FEMS Microbiology Letters, 366, 2019, fnz235

doi: 10.1093/femsle/fnz235
Advance Access Publication Date: 23 November 2019
Research Letter

RESEARCH LETTER – Virology

Inactivation of human norovirus and its surrogate by the disinfectant consisting of calcium hydrogen carbonate mesoscopic crystals

Hidekatsu Shimakura[1], Fumihiro Gen-Nagata[2], Makoto Haritani[3], Koichi Furusaki[4], Yusei Kato[1], Nanako Yamashita-Kawanishi[1], Dung T. LE[1], Masano Tsuzuki[1], Yukinobu Tohya[5], Shigeru Kyuwa[6], Hiroyuki Saito[7], Taisuke Horimoto[8], Takashi Onodera[3] and Takeshi Haga[1,*]

[1]Division of Infection Control and Disease Prevention, Department of Veterinary Medical Science, Graduate School of Agricultural and Life Sciences, The University of Tokyo, 1-1-1 Yayoi, Bunkyo-ku, Tokyo 113-8657, Japan, [2]Division of Biomedical Food Research, National Institute of Health Sciences, 3-25-26 Tonomachi, Kawasaki-ku, Kanagawa 210-9501, Japan, [3]Research Center for Food Safety, The University of Tokyo, 1-1-1 Yayoi, Bunkyo-ku, Tokyo 113-8657, Japan, [4]Mineral Activation Technical Research Center, 434 Ohkura, Tamana-shi, Kumamoto 865-0023, Japan, [5]Laboratory of Veterinary Microbiology, Department of Veterinary Medicine, College of Bioresource Sciences, Nihon University, 1866 Kameino, Fujisawa, Kanagawa 252-0880, Japan, [6]Laboratory of Biomedical Science, Department of Veterinary Medical Science, Graduate School of Agricultural and Life Sciences, The University of Tokyo, 1-1-1 Yayoi, Bunkyo-ku, Tokyo 113-8657, Japan, [7]Department of Microbiology, Akita Prefectural Research Center for Public Health and Environment, 6-6 Senshu-Kubotamachi, Akita 010-0874, Japan and [8]Laboratory of Veterinary Microbiology, Department of Veterinary Medical Science, Graduate School of Agricultural and Life Sciences, The University of Tokyo, 1-1-1 Yayoi, Bunkyo-ku, Tokyo 113-8657, Japan

*Corresponding author: Division of Infection Control and Disease Prevention, Department of Veterinary Medical Science, Graduate School of Agricultural and Life Sciences, The University of Tokyo, 1-1-1 Yayoi, Bunkyo-ku, Tokyo 113-8657, Japan. Tel/Fax: +81-3-5841-7573; E-mail: ahaga@mail.ecc.u-tokyo.ac.jp

One sentence summary: The disinfectant with calcium hydrogen carbonate mesoscopic crystals inactivated noroviruses along with the destruction of viral capsids.

Editor: Arnoud van Vliet

ABSTRACT

Human norovirus is one of the major causes of foodborne gastroenteritis, and it can be easily transmitted from infected person, virus-contaminated foods and environmental surfaces. Effective disinfection method is needed to stop the transmission of human norovirus. CAC-717 is a new disinfectant consisting of calcium hydrogen carbonate mesoscopic crystals. We aimed to evaluate the efficacy of CAC-717 against human norovirus. This study used human norovirus derived

Received: 19 July 2019; **Accepted:** 22 November 2019

from fecal specimens and cultured murine norovirus, which is one of the surrogate viruses for human norovirus. The disinfection effect against murine norovirus was estimated by infectivity assay and transmission electron microscopy. The inactivation effect against human norovirus was assessed by reverse transcription polymerase chain reaction. Disinfection effect of CAC-717 against the infectivity of murine norovirus was shown within 100 s after the CAC-717 treatment, presenting the destruction of viral capsids. The treatment of CAC-717 significantly reduced human norovirus genomic RNA (3.25-log reduction) by the presence of the mesoscopic structure of calcium hydrogen carbonate. CAC-717 stably inactivated human norovirus in stool suspensions. The inactivation effect of CAC-717 against human norovirus was less susceptible to organic substances than sodium hypochlorite. CAC-717 would be a useful alternative for disinfecting human norovirus in contaminated environmental surfaces.

Keywords: calcium hydrogen carbonate mesoscopic crystals; infectivity assay; norovirus; RT-qPCR; transmission electron microscopy

INTRODUCTION

Human norovirus (HuNV) is a non-enveloped single stranded positive-sense RNA virus, and it is one of the major causes of foodborne gastroenteritis. HuNV infection causes severe vomiting, diarrhea and abdominal pain in 24 to 48 h after the intake of the virus and may become lethal in infants and elderly people. The infected person can shed billions of HuNV particles in feces, and the infection to others can be easily established by intake of only a small number of HuNV particles through several transmission routes: person to person, airborne, foodborne and waterborne (Teunis *et al*. 2008; Alsved *et al*. 2019). The outbreak of HuNV infection occurs due to the high transmissibility of the virus (Sakon *et al*. 2018). Genotypes of HuNV are classified based on the genomic sequence (Kroneman *et al*. 2013), and genogroup II (GII), and especially genotype GII.4 is the predominant strain for the recent HuNV outbreaks (Beek *et al*. 2018). Emerging HuNV strain can rapidly replace the predominantly existing strains by escaping the population immunity, causing an outbreak.

