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UV Disinfection of *Giardia lamblia* Cysts in Water

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The human and animal pathogen *Giardia lamblia* is a waterborne risk to public health because the cysts are ubiquitous and persistent in water and wastewater, not completely removed by physical-chemical treatment processes, and relatively resistant to chemical disinfection. Given the recently recognized efficacy of UV irradiation against *Cryptosporidium parvum* oocysts, the inactivation of *G. lamblia* cysts in buffered saline water at pH 7.3 and room temperature by near monochromatic (254 nm) UV irradiation from low-pressure mercury vapor lamps was determined using a "collimated beam" exposure system. Reduction of *G. lamblia* infectivity for gerbils was very rapid and extensive, reaching a detection limit of >4 log within a dose of 10 JM^{-2}. The ability of UV-irradiated *G. lamblia* cysts to repair UV-induced damage following typical drinking water and wastewater doses of 160 and 400 JM^{-2} was also investigated using experimental protocols typical for bacterial and eucaryotic DNA repair under both light and dark conditions. The infectivity reduction of *G. lamblia* cysts at these UV doses remained unchanged after exposure to repair conditions. Therefore, no phenotypic evidence of either light or dark repair of DNA damage caused by LP UV irradiation of cysts was observed at the UV doses tested. We conclude that UV disinfection at practical doses achieves appreciable (much greater than 4 log) inactivation of *G. lamblia* cysts in water with no evidence of DNA repair leading to infectivity reactivation.

Introduction

Giardia lamblia is one of the most important health-related waterborne pathogens. It has been associated with numerous waterborne outbreaks over the last several decades, many of which were associated with municipal drinking water supplies which had met regulatory standards for turbidity and coliforms (*1*). Cysts of *G. lamblia* are consistently present at high concentrations (10^2−10^4/L) in wastewater (*2*), highly prevalent in surface source waters (*3*), and relatively resistant to conventional water and wastewater treatment processes, including chemical disinfection (*4*). A properly operated conventional filtration system achieves some physical removal of this microorganism cyst (about 99−99.9%), but most chemical disinfection processes achieve of only modest inactivation of *G. lamblia* at practical doses and contact times (*4*−*6*). On the basis of the in vitro viability assays of excystation and vital dye staining, Rice and Hoff (*7*) reported that *G. lamblia* cysts are also very resistant to UV irradiation. Craik et al. (*8*), using an in vivo mouse animal infectivity assay, reported that polychromatic UV radiation from medium-pressure (MP) UV mercury lamps extensively inactivates *Giardia muris* cysts at relatively low UV doses. However, the majority of drinking water and wastewater treatment plants worldwide now practicing UV disinfection, employ monochromatic low-pressure (LP) UV lamps for irradiation, not polychromatic MP UV lamps. Furthermore, *G. muris* is not a human pathogen, and significant physiological differences exist between *G. lamblia* and *G. muris*. Therefore, the extent of UV inactivation of the human pathogen *G. lamblia* as measured by an animal infectivity assay has not been reported.

In addition to a need for evaluating the effect of UV irradiation on *G. lamblia* infectivity, the capability of this parasite to repair UV-mediated DNA damage and restore infectivity also requires investigation. Knowledge of DNA repair capability will aid in proper design of UV disinfection systems to achieve protozoan inactivation. Many organisms have the ability to repair DNA lesions caused by either exogenous (UV light, ionizing radiation, and environmental chemicals) or endogenous (oxidative damage and structural instability) mechanisms (*9*). It is well-known that some of the health-related microorganisms have one or more of these repair pathways (i.e., photoreactivation), such as most of the indicator bacteria (*10*−*12*) and some pathogenic bacteria (*13*, *14*). However, it was recently reported that UV-inactivated *Cryptosporidium parvum* did not exhibit restoration of cell culture infectivity following specific light and dark repair protocols (*15*). It is not known whether *G. lamblia* cysts have DNA repair pathways capable of reversing DNA damage caused by UV irradiation and thus are able to restore infectivity.

The objectives of this research were to (1) determine the kinetics and extent of inactivation of *G. lamblia* cyst infectivity for Mongolian gerbils by various doses of UV irradiation from LP mercury UV lamps and (2) determine the kinetics and extent of DNA repair of UV-irradiated *G. lamblia* cysts under the conditions of dark and light repair typically employed for bacterial, eucaryotic, and mammalian cell DNA repair studies.

