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Increased p53 and decreased p21 accompany apoptosis induced by ultraviolet radiation in the nervous system of a crustacean

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ABSTRACT

Ultraviolet (UV) radiation can produce biological damage, leading the cell to apoptosis by the p53 pathway. This study evaluated some molecular markers of the apoptosis pathway induced by UVA, UVB and UVA+ UVB (Solar Simulator, SIM) in environmental doses, during five consecutive days of exposure, in the brain of the crab *Ucides cordatus*. We evaluated the central nervous system (CNS) by immunoblotting the content of proteins p53, p21, phosphorylated AKT, BDNF, GDNF, activated caspase-3 (C3) and phosphohistone H3 (PH3); and by immunohistochemical tests of the cells labeled for PH3 and C3. After the fifth day of exposure, UVB radiation and SIM increased the protein content of p53, increasing the content of AKT and, somehow, blocking p21, increasing the content of activated caspase-3, which led the cells to apoptosis. The signs of death affected the increase in neurotrophins, such as BDNF and GDNF, stimulating the apoptotic cascade of events. Immunohistochemical assays and immunoblotting showed that apoptosis was present in the brains of all UV groups, while the number of mitotic cells in the same groups decreased. In conclusion, environmental doses of UV can cause apoptosis by increasing p53 and decreasing p21, revealing an UV-damage pathway for *U. cordatus*.

1. Introduction

Crustaceans are important models for the understanding of evolutionary mechanisms of biological regulation, including cell proliferation and programmed cell death (Schmidt, 2007; Li et al., 2012; Menze et al., 2010; Leu et al., 2013). Brachyuran crabs such as *Ucides cordatus* are an important food resource for many low-income communities (Muñoz de Chávez et al., 2000; de Magalhães et al., 2012). *U. cordatus* live in mangroves (Costa, 1972; Zepp et al., 2003; Casiccia et al., 2003), where they are continuously subject to damage induced by solar ultraviolet (UV) radiation (Diffey, 2002). The progressive changes in the mangrove ecosystem due to the depletion of stratospheric ozone and the consequent increase in the amount of UV radiation that reaches the Earth (Manney et al., 1994; Zepp et al., 2003; Gareau, 2010) will have multiple biological and ecological consequences for these crabs and other mangrove inhabitants, with resulting economic effects on humans, because these crabs are a fishery resource.

Approximately 95% of the solar UV radiation that reaches the Earth's surface is classified as UVA (320–400 nm), and less than 5% as UVB (290–320 nm) (Palancar and Toselli, 2004); lower wavelengths (UVC) are blocked by the ozone layer. Damaging effects induced in animals by UV include the production of reactive oxygen species (ROS) (Heck et al., 2003), apoptosis (Finkel and Holbrook, 2000), and immunosuppression (Timares et al., 2008). UVA induces mainly oxidative stress, which promotes the oxidation of biomolecules such as lipids and protein, while UVB acts directly on DNA (Petit-Frère et al., 2000), creating a highly reactive microenvironment.

UV radiation may induce either cell-cycle arrest or apoptosis (Ishikawa et al., 2006; von Thaler et al., 2010; Darzynkiewicz et al., 2012; Hein et al., 2014). Its effects depend on a variety of mechanisms, including the involvement of trophic factors, signaling through p53-mediated pathways, and activation of caspases (Pincelli and Yaar, 1997; Marconi et al., 2003; Weinkauf et al., 2012; Goh et al., 2011; Reichrath and Rass, 2014; Talebizadeh et al., 2014; Amar et al., 2005). As yet, few studies have examined the effects of UV radiation on the central nervous system (CNS) of crustaceans (Miguel et al., 2002, 2007; Gouveia et al., 2005).

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Specific responses of the cell type may require a cooperative events in which the protein buildup downstream of p53 may favor either survival (due to reversible arrest of the cell cycle and attempted repair) or apoptosis (Liu and Kulesz-Martin, 2001). Neurotrophins are synthesized as precursor molecules that can be cleaved intracellularly by enzymes (Seidah et al., 1996), or may be secreted as pro-neurotrophin (Lee et al., 2001). Neurotrophins bind to two different types of receptor: Trk (tyrosine kinase receptor) and $p75^{NTR}$ (neurotrophin receptor). The more definite role for $p75^{NTR}$ is in apoptosis signaling (Coulson et al., 1999; Friedman, 2000), particularly in response to pro-neurotrophin (Lee et al., 2001; Beattie et al., 2005).

