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In Vitro Virucidal Effect of Mouthrinse Containing C31G on Seasonal Influenza Viruses

Dong-Hun Lee[1], Ha-Na Youn[1], Jae-Keun Park[1], Byung-Hwa Kang[2], Jae-Hoon Kang[2], Joong-Bok Lee[1], Seung-Yong Park[1], In-Soo Choi[1], Sang-Won Lee[1], and Chang-Seon Song[1]*

[1]*College of Veterinary Medicine, Konkuk University, Seoul 143-701, Republic of Korea*
[2]*Research Laboratories, Ildong Pharmaceutical Co., Ltd., Hwaseong 445-170, Republic of Korea*

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**Corresponding author*
Phone: +82-2-450-3712;
Fax: +82-2-455-3712;
E-mail: songcs@konkuk.ac.kr

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C31G is a potent antimicrobial agent and can disrupt the microbial membrane by the alkyl portion of the molecule. The objective of this study was to evaluate the virucidal effectiveness of C31G and mouthrinse containing C31G (Sense-Time) on seasonal influenza viruses. Evaluation of the virucidal activity against influenza viruses was performed with end-point titration in 10-day-old chicken embryos and Madin-Darby canine kidney cells. *In vitro* studies demonstrated that C31G and Sense-Time inhibited the growth of seasonal influenza viruses even in the presence of 5% organic material. Gargling with C31G or Sense-Time would enhance oropharyngeal hygiene, which would be helpful for reducing influenza transmission.

Keywords: Mouthrinse, C31G, gargle, influenza virus

On 11 June 2009, the World Health Organization (WHO) declared a current influenza pandemic. Laboratory-confirmed cases of pandemic influenza A (H1N1) 2009 virus infection, claiming the life of more than 18,449 people, had been reported in 214 countries across five continents [11]. Viral transmission could occur through direct contact or indirect contact with respiratory secretions containing influenza viruses [1].

Because of the worldwide shortage of vaccine and antiviral agents against pandemic influenza, the WHO recommends non-pharmaceutical public health interventions to reduce the transmission of pandemic influenza. In particular, the current guidelines of WHO recommend hand sanitization, and oral and respiratory hygiene to prevent the transmission of influenza virus in public [1]. During the pandemic influenza outbreak in 1918, a reliable method that helped to prevent viral infection was the gargling and rinsing out of the nasopharynx with antiseptic solution [5]. In addition, previous studies reported that gargling was beneficial for the prevention of upper respiratory tract infections, including influenza virus infection [4, 9]. In order to reduce the transmission of influenza virus, safe and effective antiseptic solutions for gargling would be required.

Antiseptic mouthrinses are used in many clinical situations for prophylactic and therapeutic purposes. There have been previous studies describing the antimicrobial and virucidal effectiveness of antiseptic mouthrinses [7, 10]. The virucidal efficacy of mouthrinses, although assumed in many instances, has not been adequately evaluated in terms of clinical and practical significance to prevent the transmission of influenza viruses. C31G is a potent antimicrobial agent composed of a buffered equimolar mixture of two amphoteric surface-active agents. It can bind to microbial surfaces *via* the polar head group of the amine oxide-betaine mixture and subsequently disrupt the microbial membrane with the alkyl portion of the molecule [8]. It has been shown that C31G has potent antimicrobial and virucidal activities against enveloped viruses, bacteria, fungi, and yeasts [2, 3].

The purpose of this study was to assess the *in vitro* virucidal effectiveness of C31G and a commercially available mouthrinse containing C31G against seasonal influenza viruses. Our data showed that C31G and mouthrinse containing C31G appeared to be effective in reducing and inhibiting the infection of seasonal influenza viruses *in vitro*.

A 3.0% solution of C31G and a mouthrinse containing 3.0% C31G, Sense-Time (ILDONG Pharmaceutical, Korea), were obtained from the manufacturer. The 3.0% solution of C31G used in this study possessed two compounds, 9.372 g of cetyl betaine and 4.377 g of myristamine oxide in 1 liter of distilled water (DW). The 1:5 diluted C31G was serially diluted 2-fold from 1:5 to 1:320 using DW, hard water (HW), or HW containing 5% fetal bovine serum (HWF, simulating 5% organic material). Sense-Time was serially diluted 2-fold from 1:2 to 1:128 using DW, HW, and HWF.

