CLEANTOP WM-1, Kaigen Co. Ltd. Osaka, Japan). The principle of the apparatus is shown in Fig. 1. The apparatus consists of two wells separated by a cationic membrane (Naion 450, Dupont, New York, USA), with positive and negative electrodes installed in each well (Fig. 1). Ten liters of 0.05% NaCl in tap water were electrolyzed for 45 min at room temperature using a 3 A current. This solution, whose ORP, pH, free chlorine content and temperature were 1053 mV, pH 2.34, 4.20 ppm and 27.5°C, respectively, was obtained from the well with the positive electrode. Alkaline water,

whose ORP, pH and available chlorine content were −680 mV, pH 11.45 and 0 ppm, respectively, was obtained from the well with the negative electrode. ORP, pH and available chlorine content were measured with an ORP meter (D-14, Horiba, Kyoto, Japan), pH meter (D-14, Horiba) and free chlorine meter (Hach, Colorado, USA), respectively.

2.2. Neutralization of pH and inactivation of free chlorine

For neutralization of pH and inactivation of available chlorine in the solution, was titrated with alkaline water from the negative electrode and various concentrations of bovine serum albumin (BSA; Sigma, St. Louis, USA) solution in distilled water. Briefly, the solution was mixed with the alkaline water and the pH of the mixture was measured using a pH meter. The solution was then mixed with the same volume of BSA solution, and the free chlorine content in the mixture was measured using the free chlorine meter.

2.3. The efficacy of this solution against of human hepatitis B virus surface antigen (HBsAg)

HBsAg, purified from human plasma (HBs; CND Co. Ltd., Beijing, China), was purchased from ATEST Inc. (Kyoto, Japan), and resuspended at 1 μg/ml in 50 mM phosphate-buffered saline (pH 7.2). Twenty microliters of the HBsAg suspension were mixed with 175 μl of the solution and allowed to react at room temperature for a designated time. The final concentration of the HBsAg in the mixture was ≈0.1 μg/ml. To neutralize free chlorine and pH, 300 μl of 3% BSA solution and 125 μl alkaline water were added to the mixture. The residual antigenicity of the protein was measured using a HBsAg capture

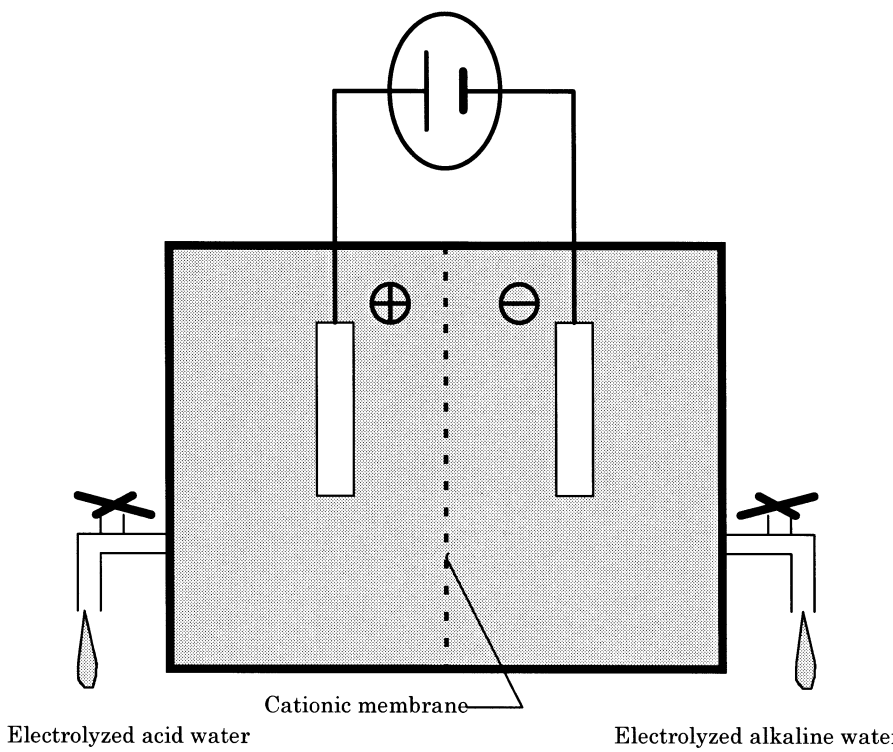


Fig. 1. Principle of electrolysis apparatus. The apparatus consists of two wells separated by a cationic membrane. Positive and negative electrodes were installed in each well. Electrolyzed strong acid and alkaline waters were obtained from positive and negative wells, respectively.