Materials and Methods

Parasites. *G. lamblia* (CH-3) cysts were purchased from Parasitology Research Labs, Neosho, MO. Shed cysts collected from experimentally infected Mongolian gerbils were screened to remove large debris, then mixed with zinc sulfate solution ($ZnSO_4$, 1.2 specific gravity), and centrifuged at 1500 rpm for 5 min. Cysts recovered in the supernatant were washed with distilled water, resuspended in deionized buffer water solution containing antibiotics (Gentamycin at 1 mg/mL), and stored at 4 °C.

LP UV Irradiation System and Radiometry. A "collimated beam" bench scale UV apparatus consisted of two 15-W LP mercury vapor germicidal lamps emitting nearly monochromatic UV radiation at 253.7 nm that was directed through a circular opening to provide incident radiation normal to

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the surface of the test suspension in a 60 × 15 mm cell culture Petri dish. UV irradiance (W M^{-2}) at 253.7 nm was measured with a radiometer and UV 254 detector (International Light IL1400, Newburyport, MA) that had been factory-calibrated, traceable to National Institute of Standards and Technology standards.

UV Dose Determination. The measured incident irradiance at the surface of the test liquid (approximately 0.6 W M^{-2}) was corrected for any nonhomogeneity of irradiation across the surface area of the Petri dish to provide a value for the average incident irradiance. The average irradiance in the mixed suspension was determined mathematically from an integration of the Beer−Lambert Law over the sample depth, accounting for UV absorbance of the *G. lamblia* test suspension and incident average irradiance (*16*). Target doses were computed as the product of average irradiance and time (in seconds), and required exposure times were calculated by dividing the desired UV dose by the average UV irradiance. Note that units for UV dose are presented as J M^{-2} where 10 J M^{-2} = 1 mJ cm^{-2}. The UV absorbance of the *G. lamblia* test suspension ranged from 0.25 to 0.46 cm^{-1} at 254 nm.

UV Disinfection Experiments. In each experiment, 5-mL volumes of phosphate buffered saline (PBS) containing purified *G. lamblia* cysts at concentrations of ~10^6 organisms/mL in 60 × 15 mm cell culture Petri dishes were irradiated using the bench scale UV "collimated beam" system while magnetically stirring the samples slowly at room temperature (23−25 °C). After predetermined exposure times necessary to achieve target delivered UV doses, samples were removed from the UV irradiation system and diluted serially 10-fold for subsequent infectivity assays.

DNA Repair Experiments. Experimental samples of *G. lamblia* cysts were exposed to various doses of UV irradiation as described previously and then wrapped with aluminum foil immediately after UV exposure. One sample was kept at 4 °C as an experimental control. Replicate samples were transferred to 25 °C and 37 °C incubators. One dish at each temperature was illuminated by a 15 W fluorescent lamp at a distance of 25 cm with slow stirring (light repair) and the other was only stirred while kept wrapped with aluminum foil (dark repair). Conditions were 25 °C for 4 h and 37 °C for 2 h, as commonly used in DNA repair assays of UV-induced bacterial, eucaryotic, or mammalian cell DNA damage (*14, 11, 17−19*). After incubation, the samples were immediately serially diluted and dosed into animals for infectivity assays.

Infectivity Assays. *G. lamblia* infectivity assays were done in 8−10-week old female Mongolian gerbils (*Meriones unguiculatus*). The gerbils were purchased from Charles River, Canada (St. Constant, PQ). At least 10 days before experimental infection, the animals were treated once with a solution (20 mg/gerbil) of metrodinazole (Flagyl; Rhone Poulenc, Montreal, PQ) which was administered by gavage. This treatment ensured that the gerbils were free from all previous intestinal infections (including *Giardia*), as demonstrated by three consecutive examinations of feces over a period of 7 days. Following each experimental treatment, gerbils were dosed with *G. lamblia* cysts by gavage. Feces were collected daily starting 3 days after dosing until day 25. This period of collection was chosen because it represents the latent, the acute, and the elimination phase of *Giardia* infection in gerbils (*20*). The total number of cysts released in a 2-h fecal collection (10 a.m. to noon) by individual gerbils was determined by the sucrose flotation method as described previously (*21*). The presence of trophozoites in the small intestine was determined at day 15 after infection in one gerbil of each group and at the end of the experiment in all remaining gerbils after they were sacrificed in a CO_2 gas chamber. The small intestine was slit longitudinally, divided in small segments and placed in 20 mL of phosphate-buffered saline (pH 7.2). After incubation for 2.0 h in an ice bath placed on a shaking plate (100 cycles/min), the supernatant was collected and centrifuged at 1000 rpm for 5 min. The presence of trophozoites was determined by examining the sediment with a microscope at 20× magnification. The infectivity titers of *G. lamblia* cysts were calculated as a most probable number (MPN) as described in the next section.

