

Short communication

Reduction of *Staphylococcus aureus* in atopic skin lesions with acid electrolytic water – a new therapeutic strategy for atopic dermatitis

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The subjects studied were 22 pediatric patients newly diagnosed with atopic dermatitis (AD); 11 were treated with acid electrolytic water (AEW), which has a strong bactericidal activity (AEW group), and the other 11 with tap water (placebo group). AEW or tap water, 1 ml/cm² (body surface area), was sprayed on their skin lesions with a spray gun each twice a day for a week. There were no significant differences between the two groups in regard to sex, age, serum IgE, peripheral eosinophil counts, grading scores of AD, and duration of AD. The study was designed as a randomized, placebo-controlled, double-blind clinical trial. Colony counts of *Staphylococcus aureus* on skin lesions in the AEW group, both 3 min after spraying ($P < 0.05$) and after 1 week of skin treatment ($P < 0.01$), were significantly decreased as compared with colony counts before treatment, while there was no significant difference in the placebo group before and after treatment. Grading scores of AD also decreased in the AEW group ($P < 0.01$), but not in the placebo group. Both the subjects' guardians' evaluation and a referee physician's evaluation of treatment effect were significantly higher in the AEW group than in the placebo group ($P < 0.01$). AEW may be potentially effective in preventing a staphylococcal chronic inflammation in AD because of its strong bactericidal activity.

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Atopic dermatitis (AD) is a chronic pruritic inflammation of the skin. Although it is thought that immunologic mechanisms contribute to the pathogenesis of AD, the mechanism of allergic inflammation is poorly understood (1). The pathogenesis of AD is influenced in part by environmental factors, including infection of the skin with *Staphylococcus aureus* (2). Leyden et al. (3) demonstrated that *S. aureus* was isolated from the affected skin of over 90% of patients with AD. It has been well established that *S. aureus* may be an important trigger of the exacerbation of AD (4–7) because AD is associated with superficial *S. aureus* skin

infection and colonization (8). Therefore, reduction of *S. aureus* in the skin lesions of AD may be expected to result in symptomatic improvement of AD.

Acid electrolytic water (AEW) has unique characteristics, such as a high positive oxidation-reduction potential (ORP) of more than 1000 mV and strong acidity (pH of less than 2.7), which far exceed those of the living environment of microorganisms. AEW exerts a powerful sterilizing effect by synergism of active chlorine and active oxygen (9). An *in vitro* bacteriologic study disclosed that AEW has strong bactericidal activity, as evidenced by the observation that *S. aureus*, *Pseudomonas*

aeruginosa, and *Escherichia coli* can be exterminated in the solution within 5 s (10). AEW is rapidly neutralized by surrounding proteins and returns to regular water, and the residuum is much less than with other chemicals. Therefore, we attempted to determine whether AEW may be useful in the skin treatment of AD patients.

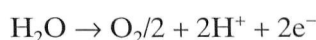
Material and methods

Subjects

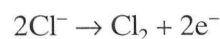
Twenty-two subjects with untreated AD were enrolled in the study and arbitrarily divided by a referee physician into two groups of 11 patients each; i.e., the AEW group and the placebo group. Their ages ranged from 2 to 56 months (Table 1). The diagnosis was based on the criteria of Hanifin & Rajka (11). Informed parental consent was obtained. During the study period of 1 week, none of the subjects was receiving systemic or topical steroids, or antihistamines. Grading scores of AD were assessed by the scoring system proposed by Kimata & Igarashi (12). Briefly, grading of skin scores was 0–2 in the ascending order of severity with respect to inflammation, lichenification, and cracking, respectively. These signs were assessed on four areas of the body, i.e., face, trunk, arms, and legs, and the maximum possible score was 24. Scores for itching and sleep disturbance were graded 0–3 in the ascending order of severity, respectively. Therefore, the maximum possible total score was 30. The present study comprised the patients of mild (grading scores: less than 10 points) and medium (grading scores: 11–20 points) groups, but not the severe group (grading scores: more than 21 points). Incidentally, we did not have patients who should be included in the severe group at the start of the present study. The ratio of mild to medium groups was 7 : 4 in the AEW group and 8 : 3 in the placebo group (Table 1).