EIA kit (Auszyme Monoclonal; Abbott Laboratories, USA) in accordance with the manufacture's instructions. Absorbance at 490 nm (reference 620 nm) of the reactants was measured in an EIA reader (Immunoreader, Nargen Nunc Japan, Tokyo, Japan). As positive control, the HBsAg solution was mixed with 300 μl of BSA solution and 300 μl of unelectrolyzed 0.05% NaCl solution. The residual antigenicity of the HBsAg protein was expressed in terms of the OD value of the suspension. Each experiment was performed in duplicate, and the mean values of the OD were plotted. In some experiments, the HBsAg was suspended in BSA solution at a final concentration of 0.1–100 μg/ml, and the residual antigenicity was measured as described above.

2.4. Infectivity assay of HIV

The residual infectivity of the final mixture was measured by microcultivation with Molt-4 cells at 2500 cells/well. In brief, 25 μl of HIV-1 (Barre-Sinoussi et al., 1983) suspension, whose $TCID_{50}$ was 1×10^4/50 μl, was mixed with 175 μl of electrolyzed strong acid water with low concentration of sodium chloride, and gently shaken for 30 s. For neutralization of the free chlorine and pH, 270 μl of 3% BSA and 125 μl alkaline water were added to the mixture. Fifty microliters of a Molt-4 cell suspension of 5×10^4 cell/ml and 100 μl of fresh RPMI-1640 medium supplemented with 10% fetal bovine serum (FBS) were added to the same volume of the final mixture in a 96-well microtiter plate (Becton Dickinson, NJ, USA). The cells were incubated at 37°C for 7 days without changing the medium. The residual infectivity of the mixture was determined by measuring the reverse transcriptase (RT) activity in the culture supernatant with a non-RI RTA kit (Asahi Chemical Industry, Shizuoka, Japan) (Sano et al., 1995).

2.5. The efficacy of the disinfection solution on HIV-1 recombinant reverse transcriptase (HIV-1 rRT) and the RT in HIV-1 particles

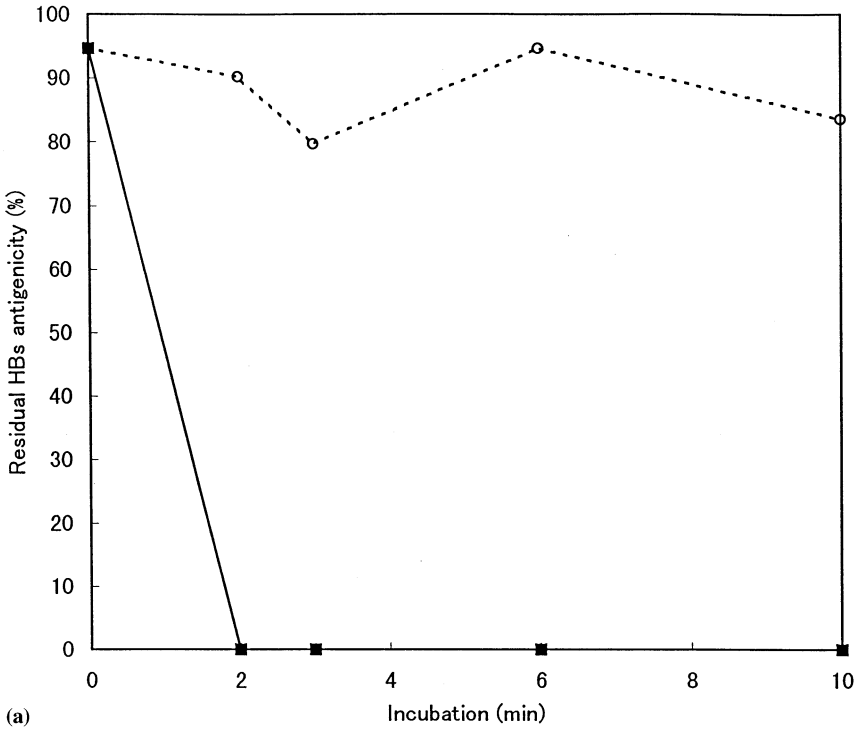
Twenty-five microliters of 1000 μU/ml HIV-1 rRT (27 Saitoh et al., 1990) solution in 0.05 M Tris–HCl buffer (pH 7.8) and the same volume of HIV-1 suspension containing $\approx 1 \times 10^4$ $TCID_{50}$/50 μl of the virus in 10% FBS RPMI-1640 medium were mixed separately with 175 μl of the solution, and allowed to stand at room temperature for a designated time. The residual free chlorine and pH in the mixture were neutralized by adding 180 μl of 3% BSA and 125 μl of alkaline water. The RT activity in the final RT solution and the virus suspension were measured with a nonradioisotopic RT assay kit (Sano et al., 1995).

