Inactivation of human adenovirus by sequential disinfection with an alternative UV technology and free chlorine

Jung-Keun Lee and Gwy-Am Shin

ABSTRACT

There has been growing concern over human exposure to adenoviruses through drinking water due to the extreme resistance of human adenoviruses to the traditional UV technology (low-pressure (LP) UV). As an effort to develop an effective treatment strategy against human adenoviruses in drinking water, we determined the effectiveness of sequential disinfection with an alternative UV technology (medium-pressure (MP) UV) and free chlorine. Human adenovirus 2 (Ad2) was irradiated with a low dose of MP UV irradiation (10 mJ/cm^2) through UV collimated apparatus and then exposed to a low dose of free chlorine (0.17 mg/L) at pH 8 and 5°C using a bench-scale chemical disinfection system. A significant inactivation (e.g. 4 $\log_{10}$) of Ad2 was achieved with the low doses of MP UV and free chlorine within a very short contact time (~1.5 min) although there was no apparent synergistic effect on Ad2 between MP UV and free chlorine. Overall, it is likely that the sequential disinfection with UV irradiation and free chlorine should control the contamination of drinking water by human adenoviruses within practical doses of UV and free chlorine typically used in drinking water treatment processes.

Key words | Drinking water, free chlorine, human adenoviruses, low-pressure UV, medium-pressure UV, UV disinfection

Jung-Keun Lee
Department of Pathobiology and Population Medicine,
Mississippi State University,
240 Wise Center Drive,
PO Box 6100,
Mississippi State, MS 39762,
USA

Gwy-Am Shin (corresponding author)
Department of Environmental and Occupational Health Sciences,
University of Washington,
4225 Roosevelt Way NE,
Suite 100,
Seattle, WA 98105,
USA.
Telephone: (206) 543-9026.
Fax: (206) 543-8123
E-mail: *gwyam@u.washington.edu*

INTRODUCTION

There is a growing interest in ultraviolet (UV) irradiation in drinking water treatment processes due to its remarkable effectiveness against highly chlorine-resistant protozoan parasites and minimal production of disinfection byproducts. First, UV irradiation is remarkably effective against highly chlorine-resistant *Cryptosporidium parvum* oocysts and *Giardia lamblia* cysts (Clancy *et al.* 2000; Craik *et al.* 2000; Shin *et al.* 2001; Linden *et al.* 2002). In addition, it has been shown that UV irradiation does not produce appreciable quantities of harmful disinfection byproducts (Oppenheimer *et al.* 1997). Furthermore, recent technological improvements–such as improved reactor design and advances in lamp technology–make UV technology increasingly cost-competitive and efficient. In fact, UV irradiation is one of the most promising candidate technologies for compliance with recent United States (US) federal regulations – the Long Term 2 Enhanced Surface Water Treatment Rule (LT2ESWTR) and Stage 2 Disinfectants and Disinfection Byproduct Rule (D/DBPR).

However, there are still several critical issues that are slowing down widespread adoption of UV irradiation as a primary disinfectant in drinking water treatment processes. One of the issues is the presence of waterborne pathogens that are considerably resistant to UV irradiation, especially some important human enteric viruses such as adenoviruses (Meng & Gerba 1996; Gerba *et al.* 2002; Nwachuku *et al.* 2005). According to the recent US drinking water regulation

doi: 10.2166/wh.2010.044

(LT2ESWTR), the UV dose to achieve a 4 $\log_{10}$ inactivation of adenoviruses is 186 mJ/cm^2, which is at least an order of magnitude higher than the UV doses for other health-related microorganisms. In fact, it is reportedly impossible to achieve reasonable inactivation of these adenoviruses within a practical range of UV dose (~40 mJ/cm^2) in drinking water treatment processes.

As one of the effective treatment strategies against human adenoviruses in drinking water, sequential disinfection with UV irradiation and chemical disinfectants has been suggested because some chemical disinfectants are relatively effective against many human enteric viruses. In fact, there is one previous study on sequential disinfection with UV irradiation and free chlorine on human adenoviruses (Ballester & Malley 2004). However, the previous sequential disinfection study was performed with traditional UV technology (low-pressure (LP) UV) and nothing is known about the effectiveness of sequential disinfection with alternative UV technologies such as medium-pressure (MP) UV or pulsed UV that have shown an enhanced effectiveness against human adenoviruses (Linden *et al.* 2007; Eischeid *et al.* 2009; Shin *et al.* 2009). In fact, these recent studies indicated that alternative UV technologies are 2–3 times more effective (in terms of $\log_{10}$ inactivation) against human adenoviruses than traditional UV technology in the UV dose ranges typically used in water and wastewater treatment processes.

