# Evaluation of calcium hydrogen carbonate mesoscopic（CAC-717) crystals as a disinfectant for prions.

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**Introduction.** Prion diseases or transmissible spongiform encephalopathies (TSEs) are fatal neurological disorders that include Creutzfeldt-Jakob disease (CJD) and kuru in humans, scrapie in sheep and goats, bovine spongiform encephalopathy (BSE) in cattle, and chronic wasting disease (CWD) in cervids. Current prion decontamination recommendations include incineration or harsh chemical treatments such as 1-2 N sodium hydroxide, 20~40% household bleach alone or in combination with prolonged autoclaving to treat relevant materials or surfaces. At the moments, safer and more broadly applicable anti-prion reagents are needed. In this study, the novel disinfectant ability of CAC-717; produced by Mineral Activation Technical Research Center, Omuta, Fukuoka 836-0041, Japan, was tested against the 263K strain from intracerebrally inoculated hamsters. The CAC-717 solution abolished prion infectivity completely within 1 h. After treatment on a tissue surface, the solution's pH normalized to almost physiological level via electric discharge.

**Materials and methods.** We continuously applied an electric field to mineral water containing calcium hydrogen carbonate and obtained a new electrically charged material, CAC-717. A Teflon insulation-coated electrostatic field electrode (N-800N, Mineral Activation Technical Research Center, Kumamoto, Japan; Japan Patent No. 5864010) was used to create the electric field, and a voltage of $2 \times 10^4$ V was applied for 48 h. CAC-717 solution in distilled water (Japan Patent No. 5778328) has a pH of 12.39 $\pm$ 0.03, and contains calcium hydrogen carbonate particles (1,120 mg/ℓ) and carbon complex microparticles (50–500 nm) with a mesoscopic structure (Figure 1).

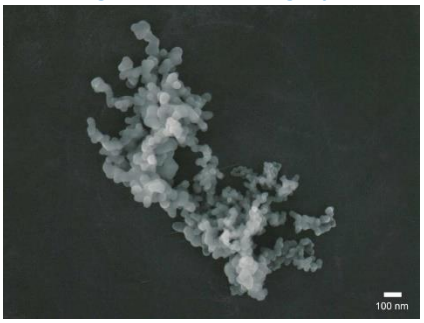
CAC-717 solution was sprayed onto the glass on the hot plate, and dried for 1 min at 100°C. Dried glass was recovered from the hot plate. The CAC-717 powder was coated with a thin layer of platinum in preparation for scanning electron microscopy. The samples were examined with a scanning electron microscope (JSM-7500, JEOL). Scanning electron microscope examination of samples revealed a spheroid mesoscopic structure. However, these tiny structures were not observed in non-electrically charged solutions of calcium hydrogen carbonate.

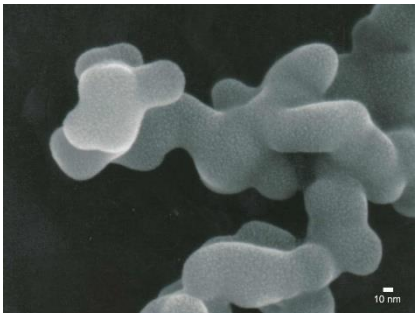
Scrapie strain 263K was isolated from sheep and propagated in hamster brain for 60 days, aliquoted, and then stored at -80 ° C until used or titrated. Prions amount before and after treatment with disinfectants were titrated in sandwich ELISA using monoclonal antibodies. Experiments were repeated in triplicate. Each prion sample was mixed with 9 times the volume of disinfectant. As a negative control normal brain homogenates or recombinant normal cellular prion protein was mixed with 9 times volume distilled water. Prion samples mixed with 9 times volume distilled water were used as another negative control. Mixtures were incubated at room temperature for 1 h. Following incubation, specimens were immediately diluted in ELISA buffer and prion amounts were determined. Western blotting was applied to detect the amount of $PrP^{Sc}$.

To examine the reduction of prion infectivity, the bioassay using a mixture of prion suspensions and CAC-717 solution was measured at 64 and 72-day post-inoculation.