MPN Calculation. The infectivity titers of *G. lamblia* cysts in samples were calculated as most probable number (MPN) values based on the presence or absence of cysts and trophozoites in individual animals that had been inoculated with sample dilutions containing specified numbers of cysts. Results for the numbers of animals positive or negative for infection out of the total number of animals inoculated with a given sample dilution (cyst concentration) were used to estimate the MPN using the Thomas equation. Because the number of cysts was used for the MPN calculation, the unit of the MPN is the number of infectious cysts/total number of cysts (counted microscopically by hemocytometer during each experiment) rather than MPN/mL.

Data Presentation. For each test, *G. lamblia* infectivity in control samples was computed as an MPN and taken as N_0, the initial concentration. The average infectivity concentrations of UV-irradiated samples were similarly computed as MPNs and taken as N_d. The proportions of initial infectious *G. lamblia* remaining at each dose (d) were computed by dividing the concentration at each UV dose (N_d) by the initial concentration (N_0). These values for infectivity reduction were then transformed to log (N_d/N_0) and plotted against their corresponding UV doses (semilog plots) for linear regression analysis.

Results and Discussion

The raw data for gerbil infectivity assays, the computed MPN values, and the MPN log reductions of each of the 6 UV disinfection experiments (trials) performed are shown in Table 1. In the first and second trials, approximately equal numbers of cysts were used in both control and UV-irradiated samples and high UV doses up to 1500 J M^{-2} were tested because only moderate levels of inactivation of *G. lamblia* cysts infectivity by LP UV were expected. As a result, the detection limit of the gerbil infectivity assay in these trials was only about 2.5−2.6 log; therefore, the reductions were already beyond the detection limits, even at the lowest UV doses tested (20 and 50 J M^{-2}). To better determine the amount and kinetics of inactivation, both lower numbers of cysts in untreated control samples (for determining gerbil infectivity titer endpoint) and lower UV doses (to give less infectivity reductions) were used in the subsequent trials. The cysts used in these experiments were highly infectious such that doses of only 5−10 cysts were capable of infecting gerbils, and infectivity reductions of up to ~5 log could be followed. Despite this greater limit for detection of UV infectivity reduction, it was not until an extremely low UV dose of only 5 J M^{-2} was delivered that some infectious *G. lamblia* cysts remained in the UV-irradiated samples.

The reduction kinetics of *G. lamblia* infectivity for several different doses of LP UV irradiation applied to cyst suspensions in PBS at pH 7.3 and room temperature are shown in Figure 1. Only the results of trials 3−6 are presented because of the poor detection limits for infectivity reduction in the first two trials. The reduction of *G. lamblia* cyst infectivity for gerbils was extensive at very low doses and reached >4 log within a dose of 10 J M^{-2} in these trials. Inactivation kinetics appeared to be approximately first-order through at least 4 log (99.99%) reduction, with no "tailing" (retardant or decreasing rate) inactivation kinetics. This result is in contrast to the findings of a previous study reporting a "plateau" of inactivation at about 2.5 log reduction for UV-

TABLE 1. Raw Data for Animal Infectivity Testing and Computed Inactivation for UV Disinfection of *G. lamblia* Cysts