Acid electrolytic water (AEW)

An AEW production apparatus (Shionogi Pharmaceutical, Osaka, Japan) consists of two chambers separated by a porous barrier which passes only ions. Each 2 l of 0.1% NaCl solution was decanted into the two chambers, i.e., cathode and anode chambers, and a direct current (100 V, 0.7 A) was applied between the electrodes. The reaction which occurs in the anode chamber is as follows:



The concentration of H^+ increases, and the solution becomes acidic. Another reaction also occurs where salt is used as an electrolyte:



The reaction which occurs in the cathode chamber is as follows:



As the concentration of H^+ decreases, the solution becomes alkaline.

AEW thus produced has a pH of less than 2.7, a high ORP of more than 1000 mV, and high concentrations of residual chlorine. pH and ORP were measured by a field type pH meter (Horiba Manufactory, Kyoto, Japan). The concentration of residual chlorine was measured by the *N,N*-diethyl-*p*-phenylenediamine method (13).

Medium for isolation of *S. aureus*

We used food stamp TGSE (tellurite-glycine-salt-egg yolk) agar (14) specifically prepared for isolation of *S. aureus* only (Nissui Pharmaceutical, Tokyo, Japan).

Methods

Study design. The study was designed as a randomized, placebo-controlled, double-blind clinical trial. Before the study, the protocol was reviewed and approved by the institutional review board of Kansai Medical University.

Procedure. First, the food stamp TGSE agar was stamped on the site when the skin lesion was severest to evaluate the colony counts of *S. aureus* (stamp 1). Subsequently, 5 ml of AEW or tap water

Table 1. Characteristics of patients with atopic dermatitis

	AEW group	Placebo group	P
No. of subjects	11	11	1.00
(Male/Female)	(5/6)	(5/6)	
Age (months)	13.7 ± 16.2 (3–56)	14.8 ± 15.7 (2–55)	0.79
Serum IgE (IU/ml)	69.2 ± 67.1 (3.4–187.8)	43.7 ± 46.5 (11.6–173.6)	0.81
Peripheral eosinophil counts (/mm ³)	1113.3 ± 755.2 (93–2496)	787.3 ± 661.1 (260–2256)	0.34
Grading scores of AD before treatment	9.8 ± 4.3 (4–16)	7.0 ± 2.2 (4–11)	0.15
group: mild/medium	7/4	8/3	
Duration of AD (months)	10.8 ± 13.6 (2–44)	10.5 ± 15.0 (2–52)	0.55

AD: atopic dermatitis. Data were expressed as mean ± SD. Figures in parentheses indicate ranges.

was sprayed with a spray gun on skin lesions. They were air-dried for 3 min, after which the next food stamp was obtained (stamp 2). The patients continued to undergo the skin treatment; i.e., spraying of the skin lesions with a spray gun containing 1 ml/cm² (body surface area) of AEW or tap water each twice a day for 1 week. After 1 week, the food stamp was obtained from the same site (stamp 3). Eight patients in the AEW group further continued the therapy, and three of them were evaluated at 2 months for colony counts and grading scores. Three stamps were taken from one region from each patient, where the skin lesion was the severest. Anatomic sites studied were, for example, cheek, chest, arm, jaw, and leg, and were therefore different from one patient to another. Bacterial colony counts were done in practically the same skin lesion, and standard deviations ranged from 8 to 10% of respective mean counts. Computerized image analysis was used to calculate the bacterial colony counts on the food stamp after incubation at 37°C for 48 h, by the method previously reported by Namura et al. (15). pH, ORP, and concentration of residual chlorine of AEW did not change during the period of 1 week. The grading scores of AD were recorded before and after the skin care. Another referee physician, who was not informed of the patients' group, and the guardians, respectively, evaluated the change of skin lesions after 1 week of treatment as follows: improvement, no change, and aggravation.

Image analysis (15). A PC-9801 FA (personal computer), an AV-M 150S (multimedia color monitor), an XC-77 (ccd video camera), and ASPECT, a scientific image-processing system, were used in this study. Images of the food stamp plates were displayed on a video monitor in the black-and-white mode, and pseudocolor enhanced. Then the bacterial colony-forming units and the agar background were segmented into binary images to determine the threshold values separating the image on the video screen. After segmentation of the binary images, a colony count was performed by measuring the areas of the colonies.

