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Bactericidal Activity of Manganese and Iodide Ions against Staphylococcus aureus: A Possible Treatment for Acute Atopic Dermatitis

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We reported previously that balneotherapy using Kusatsu hot-spring water is useful for controlling the skin symptoms of acute flares/exacerbations of refractory cases of atopic dermatitis. As Staphylococcus aureus on the skin surface decreased in number or disappeared after balneotherapy, the hot-spring water was suspected to act against the microorganism. The hot-spring water showed strong bactericidal activity against S. aureus *in vitro*. In order to clarify the mechanism further, the bactericidal activity of the hot-spring water was examined by adding back cations and anions in same concentrations as those in the original hot-spring water, one at a time to cation- and anion-exchanged hot-spring water. The findings clearly demonstrated that the bactericidal activity was expressed by manganese and iodide ions in acidic conditions (pH 2.0 – 3.0). Thus, the probable mechanism for the improvement of skin manifestations through Kusatsu balneotherapy is the bactericidal activity of the hot-spring water against S. aureus. When added to water acidified with sulphuric acid (pH 2.0 – 3.0) a synergistic effect of the 2 ions was observed, so that an anti-staphylococcal effect was obtained even at low concentrations (1 mg/kg). Acidic solutions containing manganese and iodide ions may thus be clinically useful for treating skin conditions caused by S. aureus.*

Key words: Kusatsu hot-spring water.

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It is now widely accepted that patients with atopic dermatitis are prone to cutaneous Staphylococcus aureus infection during phases of acute flare/exacerbation and that the increased density of S. aureus is found to correlate well with the severity of skin manifestations (1, 2). Therefore, the reduction of S. aureus numbers by systemic and topical antibiotics or povidone-iodine improves skin symptoms in patients with atopic dermatitis (2, 3). At the Kusatsu-spa, Japan, bathing in acidic hot-spring water at a high temperature was formerly used for the treatment of leprosy and syphilis (4). We previously reported the effectiveness of Kusatsu hot-spring bathing followed by immediate application of white petrolatum in controlling skin symptoms of acute flares/exacerbations of refractory cases of atopic dermatitis (5). This treatment was without side-effects. The hot-spring water was thought to act against S. aureus, as this microorganism was found to decrease in number or disappeared from the skin surface during balneotherapy (5). In this study, we investigated the bactericidal mechanism of Kusatsu hot-spring water.

METHODS

Bacterial strains studied

S. aureus IID 671 (which is the same as FDA 209P) was chosen as a standard. In addition, 20 strains of S. aureus were isolated from the skin surface of 20 patients with atopic dermatitis. Briefly, bacteria were spread on soybean-casein digest (SCD) agar plates (Nihon Pharmaceutical, Osaka, Japan) using a cotton swab which had been in contact with the skin lesion. After incubation at 37°C for 24 h, the colonies were transferred to mannitol salt agar plates (Nihon Pharmaceutical). Colonies with a yellow halo were picked for use in this study, after incubation at 37°C for a further 24 h.

Determination of bactericidal activity

S. aureus was grown at 37°C in SCD Broth (Nihon Pharmaceutical). Cells which were in a logarithmic growth phase and in a stationary phase were harvested and suspended in 0.8% NaCl. After being mixed with a sample solution at a dilution of 1 : 100, the cell suspension was maintained at 42°C for up to 4 h. The cells were then plated on SCD agar and incubated at 37°C for a further 24 h. The number of survivors were determined by counting colonies.

Preparation of sample solution

Hot-spring water was obtained from the Kusatsu Branch Hospital and was maintained at 25°C in polyethylene tanks in dark conditions. The components found in the hot-spring water were H^+ 10.1, Na^+ 53.7, K^+ 16.0, Mg^{2+} 39.0, Ca^{2+} 72.0, Fe^{2+} 14.5, Mn^{2+} 1.4, Al^{3+} 39.0, F^- 12.0, Cl^- 343.0, SO_4^{2-} 611.0, HSO_4^- 206.0, H_2SiO_3 250.0 and HBO_2 8.2 mg/kg water, and the pH was 2.0 (5). The minor components were Zn^{2+} 0.1, V^{5+} 0.1, and I^- 0.3 mg/kg water. They were analysed by an emission spectrochemical method using a sequential plasma spectrometer (ICPS-1000 IV, Shimazu, Kyoto). From preliminary experiments, hot-spring water whose pH was adjusted to 3.0 by the addition of 1 N NaOH was prepared. One control was pH 2.0 sulphuric acid solution, which was prepared by diluting 6 N sulphuric acid solution with distilled water using a pH meter. Another control was pH 3.0 sulphuric acid solution, which was prepared by adding 1 N NaOH to a pH 2.0 sulphuric acid solution.

