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UV irradiation responses in *Giardia intestinalis*

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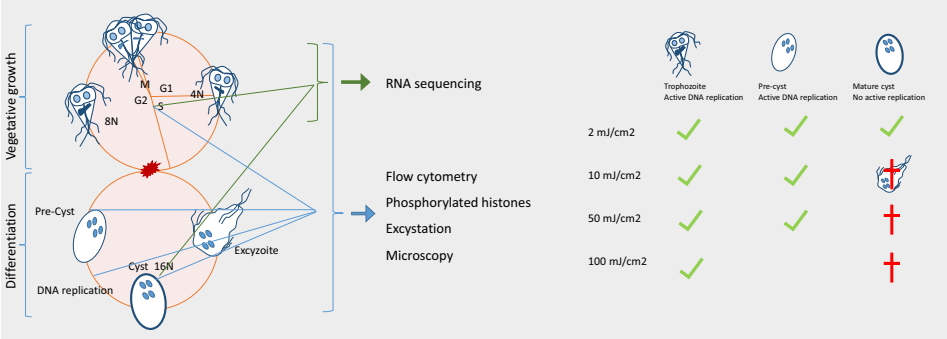
HIGHLIGHTS

- UV radiation dose of 2 mJ/cm² induces DSB in both trophozoites and cysts.
- Large differences in survival between Giardia cysts, trophozoites and encysting cells.
- DNA metabolism proteins are differentially expressed after UV treatment.
- Active DNA replication is linked to repair of UV-induced DNA lesions.

GRAPHICAL ABSTRACT



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ABSTRACT

The response to ultraviolet light (UV) radiation, a natural stressor to the intestinal protozoan parasite *Giardia intestinalis*, was studied to deepen the understanding of how the surrounding environment affects the parasite during transmission. UV radiation at 10 mJ/cm² kills *Giardia* cysts effectively whereas trophozoites and encysting parasites can recover from UV treatment at 100 mJ/cm² and 50 mJ/cm² respectively. Staining for phosphorylated histone H2A showed that UV treatment induces double-stranded DNA breaks and flow cytometry analyses revealed that UV treatment of trophozoites induces DNA replication arrest. Active DNA replication coupled to DNA repair could be an explanation to why UV light does not kill trophozoites and encysting cells as efficiently as the non-replicating cysts. We also examined UV-induced gene expression responses in both trophozoites and cysts using RNA sequencing (RNA seq). UV radiation induces small overall changes in gene expression in *Giardia* but cysts show a stronger response than trophozoites. Heat shock proteins, kinesins and Nek kinases are up-regulated, whereas alpha-giardins and histones are down-regulated in UV treated trophozoites. Expression of variable surface proteins (VSPs) is changed in both trophozoites and cysts. Our data show that *Giardia* cysts have limited ability to repair UV-induced damage and this may have implications for drinking- and waste-water treatment when setting criteria for the use of UV disinfection to ensure safe water.

1. Introduction

Giardia intestinalis, a flagellated unicellular parasite, infects the small intestine and cause watery diarrhea. It infects both humans and livestock and certain genetic variants of the parasite are considered to have zoonotic potential (Ryan and Caccio, 2013). The parasite is spread worldwide and regarded as the most common cause of protozoan diarrhea (Caccio and Sprong, 2011). *Giardia* is mainly transmitted via water and food and these transmission routes result in both sporadic cases and outbreaks. *Giardia* has been associated with numerous waterborne outbreaks over the last decades (Karanis et al., 2007; Marshall et al., 1997). Over 30% of the reported outbreaks were associated with drinking water systems contaminated, or presumably contaminated, with *Giardia* (Karanis

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et al., 2007). For drinking water outbreaks, deficiencies in water treatment processes are the most cited reasons (Karanis et al., 2007).

Giardia has two stages in its life cycle to meet changes in the surrounding environment. The trophozoite is the replicating, disease-causing stage and is found in the upper small intestine. The cyst is the infectious, dormant life form and spread of the parasite into the environment occurs through feces of humans and animals (Ankarklev et al., 2010). The cysts have a lower metabolic rate than the trophozoites (Paget et al., 1998) and are resistant to the surrounding environment, being able to survive for several weeks in cold water outside the host. The cysts are commonly found in raw sewage, wastewater and surface waters (Budu-Amoako et al., 2012a, 2012b; Lobo et al., 2009; Plutzer et al., 2008). Cysts are formed during the passage through the lower intestinal tract. During this process, encystation, the trophozoite is encapsulated in a cyst-wall and undergoes endoreplication, giving rise to a cell with four nuclei and sixteen genome copies (Bernander et al., 2001). The resulting cyst is ready to meet a hostile environment and to quickly colonize, once ingested by a new host. The parasite will meet many environmental factors once it is discharged from the host, ultraviolet (UV) light being one. This stress is present both in the environment through sun light as well UV radiation commonly used to treat drinking water.