3. Results

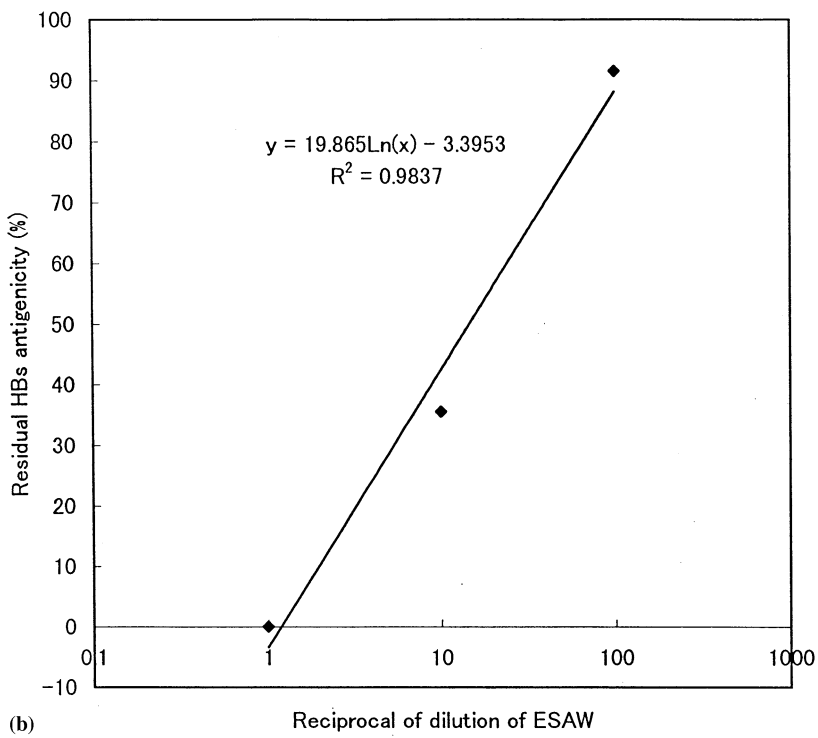
3.1. Neutralization of pH and inactivation of free chlorine

To neutralize the pH of the disinfection solution, alkaline water from the well with the negative electrode was mixed with the solution, and the pH of the mixture was measured. The pH of a mixture of 17.5 volumes of the solution and 12.5 volumes of alkaline water was 6.95, and therefore, this ratio was considered to be sufficient for neutralizing the pH of the solution. For the inactivation of free chlorine in the solution, various concentrations of BSA were added to it, and the free chlorine content in the mixtures was measured. The content of free chlorine in the solution decreased in a concentration-dependent manner with the addition of BSA solution, and addition

Fig. 2. Inactivation of HBs antigen and effect of dilution of Electrolyzed strong acid water on the inactivation. (a) Inactivation of HBs antigen. HBs antigen solution was mixed with ESW-L (solid line) or 0.05% sodium chloride solution in tap water (dotted line), and the HBsAg antigenicity of the mixture was determined. (b) Effect of diluting ESW-L on the inactivation of HBsAg by ESW-L. Decimally diluted ESW-Ls were mixed with the same amount of HBsAg solution, and the HBsAg antigenicity of the mixtures were determined. The approximate formula, its plot (solid line) and correlation of coefficient between the observed value and formula are presented in the graph.