trial number	UV dose J M^{-2}	cyst dose[a]	infectivity response[b]	cyst dose[a]	infectivity response[b]	cyst dose[a]	infectivity response[b]	infectious MPN	log reduction $-\log N_d/N_0$
1	control	350	5/5	3500	5/5	35000	5/5	>1800	
	50	380	0/0	3800	0/0	38000	0/0	<4.7	>2.6
	160	330	0/0	3300	0/0	33000	0/0	<5.5	>2.5
	500					35000	0/0	<5.7	>2.5
	1500					35000	0/0	<5.7	>2.5
2	control A[c]	600	5/5	6000	5/5	60000	5/5	>1064	
	control B[c]	500	5/5	5000	5/5	50000	5/5	>1271	
	20	600	0/0	6000	0/0	60000	0/0	<3	>2.6
	50	650	0/0	6500	0/0	65000	0/0	<2.8	>2.6
	160					60000	0/0	<3.3	>2.5
	500					85000	0/0	<2.4	>2.7
3	control	10	5/5	100	5/5	1000	5/5	>63600	
	10	100	0/0	1000	0/0	10000	0/0	<18	>3.6
	20			10000	0/0	100000	0/0	<1.8	>4.5
	30					100000	0/0	<2	>4.5
4	control	10	8/8	100	8/8			>170600	
	5	1000	3/5	10000	5/5	50000	4/5	95	>3.3
	10			10000	0/0	50000	0/0	<3.3	>4.7
5	control	5	6/6					>491800	
	10					50000	3/4	30	>4.2
6	control	5	6/7	22	4/5			160000	
	160					100000	0/0	<2	>4.9
	400					100000	0/0	<2	>4.9

[a] Cyst Dose is the number of *Giardia* cysts dosed into each animal. [b] Infectivity response is the number of animals with living stages of *Giardia* present/total number of animals infected. [c] Control A = stirred; control B = not stirred.

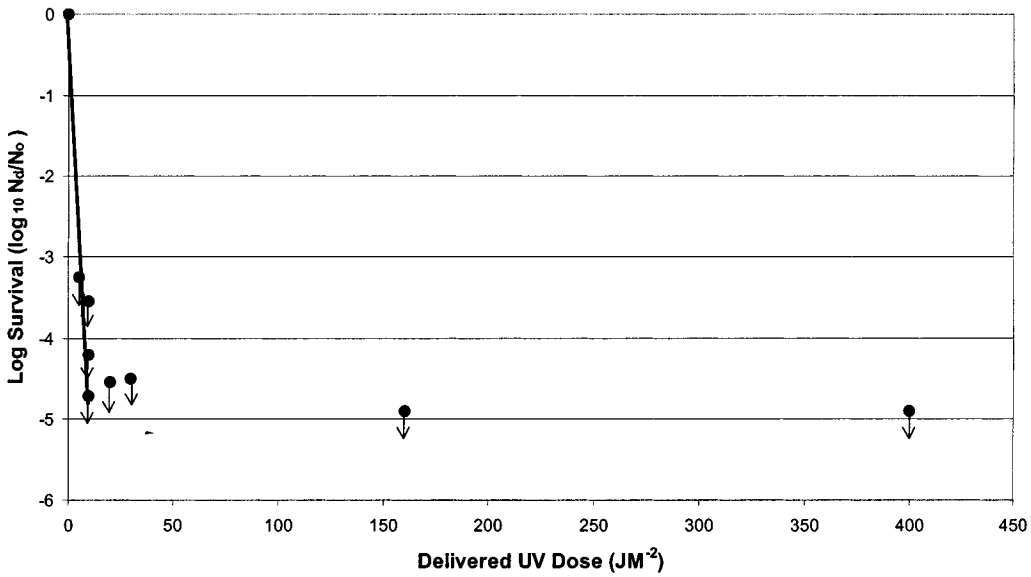

FIGURE 1. Inactivation of *G. lamblia* by LP UV irradiation in phosphate-buffered saline (pH 7.3) at room temperature for trials 3−6. All data are "greater than" values.

irradiated *G. muris* cysts (*8*). It is not certain if the discrepancy between the two studies is due to different *Giardia* species (*lamblia* vs *muris*), the relative infectivity, purity, and physical state of the cysts, the UV dosimetry conditions, the susceptibility and variability of the animals to the infection, the difference in the suspension waters used, or other factors.

As illustrated in Table 2, as compared to various health-related enteric bacteria and viruses, *G. lamblia* is much more sensitive to LP UV irradiation than other health-related waterborne enteric microbes. One possible explanation for this increased sensitivity, which was also proposed for *C. parvum* oocysts (*15*), is that compared to enteric bacteria and viruses, these two protozoan parasites have a much larger overall size and genome size, thus providing a larger nucleic acid "target" for UV photons. This target theory may also be the reason greater inactivation is achieved at lower doses for *G. lamblia* cysts than for *C. parvum* oocysts, because the latter are comparatively smaller and contain less nucleic acid.

