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Short communication

Antiviral activity of Ecasol against feline calicivirus, a surrogate of human norovirus

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Summary

Human norovirus (NoV) is a major cause of acute gastroenteritis in closed settings such as hospitals, hotels and cruise ships. The virus survives on inanimate surfaces for extended periods of time, and environmental contamination has been implicated in its transmission. The disinfection of contaminated areas is important in controlling the spread of NoV infections. Neutral solutions of electrochemically activated (ECA)-anolyte have been shown to be powerful disinfectants against a broad range of bacterial pathogens.

The active chemical ingredient is hypochlorous acid (HOCl), which is registered as an approved food contact surface sanitizer in the United States by the Environmental Protection Agency, pursuant to 40 CFR 180.940. We evaluated the antiviral activity of Ecasol (an ECA-anolyte) against feline calicivirus (FCV), a surrogate of NoV. FCV dried on plastic surfaces was exposed to Ecasol for 1, 2, or 5 min. After exposure to Ecasol, the virus titers were compared with untreated controls to determine the virus inactivation efficacy after different contact times. Ecasol was found to decrease the FCV titer by >5 $\log_{10}$ within 1 min of contact, indicating its suitability for inactivation of NoV on surfaces.

Highlights

► ECA-anolyte has been shown to be a powerful disinfectant against a broad range of pathogens. ► Evaluated antiviral activity of Ecasol (an ECA-anolyte) against feline calicivirus (FCV), a surrogate of NoV. ► A >5 $\log_{10}$ reduction in FCV titer was observed within 1 min of application of Ecasol. ► Ecasol is suitable for disinfection of NoV contaminated surfaces.

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Keywords

Ecasol; ECA-anolyte; Trustwater; Electrochemical activation; Norovirus; Feline calicivirus; Fomites

Introduction

In recent years, there have been several outbreaks of acute gastroenteritis, predominantly in closed settings, including institutionalized housing, hotels and cruise ships [1]. Epidemiological investigations have confirmed that >95% of these outbreaks, especially on cruise ships, are caused by human norovirus (NoV) [2]. NoV is a non-enveloped, single-stranded RNA virus belonging to the family *Caliciviridae* and is one of the most common causes of acute gastroenteritis in humans. This virus is shed in high concentrations (up to 11 $\log_{10}$ per gram of feces) and has a low infectious dose of <100 infectious virus particles [3]. Environmental

contamination has been implicated in the transmission of NoV because the virus is able to survive for days to months on different types of surfaces [4].

Cleaning and disinfection of contaminated surfaces are important procedures for controlling outbreaks of NoV in hospital and community settings [4]. Although the use of alcohol-based hand rubs has been promoted to control the spread of infection, alcohol has a limited effectiveness in killing NoV [5]. Various virucides are commonly used to disinfect fomites and environmental contact surfaces implicated in NoV outbreaks. The material safety data sheets and labels for these virucidal compounds rarely allow for their aerosolization, spraying, or fogging due to their toxicity and adverse health effects for given exposure durations and concentrations. Many of these chemical compounds, such as sodium hypochlorite, chlorine gas, and glutaraldehyde, have been associated with occupational illnesses. For example, exposure to glutaraldehyde is associated with contact dermatitis in health workers, and the use of quaternary ammonium compounds has been found to cause occupational asthma in users [6], [7]. For cases in which aerosolization is approved, the use of personal protective equipment and a self-contained breathing apparatus is required, which makes the use of these compounds difficult, especially in public places such as hospitals or schools.

Ecasol is a unique electrochemically activated (ECA)-neutral pH anolyte, which consists of an “activated” solution, produced by a process referred to as dilute brine electrolysis. Based on Faraday's laws of electrolysis, advanced continuous process ECA membrane cell manufacturing was pioneered in the 1970s in the former Soviet Union [8] and was then advanced to its current form by Trustwater (Clonmel, Ireland). Ecasol has been demonstrated as a powerful disinfectant and has been shown to be efficacious against a wide range of microorganisms in solution and when sprayed in the air [9], [10]. Another significant benefit of Ecasol is its lack of toxicity at ready-to-use (RTU) concentrations. It is considered safe in food processing applications by the United States Food and Drug Administration [11]. In dental procedures, Ecasol has been shown to have no adverse effects on human oral tissues [12].