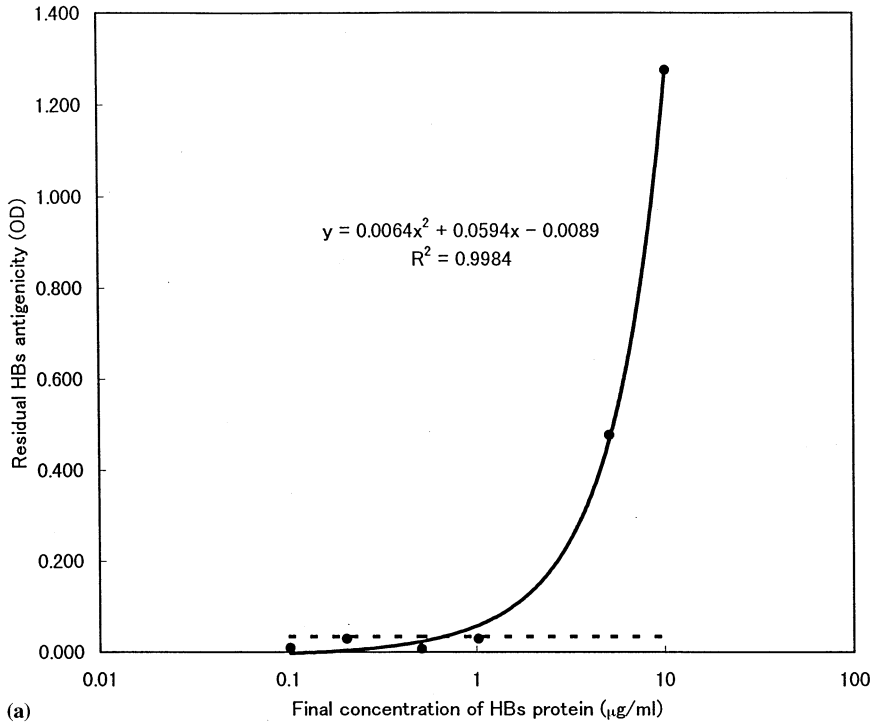

Residual HBs antigenicity (%)
100
90
80
70
60
50
40
30
20
10
0
0
2
4
6
8
10
Incubation (min)

(a)

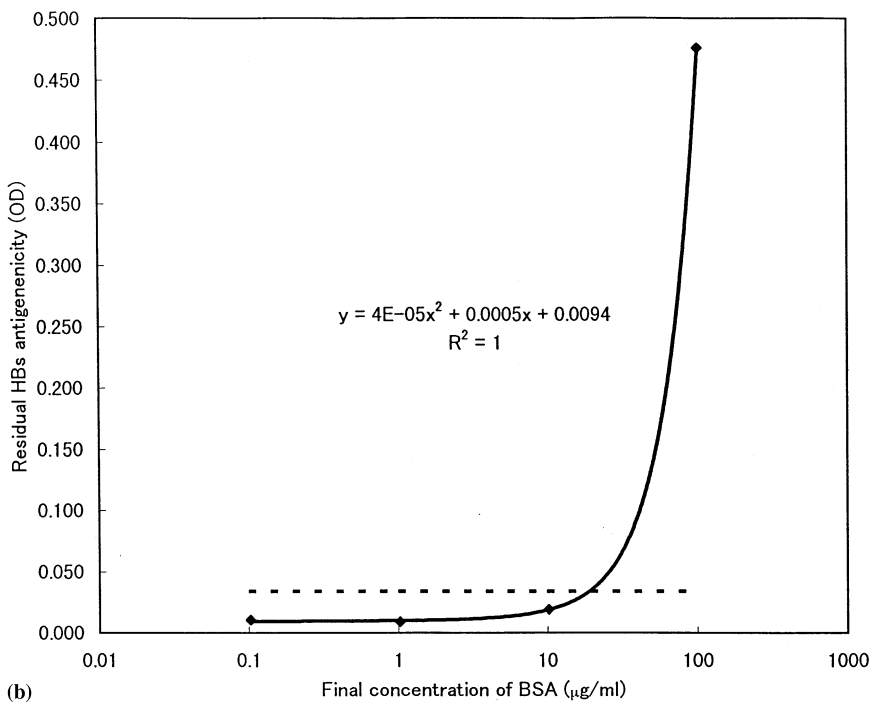

y = 19.865Ln(x) − 3.3953
R2 = 0.9837
Residual HBs antigenicity (%)
100
90
80
70
60
50
40
30
20
10
0
−10
0.1
1
10
100
1000
Reciprocal of dilution of ESAW

(b)

Fig. 2.