TABLE 2. Comparative UV Inactivation of Some Health-Related Bacteria, Viruses, and Protozoans

organism	UV dose (J M^{-2})	log reduction	ref
Escherichia coli	80	4	22
Vibrio cholera	30	4	23
Poliovirus	210	4	24
Rotavirus-Wa	500	4	25
MS2 coliphage	830	4	26
Adenovirus 40	1210	4	24
Cryptosporidium parvum	30	>3	15
Giardia muris	<250	~3	8
Giardia lamblia	10	>4	this study

The results of dark and light DNA repair experiments for UV-irradiated *G. lamblia* cysts dosed at 160 and 400 J M^{-2} are presented in Table 3. The reductions of *G. lamblia* cyst infectivity at both doses of LP UV were not restored following exposure of the UV-irradiated cysts to either light or dark

TABLE 3. Effect of Dark and Light Repair Conditions on Infectivity of *G. lamblia* Cysts Exposed to a Delivered Dose of 160 and 400 J M^{-2} of LP UV

conditions	log *G. lamblia* reduction		
	control	dark	light
	160 J M^{-2}		
37 °C, 2 h	>4.2	>4.2	>4.2
25 °C, 4 h	>4.2	>4.2	>4.2
	400 J M^{-2}		
37 °C, 2 h	>4.2	>4.2	>4.2
25 °C, 4 h	>4.2	>4.2	>4.2

repair conditions. Therefore, there was no apparent evidence of either light or dark repair of DNA damage caused by LP UV irradiation of *G. lamblia* cysts at these doses causing high (> 4.2 log) infectivity reductions.

The original objectives of this work were to determine the kinetics and extent of inactivation of *G. lamblia* under UV irradiation. However, given the nature of the animal infectivity assays coupled with the extreme sensitivity of the *Giardia* to UV irradiation, many of the data points were scored as "greater than" values. Thus, although it was clear that low doses of UV irradiation could achieve high levels of *G. lamblia* inactivation, neither the inactivation kinetics nor the absolute extent of inactivation was able to be determined. To attain these parameters in future studies, it will be necessary to either use lower UV doses such that complete inactivation of cysts does not occur, use higher titers for cyst dosing to increase the likelihood that an infectious cysts will be dosed into a host animal, or to utilize a source of *Giardia* cysts that is not so highly infectious such as to cause complete infection at low viable cyst doses in control samples.

In conclusion, UV irradiation appears to be very effective for inactivation of the human pathogen *G. lamblia* in water, based on the gerbil animal model for giardiasis. The results of this study also indicate no phenotypic evidence of either light or dark repair of UV-damaged DNA in *G. lamblia* cysts irradiated with 160 or 400 J M^{-2} of LP UV and exposed to typical conditions employed for bacterial, eucaryotic, or mammalian cell DNA repair. The UV doses tested in the reactivation experiments were typical of the UV doses used in drinking water and wastewater treatment practices. For example, the low dose of 160 J M^{-2} is the minimum UV design dose that is used on residential UV systems intended to polish municipally treated drinking water delivered to households, and the 400 J M^{-2} is a common design dose used on residential systems treating nonmunicipal drinking water and at the municipal drinking water treatment plant to disinfect water before it enters the distribution system. Thus, it appears that typical UV disinfection doses designed to polish municipally disinfected drinking water or to inactivate a full range of pathogens including bacteria and viruses at the municipal treatment plant will provide adequate disinfection and also protect against DNA repair and reactivation of *G. lamblia* cysts. However, alternative DNA repair mechanisms may exist and be activated under different environmental conditions than those used in this study. In addition, *G. lamblia* exposed to lower UV doses may potentially exhibit DNA repair capability because of the lesser levels of UV-induced DNA damage. Therefore, although UV doses much lower than 160 or 400 J M^{-2} appreciably inactivate *G. lamblia* cyst infectivity, only these higher UV doses have been experimentally demonstrated here to protect against reactivation of cyst infectivity. More thorough biochemical, genetic, and phenotypic studies are recommended to further investigate the presence of DNA repair activities in *G. lamblia* cysts under a variety of UV irradiation and subsequent DNA repair conditions. Nonetheless, because *G. lamblia* cysts have been shown to be very sensitive to low doses of LP UV irradiation, properly designed and operated LP UV disinfection systems are expected to effectively control this pathogen in wastewater effluents and drinking water supplies. The results of this study provide evidence of *G. lamblia* control at current UV design doses that are compatible with the intended use of UV to inactivate a broader range of target organisms.

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