Ultraviolet disinfection has been introduced at many water and wastewater treatment plants as a microorganism reduction method (Hijnen et al., 2006). The use of UV has proven effective especially for waterborne protozoan parasites such as *Giardia* (Linden et al., 2002) and *Cryptosporidium* (Shin et al., 2001). The latter being less sensitive to chemical treatments with for example chlorine (Betancourt and Rose, 2004). Due to its maximum absorbance at 260 nm, DNA is considered the primary target of UV radiation (Rastogi et al., 2010). UV radiation is divided into the UV-C (240–290 nm), UV-B (290–320 nm) and UV-A (320–400 nm) regions of which UV-C is the commonly used wavelength in water treatment due to its harmful effects on microorganisms. UV-C radiation of microorganisms may cause double strand breakage (DBS) or form dimers between adjacent bases in the DNA, the latter being the most prevalent photoreaction resulting from UV-C (Cadet et al., 2005). The type of DNA lesions that are induced is dependent on the wavelength of UV, the DNA sequence, and protein–DNA interactions. The biological effects of damage depend on the type of lesion induced, its genomic location and the developmental state of the injured cell. Formation of base dimers interferes with important cellular functions like DNA replication and transcription. This type of damage may be lethal but the level of sensitivity to UV radiation is highly species-specific. Many organisms have developed multiple strategies to avoid, or repair DNA lesions. If the lesions are repaired correctly, the DNA is restored to its original state and, after some delay, the cell proceeds in the cell cycle. Bacterial tolerance to UV exposure as well as mechanisms to repair DNA damage are well described (Hader and Sinha, 2005). In contrast, many studies have showed effective inactivation of cysts and oocysts (Craik et al., 2001; Hijnen et al., 2006; Linden et al., 2002). However, studies on *Giardia* inactivation have given conflicting results. Inactivation of the infectious cyst stage has been reported (Linden et al., 2002), while Li et al. (2008) showed that *Giardia* trophozoites can survive exposure to UV radiation up to 10 mJ/cm^2.

In recent years several studies have been performed studying transcriptional changes during different stress conditions in *Giardia* (for example DTT, drugs, differentiation, host-interaction and oxidative stress) (Birkeland et al., 2010; Morf et al., 2010; Muller et al., 2008; Raj et al., 2014; Ringqvist et al., 2011; Spycher et al., 2013). However, this is the first report on transcriptional profiling on *Giardia* after UV irradiation. Determining the changes in gene expression in both life stages of this parasite during UV exposure will help to understand the general stress response as well as the specific response to a naturally found stressor encountered by the parasite in the environment.

The objective of the present study was to explore the responses when *Giardia* is exposed to UV irradiation. We investigated the inactivation effect of UV radiation on *Giardia* in growing trophozoites, encysting cells and cysts by measuring the level of reactivation of radiated cells. RNA sequencing was used to identify genes participating in DNA damage response as a consequence of UV irradiation. Inactivation and reactivation was measured using *in vitro* excystation, flow cytometry and detecting DNA lesions by staining of phosphorylated histone H2AX. Our data show that there are minor transcriptional changes in *Giardia* due to UV radiation and that replicating cells recover better than dormant cysts after UV treatment.

2. Materials and methods

2.1. Reagents and cell cultivation

All reagents were obtained from Sigma Chemical Co unless indicated otherwise. *Giardia intestinalis* trophozoites of strain WB clone C6 (ATCC no. 50803) were cultivated in TYI-S-33 medium with a pH of 7.0 prepared as in Jerlstrom-Hultqvist et al. (2010). To induce encystation a slightly modified high bile protocol was used (Kane et al., 1991). In brief, the growth medium was removed from cultures that were 70–80% confluent and encystation media with pH 7.8 containing 1.25 mg/ml of bovine bile was added. *In vitro* generated cysts were harvested 30 hours post-induction of encystation by centrifugation and kept at 4 °C in water for a minimum of 48 hours prior to experiments.

2.2. UV irradiation and post-UV cell viability

Trophozoites were grown in UV transparent 15 ml polypropylene tubes (Sarstedt, cat. No. 62.554.502) and when the culture reached approximately 20% confluence the medium was removed and the cells were placed in a UV-crosslinker (UVC 500, Amersham Biosciences). The trophozoites were UV treated at irradiation doses 2, 5, 10, 20, 50 and 100 mJ/cm^2 of 254 nm UV-C and the tubes were rotated 180° in order to irradiate most cells *i.e.* the tubes were irradiated twice. The cells were radiated for 10 s each time, *i.e.* totally 20 s per sample. After irradiation, pre-warmed freshly made TYI-S-33 medium was added to the cells immediately. The confluence of the cultures was evaluated in terms of confluence 48 hours post-treatment and scored in intervals; + up to 20%, ++ 21–70%, +++ 71–100%. The viability of irradiated water treated cysts was carried out by excystation followed by monitoring if the excyzoites were able to leave the cysts and if they were able to establish a culture of trophozoites. The same procedure was carried out for the cysts generated from UV treated encysting cultures (12 and 22 hours post induction).

The cysts were kept in distilled water at 4 °C for a minimum of 2 days prior excystation as described in Boucher and Gillin (1990). The cultures were monitored daily and evaluated in terms of confluence. The confluence at day 7 post-irradiation was scored using the same intervals as for trophozoites (see discussion earlier). One week post-treatment all cultures with no detectable live cells were considered non-viable. Untreated cysts were used as controls to make sure that the excystation itself was successful and the cysts were regarded as non-viable if they were not able to establish a culture in the same time span as the controls.

For RNA seq the irradiation dose of 2 mJ/cm^2 was selected for both trophozoites and water resistant cysts. Trophozoites were grown to 80% confluency and thereafter UV treated at the selected dose as described earlier. The cells were allowed to recover for 3 hours in TYI-S-33 at 37 °C prior RNA extraction or fixation on slides for histone H2A staining. The cysts suspended in water were placed in the middle of a Petri dish and irradiated at the same settings as the

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