Seasonal influenza viruses (A/Brisbane/59/2007 (H1N1), A/Brisbane/10/2007 (H3N2), and B/Brisbane/60/2008) were propagated in 10-day-old specific pathogen-free (SPF) chicken embryonated eggs (CEEs) and the 50% egg infectious doses (EID_{50}) were determined. A/Brisbane/59/2007 (H1N1) and A/Brisbane/10/2007 (H3N2) viruses had a titer of $10^{7.0}$ EID_{50}/ml, and B/Brisbane/60/2008 virus had a titer of $10^{6.0}$ EID_{50}/ml. First, 2.5 ml of undiluted and diluted C31G and Sense-Time were added onto 2.5 ml of each virus. Phosphate buffered saline (PBS) was used for the control group. Each mixture was incubated for 30 min at 4°C and neutralized with 5 ml of 10% FBS. For A/Brisbane/59/2007 (H1N1) and A/Brisbane/10/2007 (H3N2), virus titrations were performed using end-point titration in 10-day-old SPF CEEs. For B/Brisbane/60/2008, virus titrations were performed using end-point titration in Madin-Darby Canine Kidney (MDCK) cells. Each mixture was serially diluted 10-fold using PBS or minimal essential medium and then used to inoculate SPF CEEs and MDCK cells. Infectious viral titers within the diluted mixtures were calculated from three replicates using the method of Spearman-Karber [6]. Statistical significance was determined by ANOVA and then the values were compared with the PBS control using Dunnett's test.

C31G and Sense-Time completely inactivated all of the tested viruses at their commercial concentration (Figs. 1 and 2). Up to the 1:16 dilution within DW and HW, Sense-Time completely inactivated all of the tested viruses. Sense-Time diluted in HWF completely inactivated influenza B virus up to the 1:4 dilution and the influenza A (H1N1, H3N2) viruses up to the 1:8 dilution. C31G diluted in DW and HW completely inactivated the influenza A (H1N1, H3N2) viruses up to the 1:160 dilution and influenza B virus up to the 1:40 dilution. C31G diluted in HWF completely inactivated the influenza A (H1N1, H3N2) viruses up to the 1:40 dilution and influenza B virus up to the 1:10 dilution. Although Sense-Time contained the same concentration of C31G, the C31G solution showed a higher virucidal

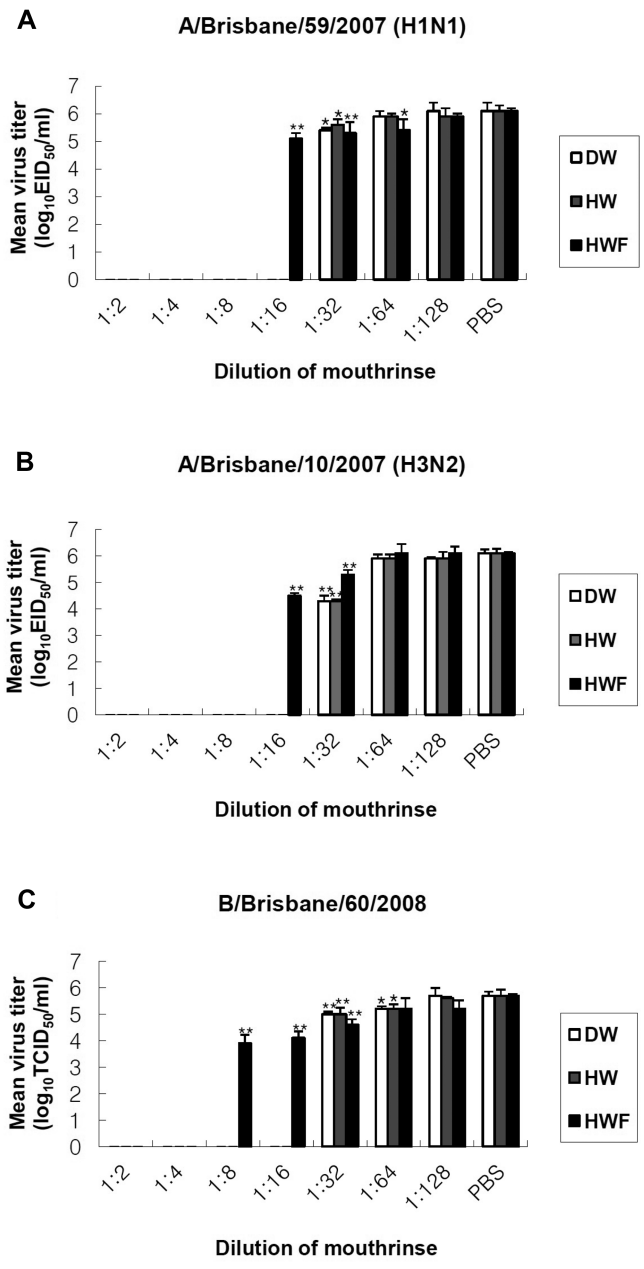

Fig. 1. Virucidal effect of mouthrinse containing C31G on seasonal influenza viruses.

