Antibacterial Activity of 1,2-Alkanediol against 
Staphylococcus aureus and 
Staphylococcus epidermidis

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Abstract: 1,2-Alkanediol exhibits antibacterial activity against several bacteria and yeast. However, few studies have reported antimicrobial tests on skin microbiome. Bacterial microbiome on the skin surface include Staphylococcus aureus (S. aureus), which causes rough skin and inflammation in atopic dermatitis and Staphylococcus epidermidis (S. epidermidis), which enhances innate immunity. In this study, the minimal inhibitory concentration (MIC) was evaluated for 1,2-alkanediol comprising 4–12 carbon atoms against S. aureus and S. epidermidis. 1,2-Alkanediol comprising 6–12 carbon atoms exhibited antimicrobial activity against both species of Staphylococcus. The antibacterial activity depended on the alkyl chain length. In addition, the minimum bactericidal concentration (MBC) on agar was evaluated for 1,2-alkanediol comprising 6–12 carbon atoms. 1,2-Octanediol and 1,2-decanediol exhibited significant bactericidal activity.

Key words: antibacterial activity, 1,2-alkanediol, Staphylococcus, MIC, MBC

1 Introduction

In many cases, preservatives are added to cosmetics to prevent the degradation of products by microbial contamination. In particular, parabens are the most widely used preservative agents. Recently, some studies have suggested that parabens are deleterious to human health, and focus on 1,2-alkanediol as an alternative ingredient of parabens.

1,2-Alkanediols have been added in cosmetics as humectants and solvents because of their low toxicity and water solubility. In addition, some of them exhibit strong antibacterial activity against Escherichia coli and Aspergillus niger.

However, few studies have reported the antibacterial activity of 1,2-alkanediol against the human skin microbiome. Staphylococcus aureus (S. aureus) causes the inflammation of atopic dermatitis and skin roughness, while Staphylococcus epidermidis (S. epidermidis) enhances the innate immunity and ensures a healthy epidermis.

In this study, the antibacterial activity of 1,2-alkanediol against S. aureus and S. epidermidis is systematically examined to reveal the effect of the carbon number of the alkyl chain length. Previously, we focused on the selective bactericidal activity of fatty acids and found that the alkyl chain length, unsaturated degree, and metal ion affect bactericidal activity. The findings of the relationship between the molecular structure and the antimicrobial activity of 1,2-alkanediols are useful for designing cosmetic formulations that control skin microbiome.

Antibacterial activity was determined using minimal inhibitory concentration (MIC), which is the minimum drug concentration required to suppress bacterial growth. In addition, the minimum bactericidal concentration (MBC) was evaluated for 1,2-alkanediols which show significant inhibitory effect.

2 Experimental

2.1 Materials

Six 1,2-alkanediols, 1,2-butanediol (≥ 98%), 1,2-pen-
tanediol (96%), 1,2-hexanediol (98%), 1,2-octanediol (98%), 1,2-decanediol (90%), respectively, were purchased from Sigma-Aldrich Co. LLC (St. Louis, USA) (Fig. 1). 0.1 mol L⁻¹ phosphate buffer solution (pH 6) were purchased from FUJIFILM Wako Pure Chemical Industries Ltd. (Osaka, Japan). Sodium chloride was purchased from Kanto Chemical Co., Inc. (Tokyo, Japan). Ethanol was purchased from Junsei Chemical Co. (Tokyo, Japan). Beef extract was purchased from Nacalai Tesque Inc. (Kyoto, Japan). Polypeptone was purchased from Nisui Pharmaceutical Co., Ltd. (Tokyo, Japan). Agar powder was purchased from Nissui Pharmaceutical Co., Ltd. (Tokyo, Japan). S. aureus (NBRC13276) and S. epidermidis (NBRC12993) were obtained from the National Institute of Technology and Evaluation (Tokyo, Japan). Water was purified using a Demi-Ace Model DX-15 demineralizer (Kurita Water Industries Ltd., Tokyo, Japan).

2.2 Evaluation of MIC and MBC of 1,2-alkanediols against S. aureus and S. epidermidis

The evaluation of MIC and MBC was performed three times for all conditions to confirm reproducibility. Before the cultivation of S. aureus and S. epidermidis, a medium containing beef extract (0.15 g), polypeptone (0.30 g), sodium chloride (0.15 g), and water (30 mL) was sterilized in an autoclave at 121°C for 20 min. The cultures of S. aureus and S. epidermidis were prepared by shaking 30 mL of the medium containing one colony for 24 h at 37°C (145 rpm). To evaluate the MIC, the medium containing beef extract (0.50 g), polypeptone (1.00 g), sodium chloride (0.50 g), and pH 6 phosphate buffer (50 mL) was sterilized in an autoclave at 121°C for 20 min. The pH of the medium containing these components was 5.8.

MIC was defined as the minimum concentration at which the turbidity did not increase when the 1,2-alkanediol was added to the bacterial dispersion. The 1,2-alkanediol ethanol solutions were diluted to prepare the following sample solutions: 1600–204800 µg mL⁻¹ (1,2-butanediol and 1,2-pentanediol), 1600–89600 µg mL⁻¹ (1,2-octanedi-ol), 400–25600 µg mL⁻¹ (1,2-octanediol), 3.13–89600 µg mL⁻¹ (1,2-decanediol), and 3.13–400 µg mL⁻¹ (1,2-dodecanediol). The MIC of 1,2-dodecanediol was not evaluated at a concentration greater than 400 µg mL⁻¹ due to the solubility of 1,2-alkanediols.