ECA technology involves the generation of electrochemically activated solutions by passing a carefully regulated electric current through a brine solution in specialized electrode compartments and separating the ions according to charge. Ecasol is a positively charged solution emerging from a Trustwater generator. It is a strong oxidizing solution, with a pH of 7.0, a redox potential of +1200 mV, and an active chlorine content of approximately ~700 mg L^{-1}. Hypochlorous acid (HOCL) is the major component of Ecasol, which also contains free radicals and a small amount of sodium chloride (NaCl). As the free radicals gradually lose energy and reform

as water, HOCL dissociates into hydrogen and hypochlorite ions, which eventually revert to NaCl (<0.2%) and water (>99.8%). The water evaporates, leaving salt crystals that can be removed by routine cleaning.

We undertook this study to evaluate the effectiveness of Ecasol for decontaminating surfaces contaminated with NoV. Because NoV is currently non-cultivable in vitro, efficacy tests of disinfectants rely on the use of surrogates, e.g., feline calicivirus (FCV) or murine norovirus (MNV). In this study, we used FCV as a surrogate for NoV.

Materials and methods

Virus

Strain 255 of FCV was propagated in Crandell-Reese Feline Kidney (CRFK) cells, and aliquots of the virus were stored at −80°C until use.

Preparation of ECA anolyte

The Ecasol anolyte solution was prepared on the day of the test using a fully automatic ECA device (Trustwater model AQ-50). This device electrolyzes dilute brine solution using a membrane-type electrolytic cell controlled to produce two metastabilized electrolyte streams in dynamic equilibrium. The catholyte stream (Aversol™ by Trustwater) has a high pH and is classified as an amphoteric surfactant, having reduced surface tension and mild detergent-like properties. Trustwater's automated process uses this solution to maintain the Ecasol stream at a neutral pH. The new Ecasol solution was titrated at 700 ppm free available chlorine (FAC), with a pH of 6.7. The solution was delivered to the lab on the day of the experiment and was used immediately upon delivery, with a time from solution generation to lab experimentation of approximately 2 h. The stability of Ecasol depends upon storage conditions because it can lose up to 10% of its activity within 3 weeks of generation if it is not stored properly.

Surface decontamination test

Two concentrations of Ecasol, 150 ppm and 500 ppm FAC, were prepared by diluting the solution with deionized water. These testing concentrations were selected because 150 ppm is the most commonly used concentration for food contact surface sanitization, based

on the recommendation of 40CFR 180.940, and the concentration of 500ppm was selected because Ecasol is a known sporocidal at this concentration.

The test was performed in 6-well tissue culture plates, and the experiments were conducted in triplicate. The six wells of the plate were labeled A through F, and the FCV suspension was uniformly applied to the bottom of the six wells at 100μL/well. The inoculum was allowed to dry for 30min at room temperature (approximately 23°C) in a type II biosafety cabinet. After the inoculum dried, the Ecasol solution was added to wells A–C at 5mL/well. Wells D–F served as controls for each treated well (well D for well A, and so on), with 5mL of phosphate buffered saline (PBS) per well. The plate was incubated at room temperature on an orbital shaker (at 120rpm) for different time periods (1, 2, and 5min for wells A and D, B and E, and C and F, respectively). After the appropriate contact times, the well contents were immediately diluted 10-fold using a maintenance medium to stop Ecasol activity at the indicated times. Serial 10-fold dilutions of these eluates were prepared in Eagle's MEM, followed by inoculation of CRFK cells grown in 96-well microtiter plates, using four wells for each test dilution. The inoculated plates were incubated at 37°C and examined daily for 4 days by microscope for FCV-induced cytopathic effects (CPE). The virus titers were calculated by the Reed and Muench method [13], and the log reductions were calculated by comparing the titers of the Ecasol-treated wells with those of the PBS-treated control wells. To determine the cytotoxicity of the Ecasol solution to the CRFK cells, 10-fold serial dilutions of Ecasol prepared in Eagle's MEM were added to monolayers of CRFK cells prepared in a 96-well plate (4 wells/dilution). Each concentration of Ecasol (150ppm and 500ppm) was tested in separate experiments, and each experiment was performed in triplicate.