1.400
1.200
1.000
0.800
0.600
0.400
0.200
0.000
Residual HBs antigenicity (OD)
y = 0.0064x2 + 0.0594x − 0.0089
R2 = 0.9984
0.01
0.1
1
10
100
Final concentration of HBs protein (μg/ml)

(a)


0.500
0.450
0.400
0.350
0.300
0.250
0.200
0.150
0.100
0.050
0.000
Residual HBs antigenenicity (OD)
y = 4E−05x2 + 0.0005x + 0.0094
R2 = 1
0.01
0.1
1
10
100
1000
Final concentration of BSA (μg/ml)

(b)

Fig. 3.

of an equal volume of BSA solution at a concentration over 0.02% (final concentration in the mixture of 0.01%) was determined to be sufficient to inactivate the free chlorine.

In order to examine the effect of the solution on Hbs antigenicity, an HBsAg suspension was mixed with the solution and the antigenicity of the protein was measured and expressed as the OD of the suspension. The disinfection solution reduced the antigenicity of the HBsAg to below detectable levels within 2 min. In contrast, the unelectrolyzed solution of 0.05% NaCl did not cause any significant reduction in the antigenicity (Fig. 2a). The residual antigenicity of the HBsAg was correlated inversely with the concentration of the electrolyzed solution, that is, dilution of the solution decreased the extent of reduction in the antigenicity in a dilution-dependent manner (Fig. 2b). To define the threshold of the inactivating potential of the solution against HBsAg, HBsAg solutions with increasing concentrations of the protein were allowed to react with the electrolyzed solution under the same conditions. Complete modificiation of the antigenicity of HBsAg was obtained at a final concentration of 1.0 μg/ml of the HBsAg protein in the mixture, but residual antigenicity was still detected at final concentrations of the HBsAg protein of over 1.0 μg/ml, in a concentration-dependent manner (Fig. 3). To clarify whether the inactivating potential is influenced by other contaminating proteins, HBsAg was allowed to react with the electrolyzed solution in the presence of BSA. Antigenicity of the HBsAg was not detectable in the presence of BSA at a final concentration of 10 μg/ml (Fig. 3b). When HBsAg was allowed to react with the electrolyzing solution in the presence of BSA at a final concentration of 100 μg/ml, $\approx 80\%$ residual antigenicity was detected (Fig. 3b).

Table 1
Inactivation of HIV-1 infectivity

	Residual $TCID_{50}$/50 μl[c] after ESW-L treatment			
	No treatment	2 min	10 min	20 min
ESW-L[a]	$1\times10^{2.5}$	$1\times10^{0.5}$	$1\times10^{-0.75}$	0
Unelectrolyzed[b]	$1\times10^{2.5}$	$1\times10^{2.5}$	$1\times10^{2.47}$	$1\times10^{1.75}$

[a] ESW-L, electrolyzed solution containing 0.05% sodium chloride.