Effective disinfection method against HuNV-contaminated environmental surfaces is needed to stop the HuNV transmission. Heat, ultra violet (UV) and chemical disinfectants are widely used as major disinfectants. The effect of disinfection methods against HuNV is evaluated by the reduction of genomic RNA using quantitative determination, because of the difficulty in culturing of HuNV by conventional cell lines. Hence, as one of the HuNV surrogates, murine norovirus (MNV) is often used to assess the infectivity, although the resistance to disinfection methods of the surrogate viruses is not the same as that for HuNV (Hewitt, Rivera-Aban and Greening 2009; Tung *et al*. 2013). Heating effectively inactivated both MNV and HuNV (Hewitt, Rivera-Aban and Greening 2009). Ethanol showed disinfection of MNV (Belliot *et al*. 2008), whereas HuNV showed resistance to ethanol (Tung *et al*. 2013). The use of chlorine bleach solution, such as sodium hypochlorite, is recommended by the Centers for Disease Control and Prevention for inactivating HuNV in the contaminated surfaces (Hall *et al*. 2011). However, these currently administrated disinfection methods have some disadvantages. For example, heating can only be used for heat-resistant materials and suitable heating facility is needed. Sodium hypochlorite can cause corrosion of metal, mucosal irritation and distinctive smell. Moreover, protein substances can attenuate the disinfection effect of sodium hypochlorite (Kobayashi *et al*. 2016). The traditional disinfection methods, therefore, have limitations depending on the situation, and an alternative disinfectant is needed that can be easily used and applicable for any situation.

Recently, a new disinfectant named CAC-717, which contains electrically charged calcium hydrogen carbonate mesoscopic crystals, showed an effect for sterilizing influenza A viruses (Nakashima *et al*. 2017). The previous report demonstrated that the electrons accumulated in calcium compound and the calcium hydrogen carbonate mesoscopic structure can serve as 'nanobatteries' (Ponrouch *et al*. 2016). This study aimed to evaluate the disinfection effect of CAC-717 as a novel candidate disinfectant for inactivating HuNV.

MATERIALS AND METHODS

Disinfectants and controls

CAC-717 (FDA/USA Regulation No. 880.6890, Class 1 disinfectant, Japan Patent No. 5778328) was produced as reported previously (Nakashima *et al*. 2017). CAC-717 contained 6.9 mM calcium hydrogen carbonate particles with a mesoscopic structure. Because mesoscopic particles have physical properties to occlude hydrogen ion, CAC-717 showed a pH of 12.6 by the formation of mesoscopic structure.

The currently administrated disinfection methods were used in this study as index of the disinfectant efficacy of CAC-717; 70% ethanol and 1000 ppm sodium hypochlorite (NaClO) were purchased from Imazu Chemical Co. Ltd (Tokyo, Japan) and Oyalox Co., Ltd (Tokyo, Japan), respectively. Heating treatment (95°C for 10 min) was performed with dry thermo unit (BI-515, ASTEC, Fukuoka, Japan).

Phosphate buffer solutions with three ranges of pH buffering capacity (2.15, 7.20 and 12.33) were prepared as pH controls for evaluating the effect of pH change against CAC-717. Disodium hydrogen phosphate 12-water ($Na_2HPO_4 \bullet 12H_2O$, 1.296 mmol; Wako Pure Chemical Industries, Ltd, Osaka, Japan) and sodium dihydrogen phosphate anhydrous (NaH_2PO_4, 2.304 mmol; Wako Pure Chemical Industries, Ltd) were mixed and then adjusted to the same pH as CAC-717. CAC-717 without the mesoscopic structure was also prepared to identify the disinfectant efficacy of the mesoscopic structure against norovirus. To abolish the mesoscopic structure, CAC-717 was stirred and treated with an external variation of the magnetic field. Due to this treatment, calcium hydrogen carbonate will form macroparticles (i.e. aggregation of mesoscopic particles) and the electric charge will be lost. As a result of the abolishment of the CAC-717 mesoscopic structure, the hydrogen ion (proton) in the particles will become liquid, resulting in the pH of 7.0.

Noroviruses

MNV strain S7 was isolated from the feces of a mouse in Japan (Kitagawa *et al*. 2010) and used as a surrogate virus for HuNV in this study. HuNVs were derived from four fecal specimens of patients with non-bacterial gastroenteritis. The genotypes of HuNV were determined by capsid sequence-based phylogenetic

analysis (Kroneman et al. 2013). The genotypes of HuNVs in four specimens were GI.2 (GenBank Accession No.: LC128710), GII.4 Sydney 2012 (AB855775), GII.5 (AB448707) and GII.17 (LC075599).

MNV culture and infectivity assay

MNV was propagated and titrated on a macrophage-like tumor cell line, RAW 264 cells, which was obtained from the RIKEN BioResource Center Cell Bank (Tsukuba, Japan). RAW 264 cells were cultured in Dulbecco's minimum essential medium (Sigma-Aldrich, St Louis, MO) supplemented with 10% fetal bovine serum (Sigma-Aldrich) and were maintained at 37°C in 5% CO_2.