Results and discussion

In this study, we evaluated the effectiveness of Ecasol solution for the disinfection of a plastic surface contaminated with FCV. To the best of our knowledge, this is the first study of the use of Ecasol for the disinfection of surfaces contaminated with a NoV surrogate. The results indicate that Ecasol at 150ppm and 500ppm inactivated >5$\log_{10}$ of FCV (>99.999% reduction in virus titer) within 1min at room temperature (Table 1). There was no additional reduction in virus titer when the contact time was increased from 1min to 5min.

Table 1. Inactivation of feline calicivirus by Ecasol.[a]

Ecasol concentration (ppm)	Time (min)	Virus titer ($TCID_{50}$/0.1 mL)[b]		Percent virus reduction
		Control	Test	
150	1	$10^{7.0}$	$10^{1.5}$	99.9996
	2	$10^{6.75}$	$10^{1.5}$	99.9994
	5	$10^{6.75}$	$10^{1.5}$	99.9994
500	1	$10^{6.875}$	$10^{1.5}$	99.9995
	2	$10^{6.625}$	$10^{1.5}$	99.9992
	5	$10^{6.75}$	$10^{1.5}$	99.9994

a The results are an average of three different experiments.

b Control, virus treated with PBS; test, virus treated with Ecasol.

NoV is a major cause of acute gastroenteritis worldwide because of its low infective dose and its ability to survive on inert objects for extended periods of time. The virus can be transferred from contaminated surfaces to other inert objects, including faucets, door handles, and telephones. It is important to implement proper cleaning and disinfecting procedures that eliminate virus particles from contaminated surfaces. However, NoV cannot be cultured in a laboratory setting, which hampers research on the development of prevention and control strategies. To overcome this problem, FCV has widely been used as a surrogate for NoV because its physicochemical and genetic properties are similar to those of NoV [14]. FCV is a respiratory virus in felines and is susceptible to high temperatures [15]. Genogroup V murine NoV (MNV) has recently been advanced as a surrogate for NoV because it is morphologically and genetically similar to NoV and can be propagated in cell cultures [16].

Many studies have reported on various compounds used for the inactivation of FCV, including acids and alcohols [17], ozone gas [18], H_2O_2 vapors, and chlorine dioxide gas (ClO_2) [19]. Whitehead and McCue [17] showed that bleach and acid-based disinfectants could inactivate FCV within 1 min (>4$\log_{10}$ reduction). The use of ClO_2 has been shown to reduce FCV titers by >3$\log_{10}$ within 10 h [19], and ozone can inactivate FCV in less than 1 h [18]. Some of these compounds are toxic, energy intensive, and expensive and require an extended time for virus inactivation. Ecasol is non-toxic, non-corrosive, relatively inexpensive to produce, and biodegradable. After Ecasol is used, only water (>99.8%) and a small amount of salt crystals remain (NaCl, <0.2%). The amount of NaCl crystals present is too low to corrode metal surfaces and can be easily removed with water. Thus, we recommend Ecasol for disinfecting surfaces contaminated with NoV. Further studies are in progress on the use of Ecasol for a number of delivery methods, including direct application and fogging, as a disinfecting agent against additional viruses and bacteria.

Conflict of interest statement

Funding: This study was funded in part by Johnson Diversified Products, MN.

Competing interests: YC, SMG, and RJR have no competing interests. ECASOL is a registered trademark of Trustwater (Clonmel, Ireland). TJ is a distributor of Ecasol in the U.S.

Ethical approval: Not required.

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