In addition to the enhanced inactivation by alternative UV technologies, it is speculated that there is some possibility of synergism between alternative UV technologies and chemical disinfectants. Compared to the traditional UV technology (LP UV) that generates a single wavelength at 254 nm, alternative UV technologies (MP and pulsed UV) generate a wider range of wavelengths (~100–1,000 nm). Some of these wavelengths may cause some additional damage not only to DNA (the primary target of LP UV) but also to various proteins (Friedberg *et al.* 1995), which would make UV-irradiated microorganisms more susceptible to subsequent chemical disinfection. Therefore, the primary purpose of this study is to determine the effectiveness of sequential disinfection with an alternative UV technology (MP UV) and free chlorine against human adenoviruses in drinking water treatment processes and investigate the possibility of synergism between the alternative UV technology (MP UV) and free chlorine against these important human pathogens.

METHODS

Microorganism

Adenovirus 2 (Ad2) (ATCC #: VR-846) was propagated in A549 cells (ATCC #: CC-185) grown in F-12 media containing 2% fetal bovine serum. Ad2 was inoculated and cultivated on confluent A549 cells for 7–10 days until the observed cytopathic effect (CPE) on the monolayers was ~95%. Infected cells were processed through three successive freeze/thaw procedures to make cell lysates. Then, viruses were extracted by homogenizing in an equal volume of chloroform. The supernatant was recovered following low speed (5,000 × *g*) centrifugation for 15 min at 4°C. Ad2 was assayed by 50% Tissue Culture Infectious Dose ($TCID_{50}$) method on confluent layers of A549 cells grown in 24-well tissue culture plates. The infectivity of Ad2 was determined by observing CPE on the confluent A549 cells for 14 days after inoculation of disinfected samples and controls.

LP UV irradiation system and radiometry

The bench-scale, LP UV collimated beam apparatus consisted of two 15-Watt germicidal lamps (Model XX-15 G; Spectroline, Westbury, NY, USA) that produce near monochromatic UV irradiation at 254 nm. The radiation was quasi-collimated through a circular opening to provide incident radiation normal to the surface of the test suspension in a 60 × 15 mm cell culture petri dish. UV irradiance was measured with a calibrated International Light IL1700 radiometer (International Light Inc., Newburyport, MA, USA) at a wavelength of 254 nm. The delivered UV dose, accounting for the depth of the suspension (0.255 cm), was calculated based on the measurement of the irradiance incident on the petri dish, a series of correction factors (petri factor (1.001), reflection factor (0.975), water factor (variable (depending on the absorbance of the sample at 254 nm)), and divergence factor (0.9826)) as described by Bolton & Linden (2003), and the exposure time in seconds.

MP UV irradiation system and radiometry

The bench-scale, MP UV collimated beam apparatus consisted of a 400-Watt medium pressure UV lamp

(Model 7825 Immersion Lamp; Hanovia Ltd., Slough, UK) mounted on a housing above a quasi-collimating cylinder. Germicidal UV irradiance emitted from the broadband MP UV lamp was measured with the International Light IL1700 radiometer mentioned earlier. The dose was weighted by the DNA absorbance spectrum according to a spreadsheet developed by Dr James Bolton (Bolton 2002). As for LP UV, the delivered UV dose, accounting for the depth of the suspension (0.255 cm), was calculated based on the measurement of the irradiance incident on the petri dish, a series of correction factors (petri factor (0.9314), reflection factor (0.975), water factor (variable (depending on the absorbance of the sample between 200 and 299 nm)), divergence factor (0.9826), sensor factor (1.206), and germicidal factor (constant values for wavelengths between 200–299 nm based on the absorbance spectrum of DNA from *Bacillus subtilis*)) as described by Bolton & Linden (2003), and the exposure time in seconds.

Experimental protocol of UV disinfection experiments

UV disinfection experiments were performed as previously described (Shin *et al.* 2001). Briefly, Ad2 was diluted in PBS (137 mM NaCl, 2.7 mM KCl, 4.3 mM $Na_2HPO_47H_20$, and 1.4 mM KH_2PO_4, pH 7.3) to give a final concentration of $\sim 10^7$ $TCID_{50}$/mL. Aliquots of 5 mL each in 60×15 mm cell culture petri dishes were irradiated with the aforementioned collimated beam type UV sources while stirring the samples slowly on a magnetic stir plate. After the predetermined exposure time, samples were removed from the UV irradiation systems and a small portion of the samples was subjected to subsequent free chlorine disinfection.

Free chlorine solutions and measurement of free chlorine residuals

Household bleach (5.25% sodium hypochlorite, Clorox Company, Oakland, CA, USA) was diluted in chlorine-demand-free (CDF) water to prepare a 100 mg Cl_2/mL stock solution. Working solutions of free chlorine at different concentrations were prepared in a 0.01 M phosphate buffer solution on the days of disinfection experiments. Chlorine residuals were measured according to the N,N-diethyl-p-phenylenediamine (DPD) colorimetric method (American Public Health Association 1995).