A conventional 50% tissue culture infectious dose ($TCID_{50}$) assay was adapted to evaluate the infectivity of MNV. The suspension of MNV was mixed with CAC-717 or phosphate buffer saline (PBS) in 1:9 ratio. These mixtures were incubated at room temperature for 1, 15, 30 and 60 min. After incubation, the mixtures were serially diluted at 1:10 using the medium, and 100 µl of each diluent was added to the 90%-confluent monolayer of RAW 264 cells on 96-well plates. The cytopathic effects of MNV were observed after 4 to 7 days. Virus titers were calculated according to the methods of Reed and Muench (1938). The detection limit of the assay is 1.5-$\log_{10}$ $TCID_{50}$/ml. Results are representative of three independent experiments.

Purification of noroviruses

Purification of the specimen including viruses was carried out to remove debris. The specimens were centrifuged at 2000 *g* for 45 min to remove gross debris. The supernatants were transferred to ultracentrifuge tubes over 27% sucrose solutions and then centrifuged at 112 730 *g* for 2 h (CP80MX; Koki Holdings Co., Ltd, Tokyo, Japan). The pellets were suspended in distilled water and incubated at 4°C overnight. The suspensions including purified viruses were used for the experiments.

Morphological observation of MNV

The morphological observation was conducted by negative-stain transmission electron microscopy (TEM). The purified virus suspensions were treated with or without CAC-717 for 100 s. The suspensions were added to hydrophilized grids coated with an acrylic thin film (Okenshoji Co., Ltd, Tokyo, Japan), and then dried. Soon after, the specimens were stained using 1% uranyl acetate and then dried. Hydrophilic treatment of the grids was performed by soft plasma etching (SEDE-GE; Meiwafosis Co., Ltd, Tokyo, Japan) just before use. The specimens were observed through a JEM-1400 Plus (Jeol Ltd, Tokyo, Japan) at 80 kV. Results are representatives of three independent experiments.

Quantitation of genome RNA of HuNVs

HuNVs in the suspensions of stools and purified specimens were quantified by quantitative reverse transcription polymerase chain reaction (RT-qPCR). RNA extraction was carried out using the QIAamp Viral RNA Mini Kit (Qiagen, Hilden, Germany). Reverse transcription to cDNA and qPCR were performed according to the manufacturer's instructions of Takara qPCR Norovirus (GI/GII) Typing Kit (Takara Bio Inc., Shiga, Japan) and StepOnePlus Real-Time PCR System (Thermo Fisher Scientific, Waltham, MA). The four HuNV suspensions were diluted to ~2.5×10^4 copies/µl (GI.2: 1-fold, GII.4 Sydney 2012: 8-fold, GII.5: 2.5-fold and GII.17: 10-fold). When examining the effects of organic substances, ~1×10^3 copies/µl of purified specimens, which were added bovine serum albumin (BSA; Sigma-Aldrich) (0.5, 2.5 and 5.0%), were used. For the assessment, 100 µl of the diluted suspensions were mixed with disinfectants at a 1:1 ratio at room temperature for 30 min or with PBS at a 1:1 ratio at 95°C for 10 min. Propidium monoazide (PMA; Biotium, Inc., Hayward, CA) was added to the mixtures at a final concentration of 50 μM for inhibiting the amplification of RNA in HuNV whose capsid has been damaged (Fittipaldi et al. 2010), and then 365 nm UV light was exposed for 15 min to the mixtures for PMA binding to RNA. After the extraction of RNA, the quantity of genomic RNA derived from HuNV harboring intact capsid was determined by RT-qPCR. The standard curve was generated using positive controls in Takara qPCR Norovirus (GI/GII) Typing Kit, and the detection limit was 4×10^{-1} copies/µl. Inactivation rate was calculated with the following formula.

$$\% = \left(\frac{\text{Copies of untreated specimen} - \text{Copies of treated specimen with BSA}}{\text{Copies of untreated specimen} - \text{Copies of treated specimen without BSA}}\right) \times 100$$

Results are representatives of three independent experiments. Since the results from the crude untreated specimens were similar with the results of the purified untreated specimen derived from the same stool, fecal matrix did not affect the measurements of this assay.

Statistical analysis

Paired *t*-test was employed to compare with two groups. Multiple groups were compared and analyzed by one-way ANOVA and Tukey post hoc test. Statistical processing was performed with GraphPad Prism 5.0 (GraphPad Software Inc., San Diego, CA). Data were considered as a significant difference at a *P* value of < 0.01.

RESULTS

The disinfection effect of CAC-717 on MNV

The treatment of CAC-717 for 60 min showed a significant $\geqq$4.5-log reduction in the infectivity of MNV compared with untreated specimen (Fig. 1A). The disinfection effect of CAC-717 was seen as early as 1 min after the treatment (Fig. 1B). On the other hand, cytotoxicity of RAW 264 cells was also observed at the highest concentration of CAC-717 in this assay. Morphologically, the destruction of MNV capsids was observed by treating CAC-717 for 100 s (Fig. 1C). Therefore, CAC-717 showed rapid disinfection effect on the HuNV surrogate virus, MNV, through physical destruction of the capsids.