Human skin pH was measured with a skin pH meter (pH905, Courage Khazaka GmbH, Cologne, Germany). Before each measurement, the instrument was calibrated with pH standard solutions (pH 4.01 and 7.00). The pH measurement for CAC-717 solution was tested in Petri dishes. The pH measurement on skin was performed by placing 0.5 mℓ of solution on a hand.

**Figure 1**
**Scanning electron micrograph of Mesoscopic structure of calcium hydrogen carbonate**




***Reference.*** *Nakashima R, Kawamoto M, Miyazaki S, Onishi R, Osaki M, Kirisawa R., Sakudo A, Onodera T. Evaluation of calcium hydrogen carbonate mesoscopic crystals as a disinfectant for influenza A viruses. J. Vet. Med. Sci. 2017 April 10. doi: 10.1292/jvms. 16-0603. [Epub ahead of print]*

**Figure 2**
**Amount of mouse recombinant PrP with or without CAC-717 treatment examined by sandwich ELISA**

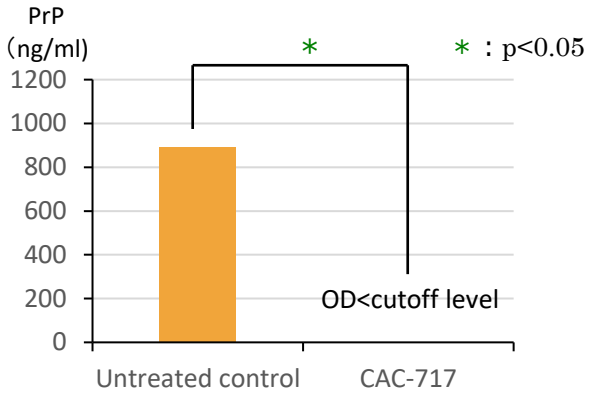



**Figure 3**
**Amount of mouse brain $PrP^{Sc}$ with or without CAC-717 treatment examined by sandwich ELISA**

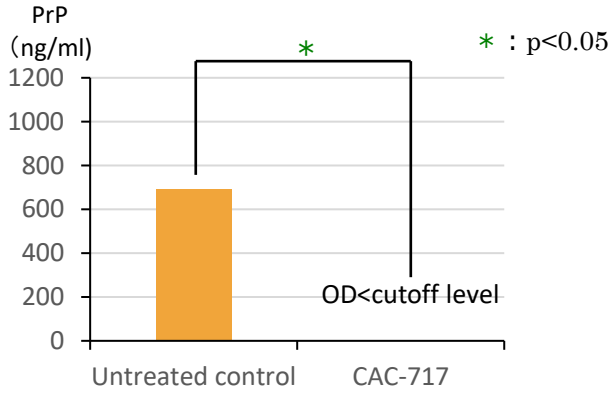
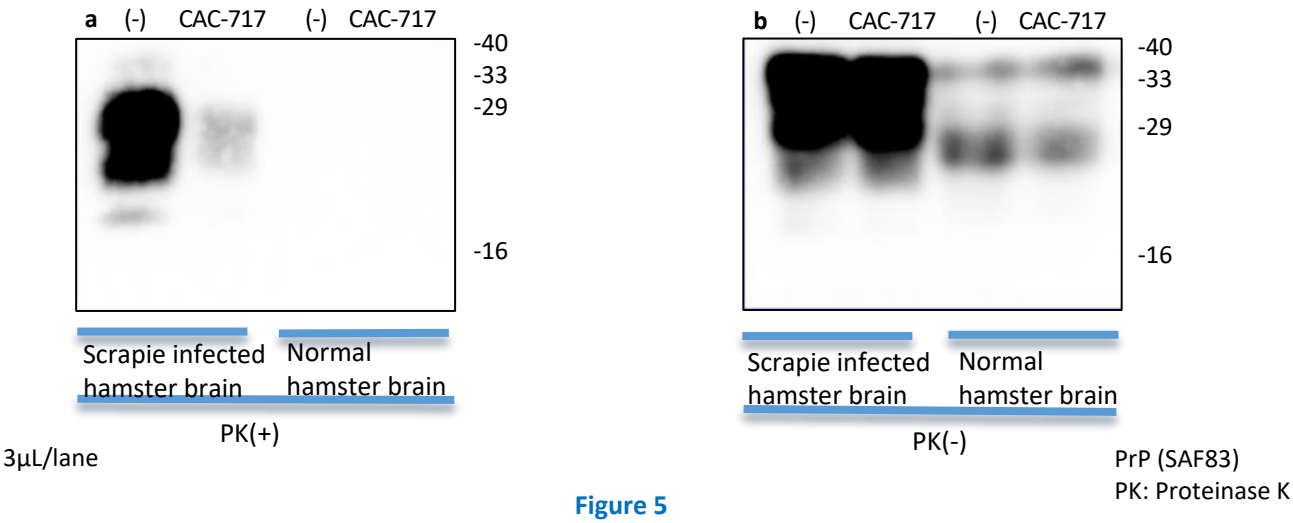


