Inactivation of Human and Simian Rotaviruses by Chlorine Dioxide

YU-SHIAW CHEN1* and JAMES M. VAUGHN2

Department of Applied Science, Brookhaven National Laboratory, Upton, New York 11973,1 and Department of Microbiology, University of New England College of Medicine, Biddeford, Maine 040052

Received 21 November 1989/Accepted 16 February 1990

The inactivation of single-particle stocks of human (type 2, Wa) and simian (SA-11) rotaviruses by chlorine dioxide was investigated. Experiments were conducted at 4°C in a standard phosphate-carbonate buffer. Both virus types were rapidly inactivated, within 20 s under alkaline conditions, when chlorine dioxide concentrations ranging from 0.05 to 0.2 mg/liter were used. Similar reductions of 106-fold in infectivity required additional exposure time of 120 s at 0.2 mg/liter for Wa and 0.5 mg/liter for SA-11, respectively, at pH 6.0. The inactivation of both virus types was moderate at neutral pH, and the sensitivities to chlorine dioxide were similar. The observed enhancement of virucidal efficiency with increasing pH was contrary to earlier findings with chlorine- and ozone-treated rotavirus particles, where efficiencies decreased with increasing alkalinity. Comparison of 99.9% virus inactivation times revealed ozone to be the most effective virucidal agent among these three disinfectants.

Human rotaviruses (HRV), members of the Reoviridae family of RNA viruses (11), are responsible for many of the reported cases of acute epidemic or endemic diarrhea affecting both children and adults (4, 12, 21, 23). The increasing number of reports associating rotaviruses with new clinical situations (5, 6, 8–10, 22) emphasizes the need to understand their epidemiology and transmission. Because these organisms may be disseminated through aquatic environments, it is important to examine the effectiveness of various modes of water disinfection on the inactivation of rotaviruses.

Traditionally, laboratory-based studies of water disinfectant efficacy have centered on the commonly used disinfectants, such as chlorine and ozone. More recent studies have addressed the inactivation potential of additional agents, including chlorine dioxide. This agent has been shown to be an effective bactericide (2, 14) and sporicide (13), as well as a potent virucide (1, 15). In addition, the use of chlorine dioxide as a water disinfectant does not result in the production of trihalomethanes (16), a problem associated with chlorine treatment of drinking water. Scarpino et al. (15) reported that chlorine dioxide inactivated poliovirus type 1 and enteroviruses more efficiently at pH 9.0 than at neutral or acidic levels. A similar pH effect was demonstrated in other experiments with poliovirus (1) and simian rotavirus SA-11 (3). To date, the only documented evidence for the inactivation of human rotavirus by chlorine dioxide was that reported by Harakeh and Butler (7). In these experiments, human rotavirus suspended in wastewater effluent was found to be somewhat less sensitive to treatment by chlorine, chlorine dioxide, ozone, and peracetic acid than the simian strain.

In the present study, the inactivation of purified, single-particle suspensions of simian (SA-11) and HRV by chlorine dioxide were compared over a range of disinfectant concentrations and pH levels. Resulting data were then compared with those from previous investigations of rotavirus inactivation by the more traditional agents, chlorine and ozone (19, 20).

*Corresponding author.
†Present address: Department of Microbiology, School of Medicine, SUNY at Stony Brook, Stony Brook, NY 11794.

MATERIALS AND METHODS

Simian rotavirus SA-11 obtained from Charles Gerba, University of Arizona, Tucson, and HRV type 2 (Wa), purchased from Biotech Research Laboratories, Rockville, Md., were used in all studies. Host cell cultures (MA-104) were purchased from Microbiological Associates, Walkersville, Md. Virus propagation, purification, and assay were carried out as previously described (19, 20). Chlorine dioxide stock solutions were prepared in a chlorine-demand-free phosphate-carbonate buffer according to the method of Benarde et al. (2), with fresh solutions (approximate concentration, 200 mg/liter) stored at 4°C in air-tight dark glass bottles for periods of up to 2 weeks. The concentration of chlorine dioxide was determined by the method of Roller et al. (14), with A267 measured in a dual-beamed spectrophotometer (model Acta III; Beckman Instruments, Inc., Fullerton, Calif.).

Prior to each experiment, chlorine dioxide stock solution was diluted to the desired concentration with chlorine-demand-free buffer. One-hundred-milliliter volumes of chlorine dioxide containing buffer were then inoculated with 1 ml of dialyzed single-particle virus stock (~107 PFU/ml) and gently mixed on a magnetic stirrer. Samples (10 ml each) were collected at intervals and placed in test tubes containing 0.1 ml of 0.5 M sodium thiouiscite to terminate the reaction. All samples were then treated with 0.5 ml of chloroform for 10 min to eliminate microbial contamination, diluted in Tris-buffered saline, and assayed as previously described (19).

To verify that host cells were both virus susceptible and contaminant free, positive and negative rotavirus controls were included in each experiment. Each experiment was repeated several times (usually two to three) to assure consistency of the results. Data were statistically analyzed according to the methods described by Sokal and Rohlf (17) and Steel and Torrie (18). Statistical analyses and graphics were performed on a Macintosh SE computer with preprogrammed statistical software.

RESULTS

The concentrations of chlorine dioxide working stocks maintained at 4°C were stable for 10 min. The dissipation of