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SCIENCE DIRECT®
Ecotoxicology and Environmental Safety 61 (2005) 53–59
Ecotoxicology and Environmental Safety
www.elsevier.com/locate/ecoenv

Rapid communication

Ultraviolet damages sperm mitochondrial function and membrane integrity in the sea urchin *Anthocidaris crassispina*

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Received 21 September 2004; accepted 22 December 2004

Abstract

Effects of ultraviolet A (UVA) and ultraviolet B (UVB) on mitochondrial function and membrane integrity of sea urchin sperm were investigated using flow cytometry and fluorescent probes. Both UVA and UVB impaired sperm mitochondrial function in a dose-dependent manner. Covariance analysis further showed that the slopes of change in mitochondrial function in relation to UVA and UVB were significantly different, suggesting that the modes of action were different. UVA did not affect membrane integrity, while membrane integrity showed a linear reduction with increasing UVB doses. Sperm mitochondria function showed significant positive correlations with sperm motility and subsequent fertilization success. Overall, our results showed that both UVA and UVB could decrease sperm motility and fertilization success through impairment of mitochondrial function, whereas UVB alone could cause additional damage through impairing the functional integrity of sperm membrane. Mitochondrial function of sperm may also offer a reliable ecotoxicological biomarker for predicting fertilization success in urchins.

Keywords: Mitochondrial function; Membrane integrity; UVA; UVB; Sea urchin; Flow cytometry

1. Introduction

In the past two decades, ozone depletion caused by anthropogenic activities has enabled more ultraviolet irradiation (UVR) to reach the Earth (Meyer-Rochow, 2000). Recent studies revealed that biologically effective levels of solar UVR can penetrate to at least 30 m (for UVB) and 60 m (for UVA) in water columns (Whitehead et al., 2000) and hence may pose a significant risk to marine organisms (Holm-Hansen et al., 1993), especially to those that discharge their gametes and complete their early life stages in shallow waters, where levels of UVR are high. Notably, gametes, embryos, and larvae are particularly susceptible to UVR, due to the absence of protective cover and their limited motility, small size, and the larger surface area to volume ratio (Hunter et al., 1982; Zagarese and Williamson, 2000). The vast majority of research on the effects of UVR carried out thus far has been restricted to eggs, embryos, and larvae of fish (e.g., Béland et al., 1999; Williamson et al., 1997; Kouwenberg et al., 1999; Battini et al., 2000; Steeger et al., 1999; Freitag et al., 1998; Lesser et al., 2001; Bass and Sistrun, 1997) and invertebrates (e.g., Saito and Taguchi, 2003; Lacuna and Uye, 2001; Anderson et al., 1993; Lesser and Barry, 2003). Only limited attempts have been made to evaluate effects of UVR on sperm (Au et al., 2002), despite the fact that fertilization capacity of sperm is crucial in assessing the environmental risk of UVR to marine organisms. Our previous study (X.Y. Lu and R.S.S. Wu, unpublished) showed that environmentally realistic levels of UVA/UVB could enhance production of reactive oxygen species (ROS) and lipid peroxidation (LPO) in sea urchin *Anthocidaris crassispina* sperm, which were closely associated with reductions in motility and subsequent fertilization success. However, the ways in which ROS production and oxidative damages can be

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doi:10.1016/j.ecoenv.2004.12.012

linked to subsequent fertilization impairments remain obscure.

Mitochondria provide energy for the movement of sperm flagella and therefore play a crucial role in determining sperm motility. This is especially important in sea urchin sperm since they have only one mitochondrion (as opposed to human sperm with many mitochondria). In human sperm, increase in ROS production correlated well with decrease in mitochondrial membrane potential (a marker for mitochondrial function) (Wang et al., 2003), which was also positively related to motility and fertility potential (Kasai et al., 2002; Troiano et al., 1998). It is likely that enhanced production of ROS in sea urchin sperm exposed to UVR (X.Y. Lu and R.S.S. Wu, unpublished) may impair the normal functions of mitochondria. Such impairments may subsequently lead to decrease in sperm motility and fertilization capacity. In addition, ROS may also affect the integrity of sperm plasma membrane, reducing the ability of the sperm membrane to fuse with that of the egg (DeBaulny et al., 1997), thereby leading to a lower fertilization rate.