The inactivation of purified HuNV by CAC-717 with the mesoscopic structure

To examine the inactivation effect of CAC-717, the specimen including GII.4 Sydney 2012, which has been the recent pandemic strain worldwide, was used as a representative (Beek et al. 2018) (Fig. 2). The specimen was purified before mixing disinfectants. CAC-717 showed a significant 3.25-log reduction of HuNV genomic RNA compared with the untreated specimen (Fig. 2A). Ethanol (70%) and the heating treatment (95°C for 10

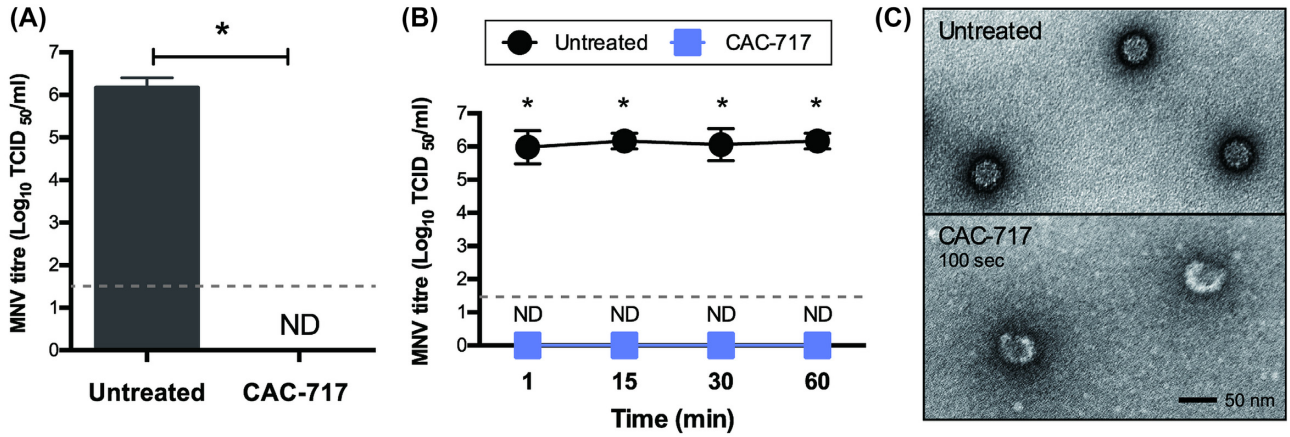

Figure 1. The disinfection effect of CAC-717 on MNV as a surrogate for HuNV. **(A)** Reduction of infectivity of MNV by CAC-717 was evaluated by $TCID_{50}$ assay. The dashed line represents detection limit of the assay. ND: not detectable. Asterisk showed a significant difference ($P < 0.01$). **(B)** Effect of CAC-717 was sequentially observed using $TCID_{50}$ assay of MNV. Infectivity of MNV after 1, 15, 30 and 60 min of CAC-717 treatment was evaluated. Circle or box indicated titers of MNV without or with treatment of CAC-717, respectively. **(C)** Morphological change of MNV was observed by TEM without or after 100 s of CAC-717 treatment.

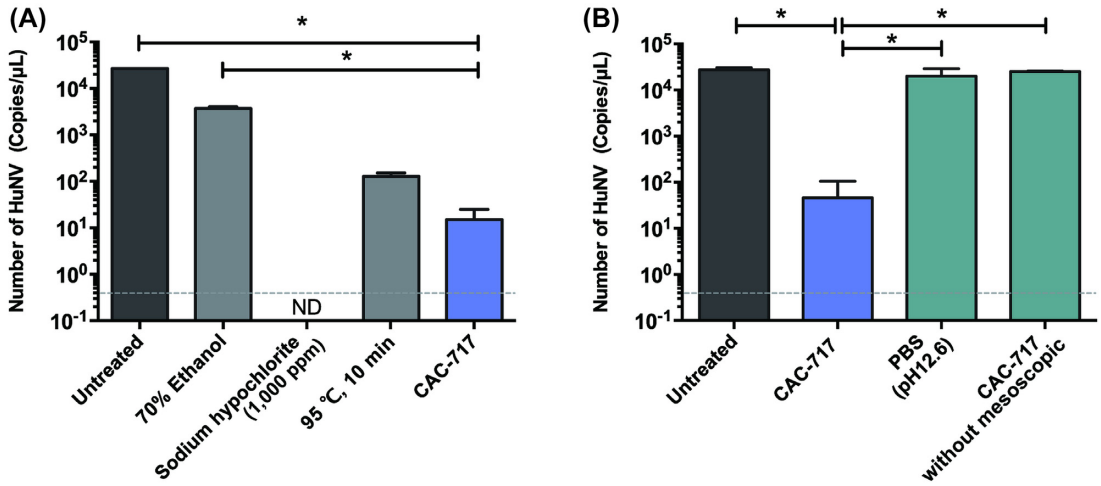

Figure 2. The inactivation of purified HuNV strain classified as GII.4 Sydney 2012 by CAC-717. The inactivation of HuNV was evaluated by RT-qPCR. **(A)** HuNV treated with CAC-717 or with other disinfection methods. **(B)** Effect of CAC-717 with mesoscopic structure (pH 12.6) was compared with a pH control (phosphate buffer, pH 12.6) and CAC-717 without mesoscopic structure (pH 7). The dashed line represents detection limit of the assay. ND: not detectable. Asterisk showed a significant difference as compared with CAC-717 ($P < 0.01$).

min) showed significant reduction of the genomic RNA showing 0.88 and 2.35-log reduction, respectively, when compared with the untreated specimen. The genomic RNA was completely undetectable by the treatment of 1000 ppm sodium hypochlorite, which has been generally used as a strong disinfectant. There was no significant difference for the inactivation effect of HuNV between CAC-717 and accepted disinfectant methods (heating treatment and 1000 ppm sodium hypochlorite).