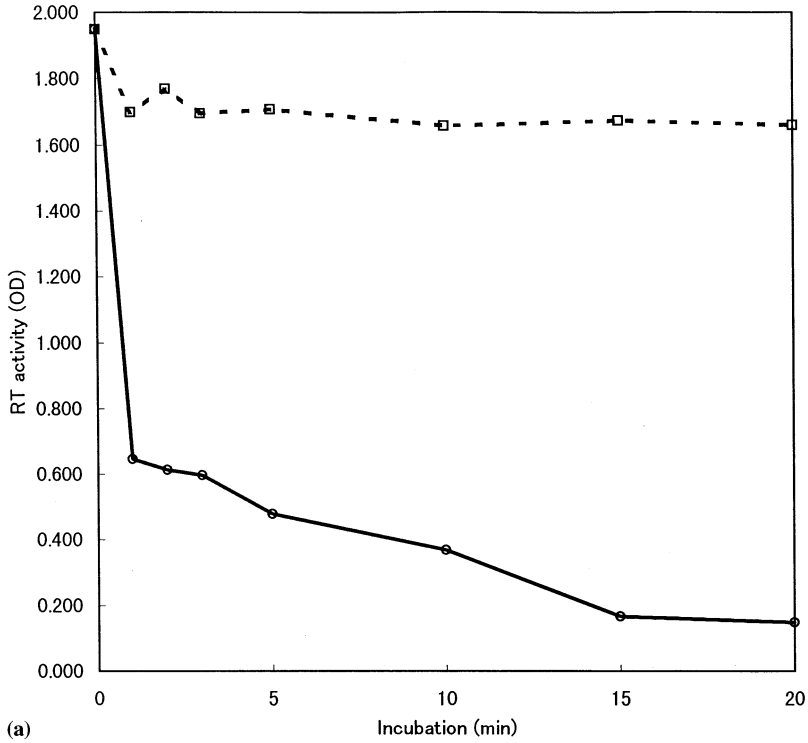
[b] Unelectrolyzed, 0.05% sodium chloride solution in tap water.

[c] $TCID_{50}$/50 μl, median tissue culture infective dose was calculated using dilution factors from three wells which showed virus growth.

3.2. Effect on HIV-1

For examination of the reduction of HIV-1 infectivity, the $TCID_{50}$ of a mixture of HIV-1 suspension and the electrolyzed solution was measured. HIV-1 infectivity was decreased in mixtures containing the solution as well as in those containing unelectrolyzed NaCl solution in a time-dependent manner (Table 1). The level of decline in the case of the solution was, however, more significant in comparison to that obtained in the case of unelectrolyzed solution. The electrolyzed solution abolished completely HIV-1 infectivity within 20 min. The attenuating potential of this solution against HIV-1 infectivity was reduced by dilution in a dilution-dependent manner (Table 2). In this study, in order to clarify the disinfective mechanisms, we also examined the effect of the electrolyzed solution on the activity of HIV-1 RT which is essential for HIV-1 replication. When the virus particles were treated with the electrolyzed solution, RT activity was reduced in a time-de-

Fig. 3. Inactivation potential of HBs antigen by Electrolyzed strong acid water containing sodium chloride at low concentration (ESW-L). (a) Inactivation potential of HBs antigen in the absence of contaminating protein. ES-LW was mixed with increasing concentrations of HBs antigen solutions, and the residual HBsAg antigenicity of the mixtures was measured. The approximate formula, its plot (solid line) and correlation of coefficient between the observed value and formula are presented in the graph. Dotted line indicates cutoff value. (b) Inactivation potential of HBsAg in the presence of contaminated protein. ESW-L was mixed with identical concentration of HBsAg solutions in the presence of increasing concentrations of bovine serum albumin (BSA) solutions, and the residual HBsAg antigenicity of the mixtures was measured. The approximate formula, its plot (solid line) and correlation of coefficient between the observed value and the formula are presented in the graph. Dotted line indicates cutoff value.


2.000
1.800
1.600
1.400
1.200
1.000
0.800
0.600
0.400
0.200
0.000
RT activity (OD)
0
5
10
15
20
Incubation (min)
(a)

