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Antimicrobial Activity of Simulated Solar Disinfection against Bacterial, Fungal, and Protozoan Pathogens and Its Enhancement by Riboflavin[▽]

Wayne Heaselgrave* and Simon Kilvington

Department of Infection, Immunity and Inflammation, University of Leicester, Medical Sciences Building, P.O. Box 138, University Road, Leicester LE1 9HN, United Kingdom

Received 18 February 2010/Accepted 29 June 2010

Riboflavin significantly enhanced the efficacy of simulated solar disinfection (SODIS) at 150 watts per square meter (W m^{-2}) against a variety of microorganisms, including *Escherichia coli*, *Fusarium solani*, *Candida albicans*, and *Acanthamoeba polyphaga* trophozoites (>3 to 4 log_{10} after 2 to 6 h; $P < 0.001$). With *A. polyphaga* cysts, the kill (3.5 log_{10} after 6 h) was obtained only in the presence of riboflavin and 250 W m^{-2} irradiance.

Solar disinfection (SODIS) is an established and proven technique for the generation of safer drinking water (11). Water is collected into transparent plastic polyethylene terephthalate (PET) bottles and placed in direct sunlight for 6 to 8 h prior to consumption (14). The application of SODIS has been shown to be a simple and cost-effective method for reducing the incidence of gastrointestinal infection in communities where potable water is not available (2–4). Under laboratory conditions using simulated sunlight, SODIS has been shown to inactivate pathogenic bacteria, fungi, viruses, and protozoa (6, 12, 15). Although SODIS is not fully understood, it is believed to achieve microbial killing through a combination of DNA-damaging effects of ultraviolet (UV) radiation and thermal inactivation from solar heating (21).

The combination of UVA radiation and riboflavin (vitamin B_2) has recently been reported to have therapeutic application in the treatment of bacterial and fungal ocular pathogens (13, 17) and has also been proposed as a method for decontaminating donor blood products prior to transfusion (1). In the present study, we report that the addition of riboflavin significantly enhances the disinfectant efficacy of simulated SODIS against bacterial, fungal, and protozoan pathogens.

Chemicals and media were obtained from Sigma (Dorset, United Kingdom), Oxoid (Basingstoke, United Kingdom), and BD (Oxford, United Kingdom). *Pseudomonas aeruginosa* (ATCC 9027), *Staphylococcus aureus* (ATCC 6538), *Bacillus subtilis* (ATCC 6633), *Candida albicans* (ATCC 10231), and *Fusarium solani* (ATCC 36031) were obtained from ATCC (through LGC Standards, United Kingdom). *Escherichia coli* (JM101) was obtained in house, and the *Legionella pneumophila* strain used was a recent environmental isolate.

B. subtilis spores were produced from culture on a previously published defined sporulation medium (19). *L. pneumophila* was grown on buffered charcoal-yeast extract agar (5). All other bacteria were cultured on tryptone soy agar, and *C. albicans* was cultured on Sabouraud dextrose agar as described previously (9). *Fusarium solani* was cultured on potato dextrose agar, and conidia were prepared as reported previously (7). *Acanthamoeba polyphaga* (Ros) was isolated from an unpublished keratitis case at Moorfields Eye Hospital, London, United Kingdom, in 1991. Trophozoites were maintained and cysts prepared as described previously (8, 18).

Assays were conducted in transparent 12-well tissue culture microtiter plates with UV-transparent lids (Helena Biosciences, United Kingdom). Test organisms (1×10^6/ml) were suspended in 3 ml of one-quarter-strength Ringer's solution or natural freshwater (as pretreated water from a reservoir in United Kingdom) with or without riboflavin (250 μM). The plates were exposed to simulated sunlight at an optical output irradiance of 150 watts per square meter (W m^{-2}) delivered from an HPR125 W quartz mercury arc lamp (Philips, Guildford, United Kingdom). Optical irradiances were measured using a calibrated broadband optical power meter (Melles Griot, Netherlands). Test plates were maintained at 30°C by partial submersion in a water bath.