Experimental protocol of chemical disinfection experiments

Chemical disinfection experiments were done in a bench scale, batch system using CDF glass test tubes placed in a water bath set at 5°C as previously described (Sobsey *et al.* 1988). Briefly, 1 mL of UV-irradiated Ad2 was added to the virus control tube containing 9 mL of phosphate buffer solution (pH 8) (virus control tube) and 3 mL of UV-irradiated Ad2 was added to the reaction tube containing 27 mL of free chlorine working solutions (pH 8) (reaction tube), and each tube was quickly vortexed. Samples of 0.6 mL were taken from the reaction tube at various time points and were immediately quenched of their free chlorine by an equal volume of 0.1% (w/v) sterile sodium thiosulfate. Samples of 0.6 mL from the virus control tube were withdrawn at the beginning and end of the experiments and similarly mixed with an equal volume of 0.1% sterile sodium thiosulfate. Samples from the reaction tube and virus control tube were diluted serially 10-fold in PBS for subsequent virus assay. At the end of the experiment, the residuals of free chlorine in the reaction tube and the disinfectant control tube were measured by the DPD colorimetric method (American Public Health Association 1995). There was no significant change in the free chlorine concentration during the experiments (the average initial concentration (0.17 mg/L) vs. the average final concentration (0.15 mg/L)) and the average of the initial and final concentration (0.16 mg/L) was used in the Ct calculation.

Data presentation and statistical analysis

For each experiment, the concentrations of Ad2 in the control sample were computed and taken as N_o, the initial Ad2 concentrations. For each test dose, the average concentrations of Ad2 were computed as N_d. The proportion of initial Ad2 remaining at each test dose (d) was computed by dividing the Ad2 concentration at each test dose (N_d) by the initial Ad2 concentration (N_o). These values were then $\log_{10}$-transformed ($\log_{10}$ (N_d/N_o)), and the values of replicate

experiments were averaged. These mean data for $\log_{10}$ (N_d/N_o) were then paired with Ct values (free chlorine concentration × exposure time) (mg/L*min) and plotted.

Statistical analysis on inactivation kinetics was performed using SAS (SAS Institute Inc., Cary, NC, USA) and EXCEL (Microsoft, Redmond, WA, USA). Linear regression analysis was used to calculate the Ct values to achieve 2–4 $\log_{10}$ inactivation with EXCEL and analysis of covariance (ANCOVA) was applied to compare the inactivation of Ad2 (slope difference) with SAS general linear model (GLM) procedure.

RESULTS AND DISCUSSION

Figure 1 shows the inactivation kinetics of Ad2 by free chlorine only (control) and sequential disinfection with LP UV and free chlorine at pH 8 at 5°C in phosphate buffer based on four replicate experiments. The sequential disinfection with LP UV and free chlorine was included in this study for comparison with the one with MP UV and free chlorine, and the treatment conditions selected was a simulation of a worst-case scenario in UV and free chlorine disinfection of drinking water treatment processes. The inactivation of Ad2 by a dose of 20 mJ/cm^2 of LP UV (the initial inactivation of Ad2 in the sequential disinfection with LP UV and free chlorine) was ~0.7 $\log_{10}$, which is similar to the ones in previous studies (Gerba *et al.* 2002; Shin *et al.* 2009). The inactivation of Ad2 by free chlorine (with prior LP UV exposure) was very fast and the Ct values to achieve 99% reduction (Ct_{99}) of Ad2 by free chlorine was 0.11 mg/L*min. However, there was no statistical difference (slope difference) between the inactivation rate of Ad2 by free chlorine with and without prior LP UV exposure (ANCOVA, p value = 0.66).

Figure 2 shows the inactivation kinetics of Ad2 by free chlorine only (control) and sequential disinfection with MP UV and free chlorine at pH 8 at 5°C in phosphate buffer based on four replicate experiments. In order to start with the same baseline for free chlorine disinfection, a lower dose (10 mJ/cm^2) was selected for MP UV because this dose of MP UV gave similar inactivation of Ad2 with 20 mJ/cm^2 of LP UV in a previous study (Shin *et al.* 2009). Again, the inactivation of Ad2 by free chlorine (with prior LP UV exposure) was very fast and Ct_{99} of Ad2 by free chlorine was 0.12 mg/L*min, but there was no statistical difference between the inactivation rate of Ad2 by free chlorine with or without prior MP UV exposure (ANCOVA, p value = 0.77).