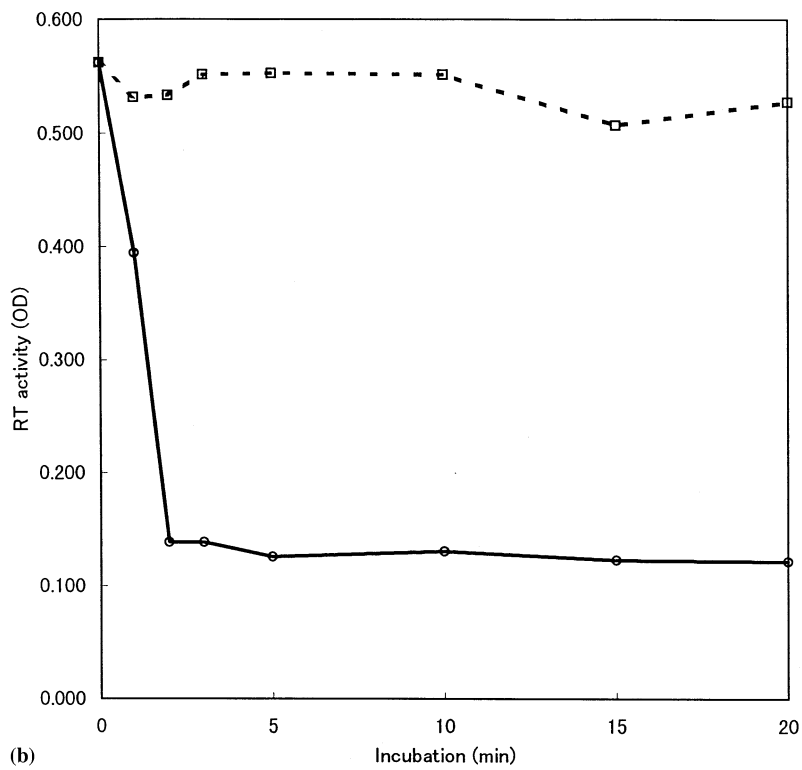

0.600
0.500
0.400
0.300
0.200
0.100
0.000
RT activity (OD)
0
5
10
15
20
Incubation (min)
(b)

Fig. 4.

Table 2
Effect of dilution of ESW-L on the inactivation of HIV-1 infectivity

Reciprocal of dilution[a]	Residual $TCID_{50}$[b] after ESW-L treatment[c]
1	0
10	$1 \times 10^{0.75}$
100	$1 \times 10^{1.5}$
1000	$1 \times 10^{1.75}$
None	$1 \times 10^{2.5}$

[a] Electrolyzed solution containing 0.05% sodium chloride (ESW-L) was decimally diluted with the unelectrolyzed solution.

[b] $TCID_{50}$, median tissue culture infective dose was calculated using dilution factors from three wells which showed virus growth.

[c] HIV-1 suspension was mixed with ESW-L and placed at room temperature for 20 min.

pendent manner and the enzyme was completely inactivated within 5 min (Fig. 4a, solid line). In contrast, unelectrolyzed NaCl solutions did not inactivate RT (Fig. 4b, dotted line). To confirm that the inactivation was the result of a direct effect on the RT molecule, a solution of HIV-1 rRT was treated with the electrolyzed solution unelectrolyzed NaCl solution, and it was found that while the electrolyzed solution reduced HIV-1 rRT activity, unelectrolyzed NaCl solution did not, under conditions of similar kinetics of the RT in the virus particles (Fig. 4b).

4. Discussion

In previous studies (Iwasawa et al., 1993; Abe et al., 1994; Yoh et al., 1994; Iwasawa and Nakamura, 1996; Wu et al., 1996; Kakimoto et al., 1997), the disinfection potential of electrolyzed NaCl solutions was examined by immunoassay and bioassay. The design of these studies was as follows: suspensions of the infective factors and pathogens were mixed with the electrolyzed solutions, and the respective mixtures were assayed for the antigenicity of the pathogens by immunoassay and for replication of the pathogens by bioassay. In this protocol, the electrolyzed solutions need to be neutralized prior to the assays, since electrolyzed solutions may influence the results of subsequent assays. Although the protocol in some of these studies did not include the neutralization step, others did, with the addition of sodium thiosulfate (Abe et al., 1994), BSA (Kakimoto et al., 1997) or FBS (Kakimoto et al., 1997). In the present study, free chlorine was neutralized and the pH of the solution obtained from the well with the positive electrode by adding BSA solution and alkaline water, respectively.