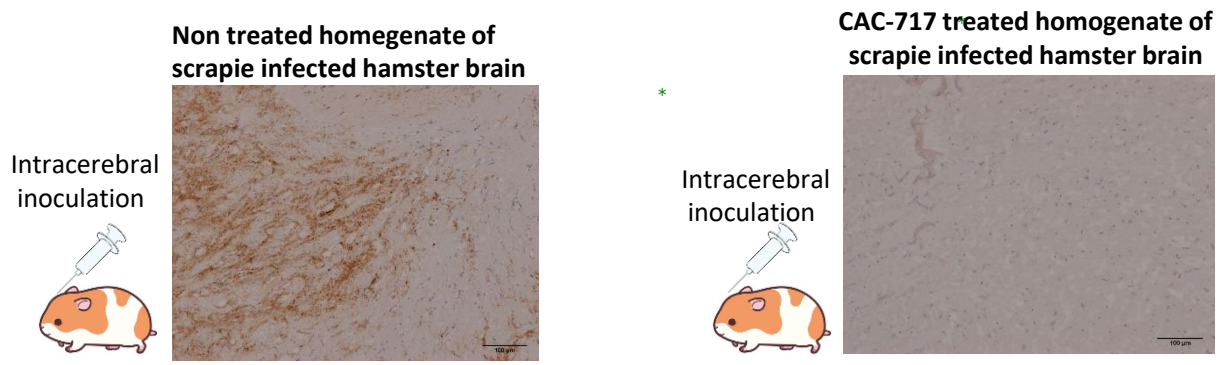

**Figure 4**
**Mouse brain $PrP^{Sc}$ and $PrP^{C}$ with or without CAC-717 treatment examined by western blotting**




3μL/lane

PrP (SAF83)
PK: Proteinase K

**Figure 5**
**CAC-717 treated homogenate of scrapie infected hamster brain**




Microphotograph of diencephalon immunostained with Ms mAb v PrP (3F4, BioLegend)

**Results.** In this study, the anti-prion effect of a novel electrically charged disinfectant, CAC-717 was investigated. CAC-717 is produced by applying an electric field to mineral water containing calcium hydrogen carbonate to generate mesoscopic crystals. Anti-prion (263K) analysis showed reduction of OD to below detectable levels by sandwich ELISA after treatment with CAC-717 for 1 h, while infectivity was also undetectable by immunohistochemistry in intracerebrally inoculated hamster brain (Figures 2 and 3). Western blotting indicated that $PrP^{Sc}$ were destroyed after CAC-717 treatment for 1 h (Figure 4a). However, most of $PrP^{C}$ remained intact after-717 treatment for 1 h (Figure 4b).

All hamsters without CAC-717 treatment died by 64-day post-infection, observed via immunohistochemistry (Figure 5). However, hamster inoculated with the suspension of 263K and CAC-717 did not show any alteration in immunohistochemistry at 72-day post-infection. CAC-717 solution abolished prion infectivity completely within 1 h.

Although CAC-717 is an alkaline solution (pH = 12.39), upon contact with human tissue the pH value normalized to almost physiological levels (pH 8.84) after an accelerated electric discharge, which enables its use as a new disinfectant. We are developing materials to substitute for human tissue in facilitating this accelerated electric discharge. Therefore, CAC-717 may be utilized as a preventative measure against prions as well as for biosecurity in the environment.