At timed intervals for bacteria and fungi, the aliquots were plated out by using a WASP spiral plater and colonies subsequently counted by using a ProtoCOL automated colony counter (Don Whitley, West Yorkshire, United Kingdom). *Acanthamoeba* trophozoite and cyst viabilities were determined as described previously (6). Statistical analysis was performed using a one-way analysis of variance (ANOVA) of data from triplicate experiments via the InStat statistical software package (GraphPad, La Jolla, CA).

The efficacies of simulated sunlight at an optical output irradiance of 150 W m^{-2} alone (SODIS) and in the presence of 250 μM riboflavin (SODIS-R) against the test organisms are shown in Table 1. With the exception of *B. subtilis* spores and *A. polyphaga* cysts, SODIS-R resulted in a significant increase in microbial killing compared to SODIS alone ($P < 0.001$). In most instances, SODIS-R achieved total inactivation by 2 h, compared to 6 h for SODIS alone (Table 1). For *F. solani*, *C. albicans*, ands *A. polyphaga* trophozoites, only SODIS-R achieved a complete organism kill after 4 to 6 h ($P < 0.001$). All control experiments in which the experiments were pro-

* Corresponding author. Mailing address: Department of Infection, Immunity and Inflammation, University of Leicester, Medical Sciences Building, P.O. Box 138, University Road, Leicester LE1 9HN, United Kingdom. Phone: 44 (0) 116 252 2950. Fax: 44 (0) 116 252 5030. E-mail: wh12@le.ac.uk.

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TABLE 1. Efficacies of simulated SODIS for 6 h alone and with 250 μM riboflavin (SODIS-R)

Organism	Condition[a]	Log_{10} reduction in viability at indicated h of exposure[b]			
		1	2	4	6
E. coli	SODIS	0.0 ± 0.0	0.2 ± 0.1	5.7 ± 0.0	5.7 ± 0.0
	SODIS-R	1.1 ± 0.0	5.7 ± 0.0	5.7 ± 0.0	5.7 ± 0.0
L. pneumophila	SODIS	0.7 ± 0.2	1.3 ± 0.3	4.8 ± 0.2	4.8 ± 0.2
	SODIS-R	4.4 ± 0.0	4.4 ± 0.0	4.4 ± 0.0	4.4 ± 0.0
P. aeruginosa	SODIS	0.7 ± 0.0	1.8 ± 0.0	4.9 ± 0.0	4.9 ± 0.0
	SODIS-R	5.0 ± 0.0	5.0 ± 0.0	5.0 ± 0.0	5.0 ± 0.0
S. aureus	SODIS	0.0 ± 0.0	0.0 ± 0.0	6.2 ± 0.0	6.2 ± 0.0
	SODIS-R	0.2 ± 0.1	6.3 ± 0.0	6.3 ± 0.0	6.3 ± 0.0
C. albicans	SODIS	0.2 ± 0.0	0.4 ± 0.1	0.5 ± 0.1	1.0 ± 0.1
	SODIS-R	0.1 ± 0.0	0.7 ± 0.1	5.3 ± 0.0	5.3 ± 0.0
F. solani conidia	SODIS	0.2 ± 0.1	0.3 ± 0.0	0.2 ± 0.0	0.7 ± 0.1
	SODIS-R	0.3 ± 0.1	0.8 ± 0.1	1.3 ± 0.1	4.4 ± 0.0
B. subtilis spores	SODIS	0.3 ± 0.0	0.2 ± 0.0	0.0 ± 0.0	0.1 ± 0.0
	SODIS-R	0.1 ± 0.1	0.2 ± 0.1	0.3 ± 0.3	0.1 ± 0.0
	SODIS (250 W m^{-2})	0.1 ± 0.0	0.1 ± 0.1	0.1 ± 0.1	0.0 ± 0.0
	SODIS-R (250 W m^{-2})	0.0 ± 0.0	0.0 ± 0.0	0.2 ± 0.0	0.4 ± 0.0
	SODIS (320 W m^{-2})	0.1 ± 0.1	0.1 ± 0.0	0.0 ± 0.1	4.3 ± 0.0
	SODIS-R (320 W m^{-2})	0.1 ± 0.0	0.1 ± 0.1	0.9 ± 0.0	4.3 ± 0.0
A. polyphaga trophozoites	SODIS	0.4 ± 0.2	0.6 ± 0.1	0.6 ± 0.2	0.4 ± 0.1
	SODIS-R	0.3 ± 0.1	1.3 ± 0.1	2.3 ± 0.4	3.1 ± 0.2
	SODIS, natural[c]	0.3 ± 0.1	0.4 ± 0.1	0.5 ± 0.2	0.3 ± 0.2
	SODIS-R, natural[c]	0.2 ± 0.1	1.0 ± 0.2	2.2 ± 0.3	2.9 ± 0.3
A. polyphaga cysts	SODIS	0.4 ± 0.1	0.1 ± 0.3	0.3 ± 0.1	0.4 ± 0.2
	SODIS-R	0.4 ± 0.2	0.3 ± 0.2	0.5 ± 0.1	0.8 ± 0.3
	SODIS (250 W m^{-2})	0.0 ± 0.1	0.2 ± 0.3	0.2 ± 0.1	0.1 ± 0.2
	SODIS-R (250 W m^{-2})	0.4 ± 0.2	0.3 ± 0.2	0.8 ± 0.1	3.5 ± 0.3
	SODIS (250 W m^{-2}), natural[c]	0.0 ± 0.3	0.2 ± 0.1	0.1 ± 0.1	0.2 ± 0.1
	SODIS-R (250 W m^{-2}), natural[c]	0.1 ± 0.1	0.2 ± 0.2	0.6 ± 0.1	3.4 ± 0.2