Statistical methods. The statistical software used to analyze the data was SAS (Statistical Analysis System) Version 6.09 (SAS Institute, Cary, NC, USA). A *P* value of less than 0.05 was considered significant. Fisher's exact test (two-tailed) and Wilcoxon's rank-sum test were used to compare the characteristics and subjective response variations of the two groups. Spearman's rank correlation was used to correlate the bacterial colony counts and grading scores of AD. The changes of the bacterial colony counts and of grading scores of AD before

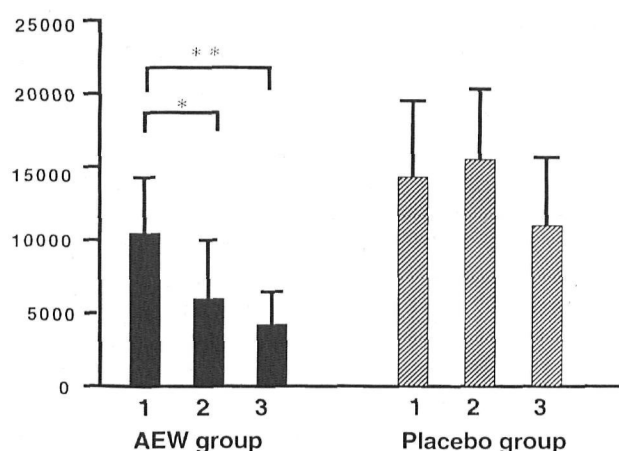
and after treatment were analyzed by Wilcoxon's signed rank test. Wilcoxon's rank-sum test was used to compare the evaluation scores of the two groups by the referee physician and patients' guardians.

Results

Characteristics of the patients are shown in Table 1. There were no significant differences between the two groups in sex, age, serum IgE, peripheral eosinophil counts, grading scores of AD, and duration of AD. A good positive correlation was observed in 22 patients between the bacterial colony counts and grading scores of AD ($r=0.49$, $P<0.05$).

As shown in the upper panel of Fig. 1, the bacterial colony counts on both stamps 2 (6139 ± 2822) ($P<0.05$) and 3 (3986 ± 2390) ($p<0.01$) in the AEW group significantly decreased as compared with stamp 1 counts (10418 ± 4216). On the other hand, there was no significant difference in

Colony counts



Grading scores

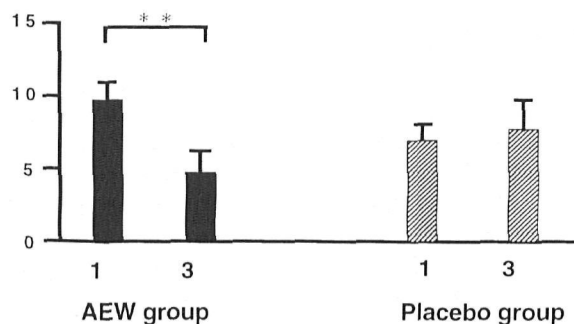


Fig. 1. Changes in bacterial colony counts on skin and grading scores of atopic dermatitis before (1), 3 min after spraying (2), and after 1 week of treatment (3) in AEW group (closed columns) and placebo group (shaded columns). Data were expressed as mean $\pm$ SE. * $P<0.05$; ** $P<0.01$.

the placebo group among stamps 1–3 (1 : 14 555±4283, 2 : 15 431±4953, 3 : 11 236±4508). The normal skin of seven adult volunteers was stamped on the food stamps, which grew no bacterial colony.

The comparison of grading scores of AD is shown in the lower panel of Fig. 1. The effect of AEW was quickly apparent. In the AEW group, grading scores of AD were significantly reduced after 1 week as compared with those before treatment ($P<0.01$). The scores in both mild and medium groups were significantly reduced after 1 week as compared with those before treatment: $P<0.05$ and $P<0.01$, respectively. All of the skin scores and scores for itching and sleep before treatment were significantly improved after 1 week ($5.8\pm3.2\rightarrow2.8\pm1.6$, $P<0.01$, $2.4\pm0.8\rightarrow1.5\pm0.8$, $P<0.01$; $1.6\pm1.3\rightarrow0.6\pm1.0$, $P<0.05$). In the placebo group, there was no significant difference in the scores before and after treatment ($4.2\pm2.0\rightarrow5.0\pm2.7$; $2.0\pm0.8\rightarrow2.0\pm0.6$; $0.8\pm1.0\rightarrow0.9\pm1.2$).