As in vitro bioassay is not available for human HBV, the activity of the electrolyzed solution against the antigenicity of the HBsAg, the viral coat protein, was examined. It was found that the electrolyzed solution affected the antigenicity of HBs protein purified from human serum in a concentration-dependent manner. These findings are similar to those reported previously (Abe et al., 1994). Bond et al. (1977) indicated that the antigenicity of the protein was retained even though the infectivity was eliminated. It is concluded therefore that the electrolyzed solution containing free chlorine, as well as sodium hypochlorite, potentially abolishes the infectivity of human HBV. Moreover, Tsiquaye and Barnard (1993) reported that sodium hypochlorite effectively inhibited the transmission of a Hepadnavirus, the duck hepatitis B virus (DHBV), in an animal model. Our preliminary data showed that the electrolyzed solution was effective in abolishing the infectivity of DHBV in the animal model (Tagawa et al., 1999). It is emphasized again that the electrolyzing solution exhibits disinfection potential against human HBV. In the present study, however, the potential of the solution to modify

Fig. 4. Inactivation of HIV-1 reverse transcriptases (RT) by Electrolyzed strong acid water containing sodium chloride at low concentration (ESW-L). (a) Inactivation of recombinant HIV-1RT by ESW-L. ESW-L (solid line) and 0.05% unelectrolized sodium chloride solution (dotted line) were mixed with recombinant HIV-1 RT solution, and the RT activity of the mixture was measured. (b) Inactivation of HIV-1RT in the virion by ESW-L. ESW-L (solid line) and 0.05% unelectrolyzed sodium chloride solution (dotted line) were mixed with HIV-1 suspensions, and the virions were solubilized. The RT activity of the solutions was measured.

HBsAg antigenicity was reduced in the presence of high concentrations of the viral protein and BSA, in a concentration-dependent manner. The results indicate that the disinfection potential of the electrolyzed solution was reduced by the presence of additional protein. It is speculated that this finding could also be attributable to the low concentration of free chlorine in the electrolyzed solution. The problem, therefore, might be overcome by adding fresh or recycled electrolyzed solution to the target material for disinfection.

In this study, in vitro bioassay showed that the electrolyzed solution abolished completely the infectivity of HIV-1. The possible mechanisms underlying this abolition are (1) inactivation of the surface protein, as observed in the experiments using HBsAg; (2) destruction of the envelope of the virus; (3) inactivation of viral enzymes; and (4) destruction of viral RNA. In the present study, efficient inactivation of HIV-1 RT by the electrolyzed solution was also observed. Since RT in the intact HIV-1 particle was inactivated by contact of the virus particle with the electrolyzed solution, we suggest that at least some components in the solution passed through the viral envelope and reacted with the RT molecules.

Freshly electrolyzed water contains sodium ions, electrons, oxygen radicals and free chlorine. Modification of HBsAg antigenicity and the disinfective potential against HIV-1 of the electrolyzed solution may be dependent on its free chlorine content. Nath et al. (1982) indicated that partial inactivation of HBV DNA polymerase was observed in the presence of 2500 ppm of free chlorine at neutral pH. Rutala and Weber (1997) reported that the infectivity of HBV and HIV are abolished in the presence of 500 and 5000 ppm for 10 min and 1 min at room temperature, respectively. In the present study, the electrolyzed solution contained only 4.2 ppm of free chlorine and showed greater efficacy against HBsAg and HIV-1 than sodium hypochlorite. It is, accordingly, difficult to explain the disinfective effects as being primarily due to the free chlorine content. The effects probably are caused by not only the free chlorine content, but also by some other factors present in the electrolyzed solution, as mentioned above.

In the late 1960s, some investigators developed methods of disinfection involving electrolysis and products of electrolyzed sodium chloride solutions, and applied them for disinfection of water (Lovtsevich and Sergunina, 1968; Shura-Bura and Gritsenko, 1968; Cherkinskii et al., 1980; Tellez-Andrade, 1984; Erusalimskaia et al., 1989; Mokienko, 1992; Brust-Carmona et al., 1998) and disinfection of apparatus such as milking equipment (Schwab et al., 1975; Iablochkin and Kurgan, 1979). Although the electrolyzed solution is not a newly discovered disinfectant, it is essential to re-evaluate its activity and efficacy in the clinical setting.

Acknowledgements

We thank A. Hanada, and K. Koumoto for their laboratory assistance, and A. Sakata for the preparation of this manuscript. This study was supported in part by a grant for AIDS prevention from The Life Insurance Association of Japan.

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