[a] Conditions are at an intensity of 150 W m^{-2} unless otherwise indicated.
[b] The values reported are means ± standard errors of the means from triplicate experiments.
[c] Additional experiments for this condition were performed using natural freshwater.

tected from the light source showed no reduction in organism viability over the time course (results not shown).

The highly resistant *A. polyphaga* cysts and *B. subtilis* spores were unaffected by SODIS or SODIS-R at an optical irradiance of 150 W m^{-2}. However, a significant reduction in cyst viability was observed at 6 h when the optical irradiance was increased to 250 W m^{-2} for SODIS-R only ($P < 0.001$; Table 1). For spores, a kill was obtained only at 320 W m^{-2} after 6-h exposure, and no difference between SODIS and SODIS-R was observed (Table 1). Previously, we reported a >2-log kill at 6 h for *Acanthamoeba* cysts by using SODIS at the higher optical irradiance of 850 W m^{-2}, compared to the 0.1-log_{10} kill observed here using the lower intensity of 250 W m^{-2} or the 3.5-log_{10} kill with SODIS-R.

Inactivation experiments performed with *Acanthamoeba* cysts and trophozoites suspended in natural freshwater gave results comparable to those obtained with Ringer's solution ($P > 0.05$; Table 1). However, it is acknowledged that the findings of this study are based on laboratory-grade water and freshwater and that differences in water quality through changes in turbidity, pH, and mineral composition may significantly affect the performance of SODIS (20). Accordingly, further studies are indicated to evaluate the enhanced efficacy of SODIS-R by using natural waters of varying composition in the areas where SODIS is to be employed.

Previous studies with SODIS under laboratory conditions have employed lamps delivering an optical irradiance of 850 W m^{-2} to reflect typical natural sunlight conditions (6, 11, 12, 15, 16). Here, we used an optical irradiance of 150 to 320 W m^{-2} to obtain slower organism inactivation and, hence, determine the potential enhancing effect of riboflavin on SODIS.

In conclusion, this study has shown that the addition of riboflavin significantly enhances the efficacy of simulated SODIS against a range of microorganisms. The precise mechanism by which photoactivated riboflavin enhances antimicrobial activity is unknown, but studies have indicated that the process may be due, in part, to the generation of singlet oxygen, H_2O_2, superoxide, and hydroxyl free radicals (10). Further studies are warranted to assess the potential benefits from riboflavin-enhanced SODIS in reducing the incidence of gastrointestinal infection in communities where potable water is not available.

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