Ion exchange treatment

For cation-exchange treatment of the hot-spring water, AG 50W-X8 (Bio-Rad Laboratories, Hercules, CA, USA) was used. In brief, 100 ml water was applied to a pencil column containing 2 ml resin (Polyprep column, Bio-Rad Laboratories) which was pre-equilibrated with diluted HCl (pH 2.0). Diluted HCl was prepared by adding distilled water to 6 N HCl using a pH meter. The initial 5 ml eluent was discarded and the following eluent was collected as cation-exchanged solution. For anion-exchange treatment, AG 1-X8 (Bio-Rad Laboratories) was used and the anion-exchanged solution was obtained similarly.

Addition of cations and anions to the ion-exchanged water

NaCl, KCl, $MgSO_4$, $CaCl_2$, $FeSO_4$, $MnSO_4$, $Al_2(SO_4)_3$, $ZnSO_4$, or V_2O_5 was dissolved in diluted pH 2.0 sulphuric acid with 1 g cation/l. Each solution was individually added back to the cation-exchanged solution to make the same cation concentration as the original hot-spring water. Then, the pH of each mixture was adjusted to 3.0 by the addition of 1 N NaOH and its bactericidal activity was determined.

Na_2SO_4, NaCl, NaF or NaI was also dissolved in diluted pH 2.0 sulphuric acid with 10 g anion/l for SO_4^{2-} or Cl^- and 1 g anion/l for F^- or I^-. Each solution was individually added back to the anion-exchanged solution to make the same anion concentration as the original hot-spring water. The pH of each mixture was then adjusted to 3.0 by the addition of 1 N NaOH and its bactericidal activity was measured.

RESULTS

The bactericidal activity against S. aureus IID 671 of the original hot-spring water (pH 2.0) and hot-spring water whose pH was adjusted to 3.0 by the addition of NaOH is shown in Fig. 1. The bactericidal activity of the original pH 2.0 hot-spring water was higher than that of the control pH 2.0 sulphuric acid solution when S. aureus either in a logarithmic growth phase or in a stationary phase was used. However, S. aureus in a stationary phase was more resistant to the control pH 2.0 sulphuric acid solution than S. aureus in a logarithmic growth phase. The bactericidal activity of pH 3.0 hot-spring water was higher than that of the original pH 2.0 hot-spring water. In contrast, control pH 3.0 sulphuric acid solution did not have any bactericidal activity. In addition, the pH 3.0 hot-spring water demonstrated almost the same bactericidal activity against all strains of S. aureus isolated from the 20 patients with atopic dermatitis (data not shown).

To investigate possible bactericidal components, hot-spring water was applied to cation and anion exchange resins.

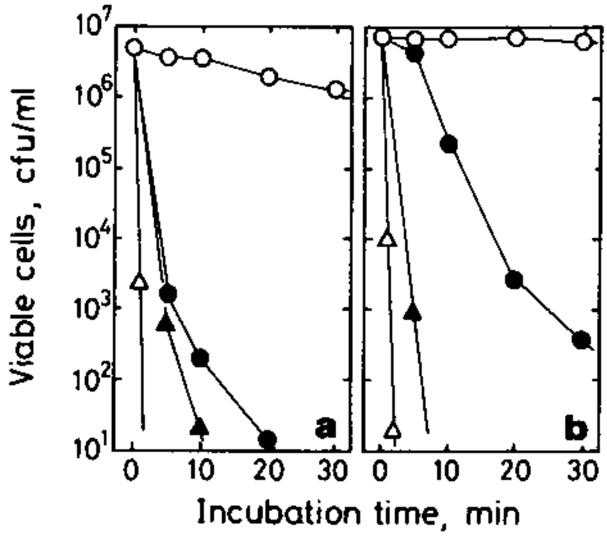

Fig. 1. Effects of hot-spring water and control sulphuric acid solution on S. aureus IID 671. Representative results are illustrated (*a*) when S. aureus is in the logarithmic growth phase and (*b*) in the stationary phase. △: hot-spring water, pH-adjusted to 3.0 by the addition of NaOH; ▲: original hot-spring water (pH 2.0); ○: control pH 3.0 sulphuric acid solution; ●: control pH 2.0 sulphuric acid solution.

Interestingly, bactericidal activity against S. aureus IID 671 was not detected in cation-exchanged nor in anion-exchanged solution. However, bactericidal activity was completely restored by mixing those two solutions again.

