Inactivation effects of calcium hydrogen carbonate mesoscopic crystals on various kinds of animal viruses including foot-and-mouth disease virus.

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Objectives

Virucidal effects of a novel electrically charged disinfectant, CAC-717, on various kinds of viruses were investigated. CAC-717 is an alkaline solution (pH 12.3) and has a self-electromotive force, but it is harmless and not irritant to humans and animals because it contains no chemicals. CAC-717 is produced by applying an electric field to mineral water containing calcium hydrogen carbonate in order to generate mesoscopic crystals (Fig. 1).

Methods

1. Sixteen kinds of animal viruses were used: foot-and-mouth disease virus (FMDV) (A, O and Asia1 types), bovine rhinitis B virus, bovine adenovirus type 7 (BAdV-7), infectious bovine rhinotracheitis virus (IBRV), bovine parainfluenza virus 3, bovine respiratory syncytial virus, bovine viral diarrhea virus (BVDV) (genotypes I and II), equine herpesvirus 1 (EHV-1), equine influenza A virus (H3N8), Aujeszky's disease virus (ADV), swine influenza A virus (pandemic, H1N1), canine parvovirus 2 (CPV-2), canine distemper virus, canine herpesvirus 1, Newcastle disease virus and bulbul orthoreovirus.
2. Nine volumes of CAC-717 were mixed with one volume of virus and incubated for 1 hour at room temperature. CAC-717 was removed by gel filtration on Sephadex LH-20. Virus titers were determined by titration in appropriate cell cultures.
3. Effects of CAC-717 against viral genomes inside virions were examined by quantifying their genomes by real-time PCR.

Results

1. Virus titration analyses showed that CAC-717 treatment achieved reductions of 2.00 $\log_{10}$ to 5.50 $\log_{10}$ and over against all of the viruses tested in this study (Tables 1 & 2).
2. The influence of fetal bovine serum (FBS) as organic matter on the antiviral effect of CAC-717 was examined. In the presence of 20% to 50% FBS, the virucidal effect of CAC-717 was abolished (Fig. 2).
3. Real-time PCR revealed that among the DNA viruses tested, CAC-717 treatment tended to reduce the amounts of IBRV, EHV-1 and BAdV-7 genomes (Table 3). Genomes of RNA viruses except for BVDV I & II were reduced drastically after CAC-717 treatment (Table 4).
4. Direct treatment of CAC-717 to ADV and CPV-2 DNAs and BVDV II RNA, the quantity of which was not reduced after CAC-717 treatment to virions, destroyed their genomes (Table 5).

Conclusion

Virucidal effects of CAC-717 on enveloped DNA and RNA viruses and on non-enveloped DNA and RNA viruses were observed. CAC-717 could inactivate all types of animal viruses including FMDV. However, organic matter influenced the virucidal effect of CAC-717. CAC-717 might act on the inside of most RNA viruses and destroy their RNA genomes. The electrical potential generated on the surface of minerals in CAC-717 and its high pH value (pH 12.3) might play key roles in the anti-viral mechanism of CAC-717. CAC-717 may be used effectively and safely as a disinfectant against various kinds of animal viruses.

Fig.1 . CAC-717: mechanism of high pH, over pH 12, — 4 steps in mesoscopic structured minerals —