To prove the inactivation effect of the mesoscopic structure, we compared CAC-717 with the controls (Fig. 2B). The phosphate buffer prepared at the same pH as CAC-717 (pH 12.6) showed no inactivation effect on HuNV. Moreover, CAC-717 including the same compositions without mesoscopic structure also did not display the inactivation of HuNV.

The inactivation effect of CAC-717 on HuNV in clinical specimens

Under actual environment conditions, various organic substances, such as fecal matrix, exist around HuNV. Therefore, four fecal specimens without the purification step were also applied (Fig. 3). Compared with the untreated specimen, CAC-717 stably showed significant log reductions at 1.36, 2.78, 1.64 and 3.52 in the GI.2, GII.4 Sydney 2012, GII.5 and GII.17 specimens, respectively. Thus, CAC-717 may be able to inactivate HuNV under the clinical conditions. Heating treatment also inactivated all of the four HuNV specimens. On the other hand, 1000 ppm sodium hypochlorite did not elicit enough inactivation effect on HuNV in the suspensions of stool, except for one specimen containing HuNV GII.17. The 70% ethanol also did not show enough inactivation effect on HuNV. Consequently, CAC-717 can stably inactivate HuNV in practice.

The influence of organic substance in the inactivation of CAC-717

Inactivation tests using all four purified specimens with the respective concentrations of BSA were performed to verify the influence of organic substances against the disinfectants (Fig. 4). CAC-717 showed 100% inactivation effect against HuNV in 0.5%

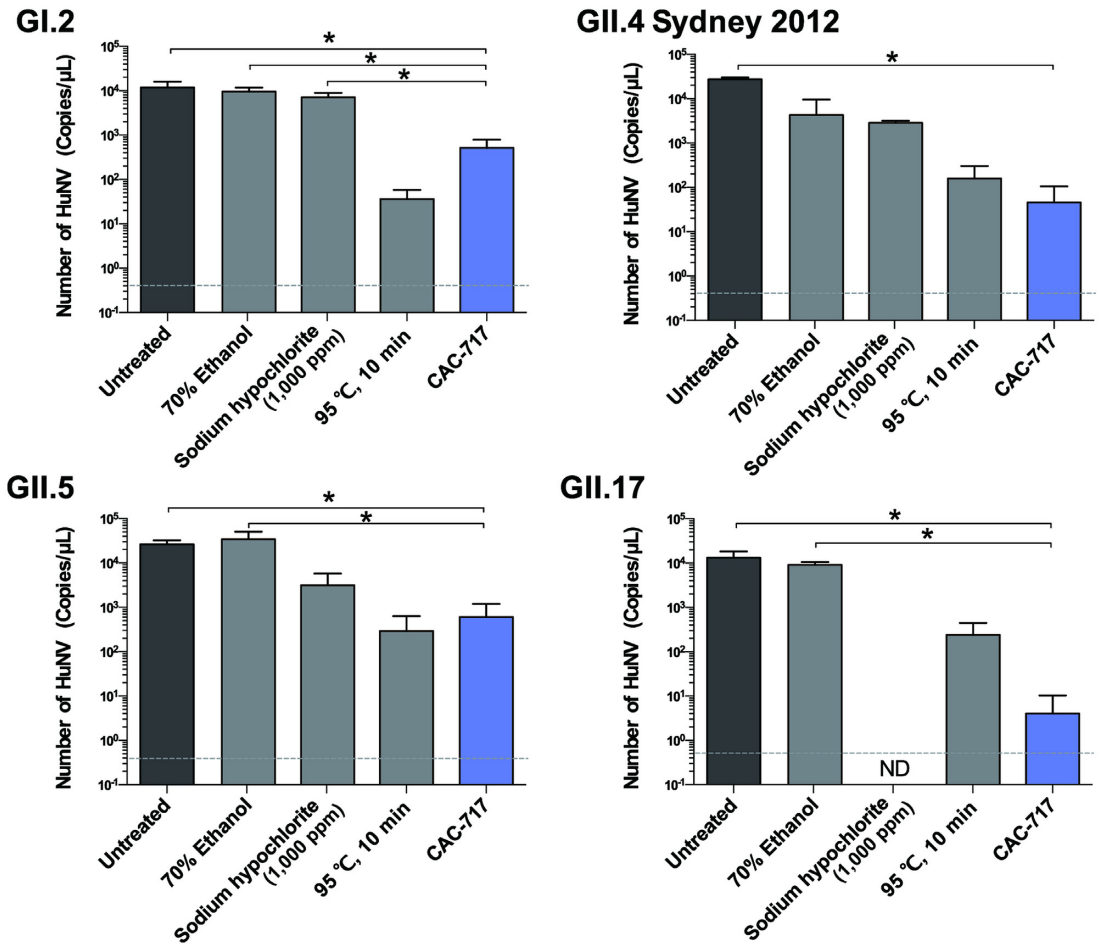

Figure 3. The inactivation of HuNV in stool derived from patients with nonbacterial gastroenteritis. Each sample contains different genotypes of HuNV, and the inactivation was assessed by RT-qPCR. The dashed line represents detection limit of the assay. ND: not detectable. Asterisk showed a significant difference as compared with CAC-717 ($P < 0.01$).