The results of this study indicate that sequential disinfection with UV (both LP and MP) and free chlorine is very effective against Ad2. Although a previous study (Ballester & Malley 2004) reported a significant inactivation of Ad2 by LP UV and free chlorine, that study was conducted with a relatively high UV dose (40 mJ/cm^2), a high free chlorine concentration (3.32 mg/L), and also at high temperatures (22–23°C). In this study, we tried to simulate a worst-case scenario in UV and free chlorine disinfection of drinking water treatment processes, so a lower dose (20 mJ/cm^2) of LP UV and a lot lower free chlorine concentration (0.17 mg/L) were tried at a very low temperature (5°C). Nonetheless, there was a significant inactivation (e.g. 4 $\log_{10}$) of Ad2 within a very short

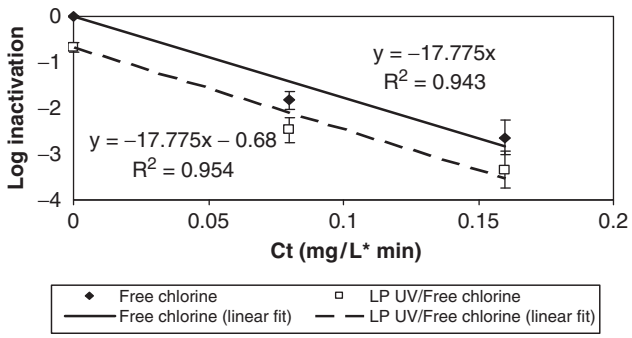

Figure 1 | Inactivation of adenovirus 2 by free chlorine only (control) and sequential disinfection with low-pressure UV and free chlorine in phosphate buffer at pH 8 and 5 °C (N = 4).

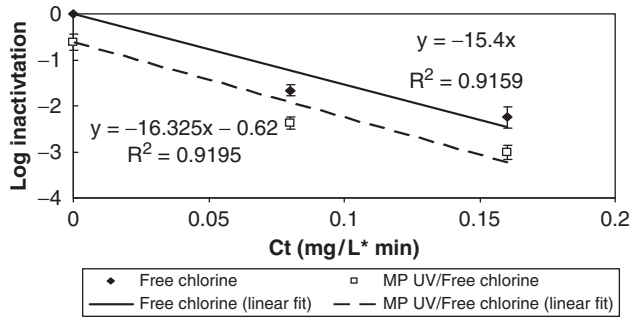

Figure 2 | Inactivation of adenovirus 2 by free chlorine only (control) and sequential disinfection with medium-pressure UV and free chlorine in phosphate buffer at pH 8 and 5 °C (N = 4).

contact time (~1.5 min) under the conditions tested. Also, it should be noted that the same level of inactivation was achieved with an even lower UV dose (10 mJ/cm^2) of MP UV. Even with the enhanced inactivation of human adenoviruses by alternative UV technologies, it is amazing that a significant inactivation (e.g. 4 $\log_{10}$) of Ad2 could be achieved with very low doses of MP UV (10 mJ/cm^2) and free chlorine (0.17 mg/L) within a very short contact time (~1.5 min) at a very low temperature (5°C).

Finally, the results of this study show that UV irradiation (both LP and MP UV) and free chlorine appear to have only an additive, not synergistic, effect on Ad2. That is, the inactivation of Ad2 by free chlorine with or without prior UV exposure (both LP and MP UV) were statistically not significantly different (p value = 0.66 and 0.77, respectively). Although there is no study on sequential disinfection with MP UV and free chlorine, there are some studies on sequential disinfection with LP UV and chlorine species (free chlorine and combined chlorine) (Cho *et al.* 2006; Baxter *et al.* 2007). The results of these studies showed that there is no synergism between LP UV and chlorine species possibly due to the different inactivation mechanism of LP UV and chlorine species. Even with the additional damages caused by alternative UV technologies, there appears to be no apparent synergistic effect between an alternative UV technology (MP UV) and free chlorine enough to alter the overall inactivation of Ad2 by MP UV and free chlorine. To our knowledge, this is the first study to address the presence/absence of synergism between an alternative UV technology (MP UV) and free chlorine against human adenoviruses.

CONCLUSION

In this study, we determined the effectiveness of sequential disinfection with an alternative UV technology (MP UV) and free chlorine against human adenoviruses in drinking water treatment processes and investigated the possibility of synergism between the alternative UV technology (MP UV) and free chlorine against these important human pathogens. The results of this study indicate that sequential disinfection with MP UV and free chlorine is very effective against Ad2 although there appears to be no apparent synergistic effect between MP UV and free chlorine. Overall, the results of this study suggest that the sequential disinfection with UV irradiation and free chlorine should control the contamination of drinking water by human adenoviruses within practical doses of UV and free chlorine typically used in drinking water treatment processes.

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First received 17 March 2010; accepted in revised form 20 May 2010. Available online 1 October 2010