In recent years, flow cytometry has been employed extensively to study various attributes of sperm, because this technique can analyze defined parameters of tens of thousands of individual cells within a few minutes (Graham et al., 1990) and provide a statistical summary for the whole population (Ormerod, 1994). Dual fluorescent staining (rhodamine 123 (Rh123) and propidium iodide (PI)) combined with flow cytometric analysis has been developed for simultaneous determination of mitochondrial function and membrane integrity of sperm in fish (DeBaulny et al., 1997, 1999), marine invertebrates (Adams et al., 2003), and mammals (Graham et al., 1990). Mitochondrial function can be assessed by staining with Rh123. Due to the negative potential of the inner membrane, Rh123 accumulates only in functional mitochondria, causing the mitochondria to produce a green fluorescence. Mitochondria of dead cells are readily distinguishable from those in live cells, since they are not able to retain Rh123 after washing (Graham et al., 1990). Membrane integrity can be evaluated by staining sperm with PI, a fluorescent dye that binds with DNA. Intact membrane of viable sperm will prevent PI from entering into the sperm and binding with cellular DNA (PI−); therefore no fluorescence will be found. Conversely, damaged membrane of dead/moribund sperm allow PI to enter and bind with DNA to produce a red fluorescence (PI+).

In this study, we examined the effects of environmentally relevant levels of UVA and UVB on sperm mitochondrial function and membrane integrity in the sea urchin *A. crassispina*. The aims were (1) to determine and compare impacts of UVA and UVB on sperm mitochondrial function and membrane integrity, (2) to relate these two parameters to sperm motility and fertilization success, and (3) to explore the possibility of using mitochondrial function or membrane integrity as sensitive biomarkers for predicting fertilization success in sperm.

2. Materials and methods

2.1. Sperm collection

Sea urchins *A. crassispina* were obtained from 5 to 10 m below chart datum at Cheung Chau, Hong Kong, during their natural breeding season in July. Urchins were acclimatized in running sea water (25 °C, 30‰, full aeration) for 48 h and then induced to spawn by injecting 0.5 M KCl into their perivisceral cavities (Vaschenko et al., 1999). Undiluted "dry" sperm collected from the aboral gonadal pore of each individual was stored separately in a microtube at 4 °C for subsequent assays.

2.2. UV exposure

UV exposure experiments were conducted in an UVA cabinet and an UVB cabinet (22 °C) within 24 h after sperm collection. Artificial UVA and UVB irradiation were generated using an UVA lamp (15 W, Cole–Parmer, VL-215L, France) or four UVB lamps (6 W, Cole–Parmer, VL-6 M, France). Urchin sperm from five male *A. crassispina* were pooled, diluted to ca. $1 \times 10^8\,mL^{-1}$ with filtered seawater, and exposed to 0, 3, 6, 9, and $15\,W\,m^{-2}$ UVA or UVB irradiance for 30 min (equivalent to 0, 5.4, 10.8, 16.2, and $27.0\,kJ\,m^{-2}$, respectively) (dose = UV irradiance × exposure time). Ultraviolet irradiance was verified using a radiometer (VLX-3 W; France) equipped with CX-312 and CX-315 sensors. Four replicates, each with 1 mL diluted sperm in a glass Petri dish (diameter: 35 mm), were prepared for each level of UVA/UVB treatment. Four replicates shielded with an UV-opaque acrylic sheet (Mitsubishi Royon Co. Ltd., Japan) were set up and served as controls ($0\,kJ\,m^{-2}$) in each UV cabinet.

2.3. Rhodamine 123 and propidium iodide staining

Sperm were transferred to a microtube containing 1 μL Rh123 (5 μg Rh123 mL^{-1} in dimethyl sulfoxide) immediately after irradiation and incubated at 4 °C in the dark for 20 min. The staining medium was then removed by centrifugation (800*g*, 10 min, 4 °C) and sperm were incubated for another 45 min in 1 mL filtered seawater to remove any Rh123 bound non-specifically to mitochondria. Samples were then diluted to obtain a final concentration of $10^6\,mL^{-1}$ and counterstained with 2.5 μL of PI (final concentration