BSA solution. The inactivation effect of CAC-717 against HuNV in 2.5% BSA solution was 99.9, 95.5, 84.3 and 87.8% for specimens of GI.2, GII.4 Sydney 2012, GII.5 and GII.17, respectively. In the 5% BSA solution, the inactivation effect of CAC-717 was 96.0, 73.2, 70.4 and 60.3% for the GI.2, GII.4 Sydney 2012, GII.5 and GII.17, respectively. In contrast, the inactivation effect of 1000 ppm sodium hypochlorite was greatly reduced by the addition of BSA. Even when 0.5% BSA was added to the purified virus, the inactivation effect of sodium hypochlorite decreased in GII.17 specimen (57.8%). In addition, the inactivation rate of sodium hypochlorite in BSA 2.5% solution was 0, 24.5, 40.0 and 17.2% for the GI.2, GII.4 Sydney 2012, GII.5 and GII.17, respectively. Similarly, inactivation rate of sodium hypochlorite in BSA 5% solution was 11.8, 34.9, 50.2 and 28.9% for the GI.2, GII.4 Sydney 2012, GII.5 and GII.17, respectively. The significant differences between CAC-717 and the sodium hypochlorite in the 0.5% BSA solution were observed in the specimens of GI.2, GII.4 Sydney 2012 and GII.5. Moreover, there were significant differences between those two disinfectants in 2.5% BSA solution in all specimens. Furthermore, the significant difference in the 0.5% BSA solution was observed in the GII.17 specimen. As a result, it was found that the inactivation effect of CAC-717 against HuNV was less susceptible to organic substances than 1000 ppm sodium hypochlorite, which is generally used as a strong disinfectant.

DISCUSSION

In this study, we used two methods to assess the efficacy: the infectivity assay and RT-qPCR. These two assays generally have different interpretation of the results (Kitajima *et al.* 2010; Park and Sobsey 2011). RT-qPCR detects the intact genomic RNA of the virus including the damaged capsids, whereas the infectivity assay does not detect the virus that has damaged capsids. We applied PMA in RT-qPCR assay to diminish the difference. PMA has been extensively used for live/dead distinction in microbiology. PMA binds to a nucleic acid through holes on a damaged capsid and then blocks DNA polymerase reaction in RT-qPCR (Fittipaldi *et al.* 2010). Thus, theoretically, PMA-treated RT-qPCR could only detect viral genomic RNA contained in intact capsid, which would retain infectivity. The intensity of UV exposure for the binding PMA should be considered in the assay because the genomic RNA of HuNV is disrupted by strong UV exposure (254 nm) (Park, Linden and Sobsey 2011). Therefore, we used the UV light with a longer wavelength (365 nm) and confirmed that there was no influence on the quantitative value of RT-qPCR in our protocol (data not shown). Consequently, the genomic nucleic acid determination by RT-qPCR with PMA is considered to be comparable with the result of infectivity assay rather than that by RT-qPCR without PMA.