Seansonal influenza viruses were serially diluted with PBS or minimal essential medium. SPF embryonated eggs and MDCK cells were inoculated with each virus-mouthrinse mixture, and infectious viral titers were calculated from three replicates using the method of Spearman-Karber. Statistical significance was determined by ANOVA and then the values were compared with the PBS control using Dunnett's test. *P* values of completely inhibited samples were lower than 0.01 and not indicated in this figure. Asterisks indicate significant differences (*$p < 0.05$; **$p < 0.01$) between partially inhibited samples and the PBS control.

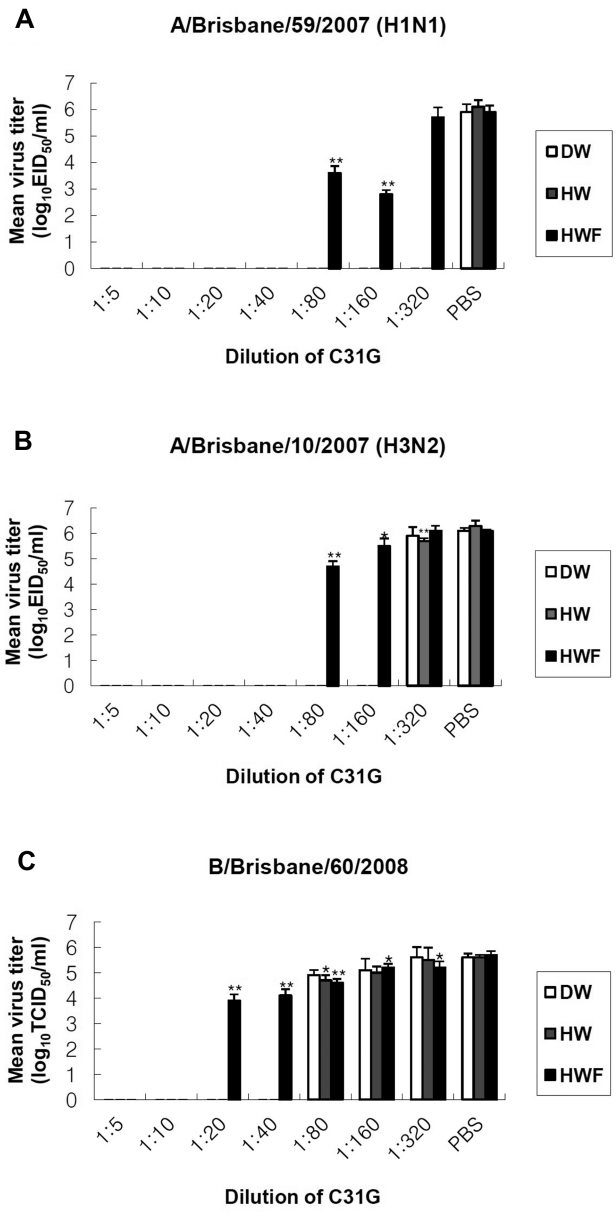

Fig. 2. Virucidal effect of C31G on seasonal influenza viruses. Seasonal influenza viruses were serially diluted with PBS or minimal essential medium. SPF embryonated eggs and MDCK cells were inoculated with each virus-C31G mixture and infectious viral titers were calculated from three replicates using the method of Spearman-Karber. Statistical significance was determined by ANOVA and then compared values with PBS control using Dunnett's test. P values of completely inhibited samples were lower than 0.01 and not indicated in this figure. Asterisks indicate significant differences (*$p < 0.05$; **$p < 0.01$) between partially inhibited samples and PBS control.

efficacy than Sense-Time in this study. It seems that the virucidal efficacy of C31G in Sense-Time might be affected by other chemical components.

In a previous study, Corner *et al*. [2] suggested that when C31G was used in the oral cavity, it had broad-spectrum antimicrobial properties as effective as chlorhexidine. Furthermore, the antimicrobial activity of C31G was not decreased when it was formulated into a mouthrinse vehicle [2]. Antiseptic mouthrinses are commonly applied to the oral cavity containing organic materials such as remained foods and saliva. In the present study, we used hard water containing 5% FBS to mimic the environment of the oral cavity containing organic materials. Sense-Time was able to completely inhibit seasonal influenza viruses up to the 1:4 dilution in HWF, representing effective virucidal activity in the oral cavity containing organic materials. Therefore, Sense-Time seems to be a reliable mouthrinse for preventing influenza infection.

In conclusion, this study has shown that C31G and Sense-Time have anti-influenza activity, for both influenza A virus and influenza B virus. Gargling with C31G or Sense-Time would enhance oral and respiratory hygiene, which could be beneficial for preventing the human-to-human transmission of influenza viruses.

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