Cations and anions involved in the original hot-spring water were added back individually to the cation-exchanged and anion-exchanged solution in the same concentrations as in the original hot-spring water. Bactericidal activity of the cation-exchanged solution against S. aureus IID 671 was recovered by the addition of manganese ions, but not by any other ions. Similarly, the addition of iodide ions restored bactericidal activity of the anion-exchanged solution (data not shown).

Various amounts of $MnSO_4$ and NaI were dissolved in diluted pH 2.0 sulphuric acid solution and the pH of the solutions was adjusted to 3.0 by the addition of 1 N NaOH. The bactericidal activity against S. aureus IID 671 of the solutions is shown in Table I. Bactericidal activity was detected only when both ions co-existed. Furthermore, such activity was recognized when the concentrations of manganese and iodide ions were similar to or higher than those in the original hot-spring water.

DISCUSSION

Kusatsu balneotherapy is a skin care method that is one of the most effective general measures for the treatment of atopic dermatitis (6). This study has shown that Kusatsu hot-spring water has bactericidal activity against S. aureus, which is expressed by manganese and iodide ions under acidic conditions (pH 2.0–3.0). This could explain our previous findings that S. aureus detected on the skin surface decreased in number or disappeared after balneotherapy in patients whose skin symptoms were improved (5). Therefore, the probable mechanism of the improvement of skin manifestations through Kusatsu balneotherapy is its anti-staphylococcal effect.

While the pH of the original hot-spring water was 2.0, hot-spring water whose pH was adjusted to 3.0 by the addition of NaOH showed higher bactericidal activity against S. aureus. To clarify whether or not the acidity of Kusatsu hot-spring water itself was responsible for the bactericidal activity, the action of sulphuric acid solution whose pH was adjusted to either 2.0 or 3.0 was examined. Interestingly, pH 2.0 sulphuric acid solution showed bactericidal activity but pH 3.0 sulphuric acid solution did not. These findings revealed that Kusatsu hot-spring water may contain one or more bactericidal substances. Experiments in which cations and

Table I. *Bactericidal activity of co-existence of manganese and iodide ions*

Manganese ion (mg/kg)	Bactericidal activity Iodide ion (mg/kg)		
	0	0.3	1.0
0	–	–	–
0.3	–	–	+
1.0	–	+	+
3.0	–	+	+
10.0	–	+	+

anions in the same concentrations as the original hot-spring water were added back one by one to cation- and anion-exchanged solutions obtained using ion-exchange resins clearly demonstrated that the bactericidal activity was expressed by manganese and iodide ions under acidic conditions (pH 2.0 – 3.0). The concentrations of manganese and iodide ions expressing bactericidal activity were 1.0 and 0.3 mg/kg, respectively, which were exactly the concentrations in the original hot-spring water. When added to water acidified by sulphuric acid, a synergistic effect of the two ions was observed so that an anti-staphylococcal effect was obtained at low concentrations (1 mg/kg). The lowest active concentration of iodide ion, 0.3 mg/kg, was lower than that of iodide included in 10% povidone-iodine including 10 mg/kg iodine, which is often used for the treatment of atopic dermatitis (3). To our knowledge, this is the first report showing improved bactericidal activity due to the co-existence of manganese and iodide ions.

Our previous paper also showed that the skin surface pH in patients with atopic dermatitis decreased from 4.6 to 3.0 after bathing in Kusatsu hot-spring water (5). On the other hand, it is suspected that the pH of the hot-spring water that contacted the skin surface increased to around 3.0 during bathing. So, bathing itself may change the pH of the original Kusatsu hot-spring water in contact with the skin surface to produce the most bactericidal situation against S. aureus. Moreover, S. aureus in a stationary phase was more resistant to the bactericidal activity of pH 2.0 sulphuric acid solution than was S. aureus in a logarithmic growth phase. As S. aureus on the skin surface of patients with atopic dermatitis is considered to be in a stationary phase, the acidity of Kusatsu hot-spring water itself was not enough to express maximum bactericidal activity.

It is well-known that Kusatsu hot-spring water has bactericidal activity, because no bacteria have been detected in the hot-spring water in repeated examinations by health officials. This study clearly demonstrated that the bactericidal activity of the hot-spring water could be reproduced by the co-existence of manganese and iodide ions in water under acidic conditions (pH 2.0 – 3.0). In addition to its application as a treatment for refractory cases of atopic dermatitis, an acidic solution containing manganese and iodide ions could be used as a disinfectant in various areas of medicine.

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