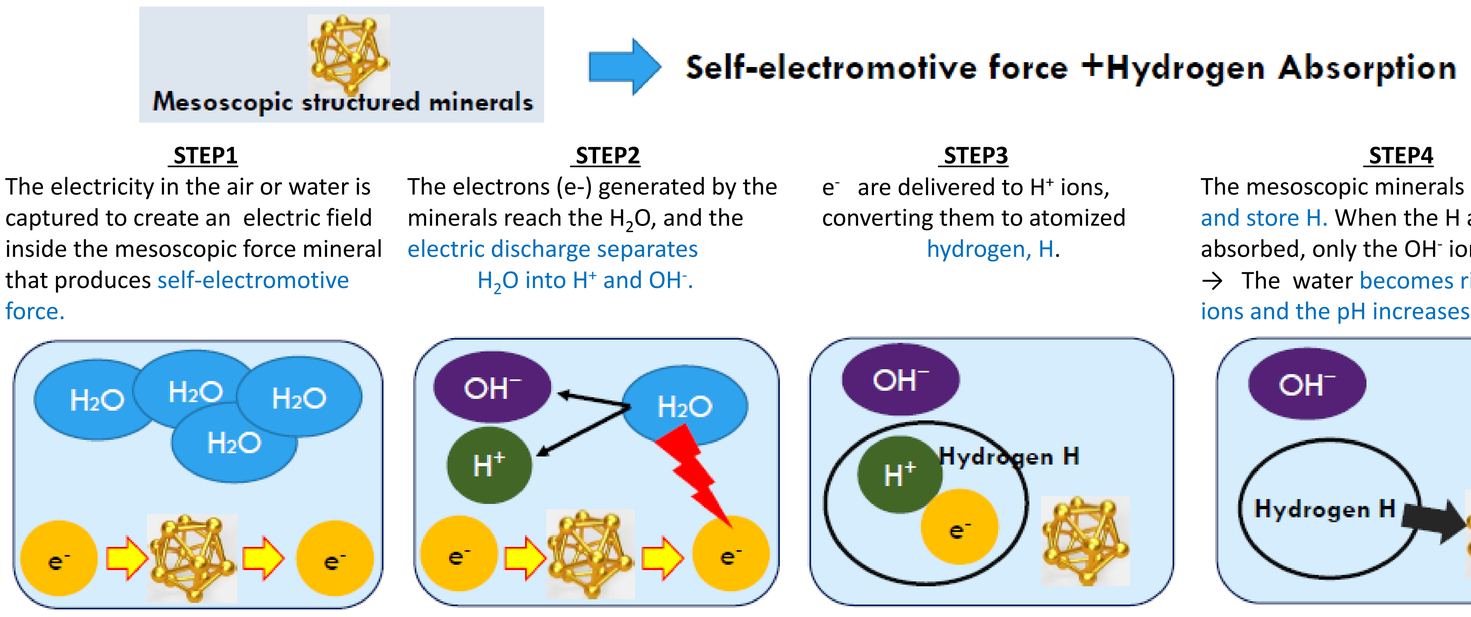

Table 1. Antiviral effect of CAC-717 on various DNA viruses

Viruses	CAC-717 *
Enveloped virus	
IBR virus	≧5.00
Aujeszky's disease virus	≧5.00
Canine herpesvirus 1	≧3.00
Equine herpesvirus 1	≧5.50
Non-enveloped virus	
Bovine adenovirus 7	≧4.50
Canine parvovirus 2	≧3.00

* Difference of log10 index against control viral titer
(Bigger number shows stronger antiviral effect)

Table 2. Antiviral effect of CAC-717 on various RNA viruses

Viruses	CAC-717 *
Enveloped virus	
Bovine RS virus	≧3.00
Bovine PI3	≧4.00
Canine distemper virus	≧2.00
Newcastle disease virus	≧4.00
SIV (Pandemic, H1N1)	≧4.00
EIV (H3N8)	≧2.00
Bovine viral diarrhea virus I	≧2.75
Bovine viral diarrhea virus II	≧3.75
Non-enveloped virus	
FMDV type A	≧4.75
FMDV type O	≧3.50
FMDV type Asia 1	≧4.00
Bovine rhinitis B virus	≧2.75
Bulbul orthoreovirus	≧3.50

* Difference of log10 index against control viral titer
(Bigger number shows stronger antiviral effect)

Fig. 2. Influence of FBS on the antiviral effect of CAC-717

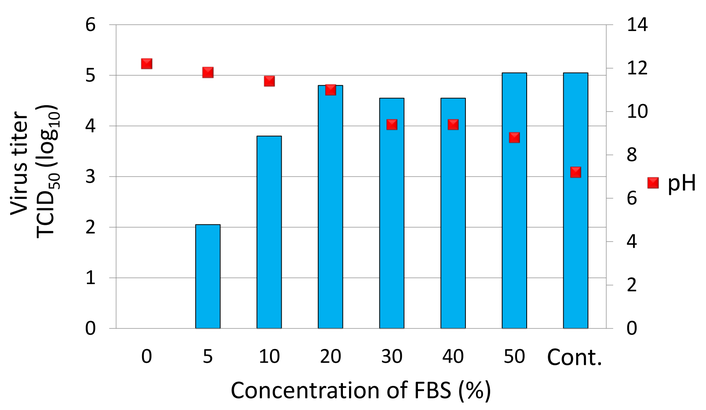

Table 3. Genome quantification of DNA viruses treated with CAC-717

DNA viruses	Solutions	
	CAC-717	Tap water
Enveloped virus		
Aujeszky's disease virus	113.75 *	101.61
Canine herpesvirus 1	95.36	93.95
IBR virus	33.61	89.14
Equine herpesvirus 1	17.10	88.81
Non-enveloped virus		
Canine parvovirus 2	93.82	82.30
Bovine adenovirus 7	37.35	106.43

* Quantity (%) relative to the control quantity after the addition of maintenance medium

Table 4. Genome quantification of RNA viruses treated with CAC-717

RNA viruses	Solutions	
	CAC-717	Tap water
Enveloped virus		
Bovine PI3	2.61 *	62.51
Bovine RS virus	0.00	114.64
Canine distemper virus	0.00	102.48
EIV (H3N8)	2.20	43.94
SIV (Pandemic, H1N1)	7.36	164.48
Newcastle disease virus	12.15	52.44
Bovine viral diarrhea virus I	105.32	182.07
Bovine viral diarrhea virus II	57.02	116.63
Non-enveloped virus		
Bovine rhinitis B virus	0.56	64.60
Bulbul orthoreovirus	0.10	134.13

* Quantity (%) relative to the control quantity after the addition of maintenance medium

Table 5. Direct treatment of CAC-717 to viral genomes, the quantity of which was not reduced after CAC-717 treatment to virions

Viral genomes	CAC-717
Aujeszky's disease virus DNA	0.07 *
Canine parvovirus 2 DNA	0.06
Bovine viral diarrhea virus II RNA	0.03

* Quantity (%) relative to the control quantity after the addition of distilled water