To prepare the sample for the MIC evaluation, 1000 µL of a liquid medium containing beef extract (0.01 g), polypeptone (0.02 g), and sodium chloride (0.01 g), 940 µL of phosphate buffer (pH 6), 40 µL of an 1,2-alkanediol ethanol solution, and 20 µL of bacterial dispersion (3 × 10⁶–2 × 10⁸ CFU mL⁻¹) were mixed in a test tube. The optical density of the prepared sample (100 µL) was evaluated by a 96-well TrueLine Cell Culture Plate (Japan Genetics Co., Ltd., Tokyo, Japan) and an absorption grating microplate reader SH-1200 Lab (Corona Electric Co., Ltd., Ibaraki, Japan). We confirmed that 2 wt% of ethanol does not affect bacterial growth in preliminary tests. The evaluated conditions were as follows: wavelength = 660 nm and number of flashes = 10. The S. aureus medium was shaken at 1000 rpm by a microplate shaker PSU-2T (Waken B Tech Co., Ltd., Kyoto, Japan) during incubation for 24 h at 37°C. On the other hand, the S. epidermidis medium was shaken for 48 h under similar conditions, because the growth rate was slower than S. aureus.

Next, 100 µL of a bacteria medium comprising 1,2-hexanediol, 1,2-octanediol, and 1,2-decanediol was smeared on an agar medium containing 2.3 wt% of agar powder to obtain MBC at 37°C. The MBC was defined as the concentration at which bacterial growth was not observed on all of the agar media. The final concentrations of 1,2-alkanediol were 89600–102400 µg mL⁻¹ (1,2-hexanediol), 4800–25600 µg mL⁻¹ (1,2-octanediol), 300–800 µg mL⁻¹ (1,2-decanediol), and 50.0–400 µg mL⁻¹ (1,2-dodecanediol), respectively. Although it is desirable to perform centrifugation prior to the evaluation of MBC, this operation was not performed because there were not enough samples.

3 Results

3.1 Antimicrobial evaluation of 1,2-alkanediol against S. aureus and S. epidermidis

The optical density at 660 nm (OD₆₆₀) was measured after the liquid medium including S. aureus (S. epidermidis) was added to the 1,2-alkanediol solution and incubated for 24 (48) h at 37°C. The turbidity did not increase when some 1,2-alkanediols exceeded a certain concentration. Figure 2 (a) and (b) show the OD₆₆₀ of the liquid medium including 1,2-octanediol and S. aureus (S. epidermidis). The optical density increased when the concentration of less than 4800 µg mL⁻¹. This result indicates that the MIC of 1,2-octanediol is 4800 µg mL⁻¹ for S. aureus and S. epidermidis. In the case of 1,2-hexanediol, 1,2-decanediol,
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and 1,2-dodecanediol, MIC was 89600, 300, and 50.0 µg mL⁻¹, respectively. Figure 3 shows the MIC of four 1,2-alkanediols against S. aureus and S. epidermidis. 1,2-Hexanediol, 1,2-octanediol, 1,2-decanediol, and 1,2-dodecanediol exhibited antimicrobial activity against S. aureus and S. epidermidis. Conversely, the MIC of 1,2-butanediol and 1,2-pentanediol were greater than 100000 µg mL⁻¹ for both S. aureus and S. epidermidis. They did not exhibit significant antimicrobial activity.

3.2 Bactericidal evaluation of 1,2-alkanediol against S. aureus and S. epidermidis

Next, MBC was measured for 1,2-hexanediol, 1,2-octanediol, 1,2-decanediol, and 1,2-dodecanediol. The liquid medium in which the OD₆₆₀ did not increase in Section 3.1 was smeared on agar plates and incubated at 37°C for 24 h. Table 1 shows the MBC when the liquid medium containing S. aureus (S. epidermidis) was smeared on agar plates. When the liquid medium containing 1,2-oktanediol was smeared, S. aureus and S. epidermidis did not grow at a concentration greater than or equal to 9600 µg mL⁻¹. 1,2-Decanediol also exhibited bactericidal activity: its MBC was 600 µg mL⁻¹. In contrast, in the case of 1,2-hexanediol and 1,2-dodecanediol, S. aureus and S. epidermidis grew under all conditions, that is, they did not exhibit significant bactericidal activity.

4 Discussion
The mechanism of the antibacterial activity of 1,2-alkanediols was considered. Although a concrete mechanism is not clear now, some researchers have suggested that membrane destruction with surfactant molecules causes the bactericidal phenomenon. Based on this finding, 1,2-butanediol and 1,2-pentanediol are extremely hydrophilic to show activity due to the short alkyl chain and two -OH groups and can not penetrate into the cell membrane. On the other hand, four 1,2-alkanediols exhibiting antimicrobial activity are more hydrophobic due to the moderately long alkyl chains. Hence, they can penetrate into the cell membrane and inhibit the growth of S. aureus and S. epidermidis. Some physical parameters support this tendency. For example, the solubility decreases with the increase of the alkyl chain length, while the octanol/water partition coefficients (log P) increases as shown in Fig. 4. The antimicrobial behavior of the four 1,2-alkanediols can explain using these parameters. 1,2-alkanediol comprising 6-12 carbon atoms (Log P = 0.25-3.441, solubility = 32.0-37000...
Conclusions

As a result of the systematic antibacterial evaluation of 1,2-alkanediol using MIC, 1,2-alkanediol comprising 6–12 carbon atoms exhibited antibacterial behavior. With the increase in the alkyl chain length of 1,2-alkanediol, the antibacterial activity increased. The MIC of 1,2-dodecanediol, 1,2-octanediol and 1,2-decanediol were 9600, 6000 and 4700 µg mL⁻¹, respectively. Furthermore, as a result of the evaluation of the bactericidal activity using MBC, 1,2-octanediol and 1,2-decanediol exhibited significant bactericidal activity. The MBC of 1,2-octanediol and 1,2-decanediol were 9600, 6000 µg mL⁻¹, respectively.

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