Although 8/11 patients in the AEW group continued to receive the therapy for over 2 months, three patients were available for the 2-month evaluation for both colony counts and grading scores, all of which were remarkably improved as follows: (before treatment, 1 week, and 2 months) 43 496→25 532→1851, 13→6→3; 16 813→0→0, 7→5→4; 1487→115→103, 8→2→2. Five patients were judged only for grading scores: 4→2→2; 14→4→6; 16→7→3; 8→7→6; 16→6→2.

The evaluation after 1 week of treatment in the two groups by the referee physician and the patients' guardians is shown in Table 2. Both the physician's and the guardians' evaluation of treatment efficacy was significantly higher in the AEW group than in the placebo group ($P<0.01$).

Discussion

The widespread nature of staphylococcal infections in AD indicates that a sufficient amount of superantigen may be released to cause T-cell activation, cytokine release, and mast-cell degranulation (16). These mechanisms could, in part, explain the exacerbation of AD associated with *S. aureus* infection

(16). On the other hand, AEW has a strong bactericidal activity, as evidenced by the observation that *S. aureus*, *P. aeruginosa*, and *E. coli* can be exterminated in the solution within 5 s (10). In this study, we tried to demonstrate the effect of AEW on AD patients; i.e., the potential effect of the bactericidal activity of AEW on AD skin lesions. As a result, treatment of skin lesions with AEW showed a significant decrease of bacterial colonies, indicating that it may have a cleaning effect on the skin in AD patients. Leyden et al. (3) showed in 13 AD patients that a mean density of 360 000 *S. aureus* per cm² decreased to 388/cm² after treatment with neomycin cream twice daily. This reduction was certainly greater than that observed with the AEW therapy; i.e., from 10 418 to 3986. However, a drop in *S. aureus* colony counts with the AEW therapy provided a significant clinical effect. Prolonged antibiotic therapy may induce colonization with resistant organisms or new pathogens, a result which could lead to clinical aggravation or relapse. In addition, the neomycin therapy may be difficult to apply to children because of its potentially adverse reactions. On the other hand, in 3/11 patients, bacterial colony counts and grading scores improved after 2 months of the AEW therapy as compared with those before treatment. As the AEW therapy may be relatively slow to induce colonization with resistant organisms, it can be used safely over a long period of time.

In addition to the above finding, grading scores of AD significantly decreased in patients treated with AEW. This observation shows that staphylococcal infections in AD patients may be closely linked to the symptoms of AD. The reliability of grading scores of AD was also supported by evaluation scores by the physician and guardians in this study, which were significantly higher in the AEW group than in the placebo group. Improvement of grading scores was closely correlated with the improvement of evaluation scores. These observations confirm that the grading scores and evaluation scores used in this study are reliable markers of AD. They also lead us to the conclusion that reduction of *S. aureus* from the skin lesions of AD

Table 2. Evaluation of treatment efficacy by guardians of patients and referee physician

	Guardians' evaluation		Physician's evaluation	
	AEW group	Placebo group	AEW group	Placebo group
Improvement	10	3	10	1
No change	0	6	1	7
Aggravation	1	2	0	3
Wilcoxon's rank-sum test	$P=0.0080$	$P=0.0002$		

by AEW therapy, because of its strong bactericidal activity, may result in symptomatic improvement of AD.

Although AEW has bactericidal activity, no remarkable adverse effects with its use were noted in this study. As it is rapidly neutralized and returns to ordinary water without the use of additional chemicals, it seems that AEW therapy is safe. AEW can thus be a potent tool in the skin treatment of AD patients. The current study with AEW in a small number of subjects has yielded a favorable clinical outcome in the management of atopic skin lesions, and it should be included in the therapeutic strategy for AD.

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