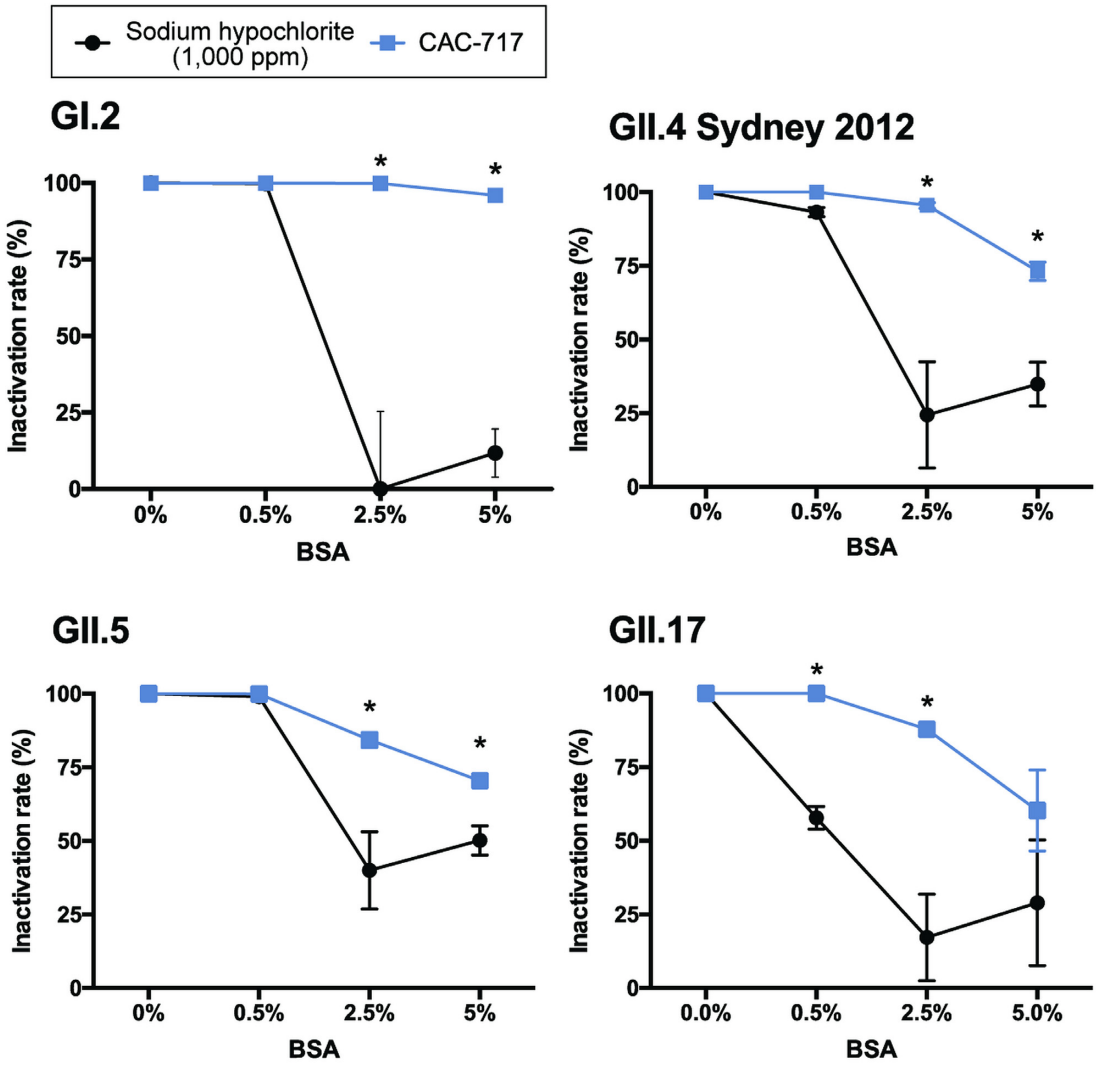

Figure 4. The influence of organic substance against the inactivation efficacy of CAC-717. The inactivation of purified HuNV, with or without BSA, was assessed by RT-qPCR. Inactivation rate was calculated according to the formula that is represented in the section 'Materials and Methods'. Asterisk showed a significant difference between CAC-717 and sodium hypochlorite ($P < 0.01$).

It is necessary to clarify the inactivation mechanism of CAC-717 in order to know the characteristics as a disinfectant. CAC-717 becomes alkaline as a result of the formation of the mesoscopic structure (Nakashima *et al.* 2017). It has been known that surrogate viruses for HuNV are inactivated by high pH condition (Cannon *et al.* 2006). However, the degree of inactivation varies with each surrogate virus. Our result indicated that there was no destruction of genome RNA of HuNV in an alkaline phosphate buffer solution with the same pH to CAC-717 (Fig. 2B). Hence, pH is not a crucial factor in its inactivation effect. On the other hand, CAC-717 without the mesoscopic structure lost inactivation effect (Fig. 2B). Therefore, the inactivation effect is likely to be caused by the mesoscopic structure of calcium hydrogen carbonate in CAC-717.

Organic substances around norovirus may reduce the efficacy of disinfectant (Takahashi *et al.* 2011). The disinfectant efficacy of sodium hypochlorite to MNV and HuNV is reduced by adding stool suspension (Park and Sobsey 2011; Tung *et al.* 2013). The similar result was observed in this study. These influences arise from the reduction of free chlorine in sodium hypochlorite by protein substance (Kobayashi *et al.* 2016). On the other hand, the previous study also demonstrated that the inactivation effect of CAC-717 was not influenced by the addition of protein substance (Nakashima *et al.* 2017). Our results revealed that the inactivation effect of CAC-717 was significantly less susceptible to organic substance than 1000 ppm sodium hypochlorite (Fig. 4). However, the inactivation effect of CAC-717 was moderately decreased in 5% BSA solution, which is the highest concentration in this study (Fig. 4). Although the pre-cleaning, such as physical removal, before using CAC-717 seems necessary when a contamination is voluminous, CAC-717 can be applied as an effective disinfectant in various situations.

The treatment period is an important factor when considering the use of disinfectants. Ethanol (70%) failed to eliminate norovirus by treating for 30 min (Duizer *et al.* 2004). Sodium hypochlorite requires an exposure time of at least 3 min to inactivate MNV (Park and Sobsey 2011). In contrast, CAC-717 showed inactivation of influenza A viruses in 1 min (Nakashima *et al.* 2017), and our results also showed that the disinfection effect of CAC-717 against MNV was seen in 1 min (Fig. 1B). Because the mesoscopic structure in CAC-717 functions as 'nanobatteries' (Ponrouch *et al.* 2016; Nakashima *et al.* 2017), the capsids

of norovirus were probably destroyed by its function when the mesoscopic particles contact the viruses. Thus, although the mechanisms of effect by CAC-717 are not entirely understood, CAC-717 would rapidly display an inactivating effect to HuNV.

Virus envelopes are associated with the resistance to disinfectants. In general, the lipid membrane of enveloped viruses can easily be disrupted by various disinfectants. One of the enveloped viruses, influenza A virus was shown to be inactivated by CAC-717 (Nakashima *et al.* 2017). In contrast to enveloped viruses, many non-enveloped viruses that are composed of genetic material tightly packed with proteins into a dense particle are highly resistant to disinfectants. In this study, CAC-717 showed inactivation effect against the non-enveloped virus, MNV and HuNV. Hence, CAC-717 might inactivate the virus with and without envelopes. Further studies are needed to assess the inactivation effect of CAC-717 against various viruses.

CAC-717 was reported to be harmless and not irritant to humans and animals, because it contains no hazardous chemicals, and its pH was reduced to 8.84 ± 1.17 when applied to human skin (Nakashima *et al.* 2017). However, in this study, cytotoxicity of RAW 264 cells was observed at the highest concentration of CAC-717. Therefore, further investigation is required for evaluating its safety when applying to humans and animals.

The duration of efficacy of CAC-717 was not investigated in this study. This is important because the versatility of the disinfectants will vary depending on its duration of efficacy. For example, chlorhexidine has a long duration and can be used to disinfect the skin in the surgical field. For use in clinical practice, further study is needed to investigate the stability of the mesoscopic structure when it is used on different types of surfaces.

In conclusion, our results suggest that CAC-717 destroys the HuNV viral capsids by its mesoscopic structure, presenting rapid and stable inactivation of HuNV in specimens under various conditions. It would be a novel candidate disinfectant for inactivating HuNV in contaminated environmental surfaces effectively. Further investigation of the safety and stability of CAC-717 is needed; however, CAC-717 will be a strong candidate for HuNV disinfectant in various fields.

FUNDING

This work was supported by the donation from Santa Mineral Co., Ltd.

Conflict of interest. Santa Mineral Co., Ltd, which supported this study, had no role in the study design (data collection, management and interpretation) manuscript writing and decision to submit it for publication.

REFERENCES

Alsved M, Fraenkel C-J, Bohgard M *et al.* Sources of airborne norovirus in hospital outbreaks. *Clin Infect Dis* 2019, doi:10.1093/cid/ciz584.

Beek JV, de Graaf M, Al-Hello H *et al.* Molecular surveillance of norovirus, 2005–16: an epidemiological analysis of data collected from the NoroNet network. *Lancet Infect Dis* 2018;**18**:545–53.

Belliot G, Lavaux A, Souihel D *et al.* Use of murine norovirus as a surrogate to evaluate resistance of human norovirus to disinfectants. *Appl Environ Microb* 2008;**74**:3315–8.

Cannon JL, Papafragkou E, Park GW *et al.* Surrogates for the study of norovirus stability and inactivation in the environment: a comparison of murine norovirus and feline calicivirus. *J Food Protect* 2006;**69**:2761–5.

Duizer E, Bijkerk P, Rockx B *et al.* Inactivation of caliciviruses. *Appl Environ Microb* 2004;**70**:4538–43.

Fittipaldi M, Rodriguez NJP, Codony F *et al.* Discrimination of infectious bacteriophage T4 virus by propidium monoazide real-time PCR. *J Virol Methods* 2010;**168**:228–32.

Hall AJ, Vinjé J, Lopman B *et al.* Updated norovirus outbreak management and disease prevention guidelines. *MMWR Recomm Rep* 2011;**60**:1–18.

Hewitt J, Rivera-Aban M, Greening GE. Evaluation of murine norovirus as a surrogate for human norovirus and hepatitis A virus in heat inactivation studies. *J Appl Microbiol* 2009;**107**:65–71.

Kitagawa Y, Tohya Y, Ike F *et al.* Indirect ELISA and indirect immunofluorescent antibody assay for detecting the antibody against murine norovirus S7 in mice. *Exp Anim* 2010;**59**:47–55.

Kitajima M, Tohya Y, Matsubara K *et al.* Chlorine inactivation of human norovirus, murine norovirus and poliovirus in drinking water. *Lett Appl Microbiol* 2010;**51**:119–21.

Kobayashi Y, Yosizawa S, Kaneko S *et al.* Bactericidal and virucidal effects of chlorous acid water and sodium hypochlorite in a suspension containing organic substances. *Jpn J Infect Prev Contr* 2016;**31**:158–64.

Kroneman A, Vega E, Vennema H *et al.* Proposal for a unified norovirus nomenclature and genotyping. *Arch Virol* 2013;**158**:2059–68.

Nakashima R, Kawamoto M, Miyazaki S *et al.* Evaluation of calcium hydrogen carbonate mesoscopic crystals as a disinfectant for influenza A viruses. *J Vet Med Sci* 2017;**79**:939–42.

Park GW, Linden KG, Sobsey MD. Inactivation of murine norovirus, feline calicivirus and echovirus 12 as surrogates for human norovirus (NoV) and coliphage (F+) MS2 by ultraviolet light (254 nm) and the effect of cell association on UV inactivation. *Lett Appl Microbiol* 2011;**52**:162–7.

Park GW, Sobsey MD. Simultaneous comparison of murine norovirus, feline calicivirus, coliphage MS2, and GII.4 norovirus to evaluate the efficacy of sodium hypochlorite against human norovirus on a fecally soiled stainless steel surface. *Foodborne Pathog Dis* 2011;**8**:1005–10.

Ponrouch A, Frontera C, Bardé F *et al.* Towards a calcium-based rechargeable battery. *Nat Mater* 2016;**15**:169–72.

Reed LJ, Muench H. A simple method of estimating fifty per cent endpoints. *Am J Hyg* 1938;**27**:493–7.

Sakon N, Komano J, Tessmer HL *et al.* High transmissibility of norovirus among infants and school children during the 2016/17 season in Osaka, Japan. *Euro Surveill* 2018;**23**:18–00029.

Takahashi H, Ohuchi A, Miya S *et al.* Effect of food residues on norovirus survival on stainless steel surfaces. *PLoS ONE* 2011;**6**:e21951.

Teunis PFM, Moe CL, Liu P *et al.* Norwalk virus: how infectious is it? *J Med Virol* 2008;**80**:1468–76.

Tung G, Macinga D, Arbogast J *et al.* Efficacy of commonly used disinfectants for inactivation of human noroviruses and their surrogates. *J Food Protect* 2013;**76**:1210–7.