The CNS of crustaceans is formed by fused ganglia: the protocerebrum, the deutocerebrum and the tritocerebrum (Sandeman et al., 1992). Nerve fibers emerging from the photoreceptors project to the optic ganglia, located in the protocerebrum in the eyestalk. The deutocerebrum comprises of the olfactory lobe (OL) and a paired structure constituted of projection neurons located in a cell cluster termed cluster 10 (cl 10), adjacent to the OL, which receives direct stimuli from the olfactory receptors in the antennae and indirect stimuli from the photoreceptors (Sandeman et al., 1992; Schmidt and Ache, 1997; Hollmann et al., 2015). In the present study, we examined the effects of environmental doses of UVA, UVB and UVA + UVB on markers of the cell cycle, neurotrophic support and apoptosis, in the brain of the crab *U. cordatus*, in order to gain insight into the sensitivity and the mechanisms of damage induced by non-ionizing radiation in crustaceans.

2. Materials and methods

2.1. Animals

Adult male *U. cordatus* were collected in mangroves of Rio de Janeiro, Brazil (S-22° 54′ 10″, W-043° 12′ 27″). We used a total of 48 crabs, previously acclimated for 7 days in aquaria, in constant conditions (water salinity 20 ppm, 25 °C and 12L:12D). The crabs were fed three times a week with small pieces of mangrove *Avicennia schaueriana*.

2.2. Artificial ultraviolet radiation

In the mangrove environment, the crabs receive natural solar radiation over a yearly range of 125–350 $J/m^2/s$, based on the daily UV index, according to the Brazilian Institute for Space Research (INPE). *U. cordatus* inhabits intermareal zones and frequently explores under the sun for feeding and mating. In the laboratory, the crabs were divided into four groups: (a) irradiated with UVA (118 $J/m^2/s$, $n = 12$) for 26 min; (b) irradiated with UVB (2 $J/m^2/s$, $n = 12$) for 23 min; (c) irradiated with UVA + UVB using a Solar Simulator (SIM) with 780 W potency (UVA = 96.5 $J/m^2/s$ and UVB = 1.2 $J/m^2/s$, $n = 12$, using a solar filter 87066) for 25 min; and (d) a control group ($n = 12$) with no UV exposure, under visible light, for 30 min. All groups were irradiated daily during 5 consecutive days. The lamps providing UVA (VL-215 L, 60 W, 365 nm; Vilber Lourmat, Marne La Valée, France), UVB (VL-215 LM, 30 W, 312 nm; Vilber Lourmat) and SIM (Newport Oriel, model 91192-1000, Stratford, CT, USA) were monitored using a radiometer/photometer (VLX, 3 W, CE, France). The UVA lamp produced an irradiance of 1.39 mW/cm^2 with 0.006 mW/cm^2 UVB contamination, and 0.928 mW/cm^2 visible light contamination. The UVB lamp produced an irradiance of 1.195 mW/cm^2 with 0.000493 mW/cm^2 UVA contamination, and 0.000113 mW/cm^2 visible light contamination. The SIM produced an irradiance of 3.575 mW/cm^2, and the visible light lamp for the control group (Phillips TLT 40 W/75, São Paulo, SP, Brazil) produced 96.0 mW/cm^2. None of the lamps showed any contamination with UVC (Table 1). The SIM lamp can produce heat, but the short exposure periods under this lamp did not increase the crabs' temperature. Until the end of the experimental period, no gross morphological or behavioral abnormalities were observed.

The doses of UVA, UVB and SIM were comparable to environmental doses measured during the summer 2013 (January) at 12:00 h (noon), in the city of Rio de Janeiro.

After exposure, the animals were cryoanesthetized and killed. The brains were removed and kept frozen at −80 °C for further analyses.

2.3. Immunohistochemistry

After the 5th day of irradiation, the brains ($n = 4$ in each experimental groups) were dissected and 10 μm-thick frozen sections were obtained using a cryostat.

The sections were washed three times before the following procedures. Heat shock antigen retrieval was done with a 0.1 M citrate buffer solution (pH 6). The slides were then incubated with 10% goat serum (Sigma® G6767) for 60 min. The polyclonal rabbit anti-phosphohistone-3 (PH3; Santa Cruz® sc8656-R, dilution 1:100) or anti-phosphorylated-caspase-3 solution (Sigma® C8487, dilution 1:50) were applied on the sections, overnight, at 4 °C. Then, the slides were incubated with the secondary antibody anti-rabbit Alexa Fluor® 488 (Invitrogen™ MG120, 1:200 dilution), for 2 h. Finally, 4′-6-diamidino-2-phenylindole (DAPI) was used to stain the cell nuclei. The solution used for washing and for diluting the antibodies was 0.1 M PBS plus 0.3% Triton-X.

Coverslips were mounted using Fluoromount™. Slides were examined in a Zeiss Axioskop 2 Plus epifluorescence microscope at 40× magnification. Images of the appropriate region were captured using a Media Cybernetics Evolution™ model MP camera. After collection, each image was analyzed and the labeled cells within cl 10 were counted with ImageJ software. We counted all cells of cluster 10 in the 10 μm thick frozen sections labeled with activated caspase 3 or PH3. The whole *U. cordatus* brain is approximately 500 μm thick, and the cl 10 around 100 μm, which allowed us to count most of the labeled cells of cluster 10.

Table 1
UV doses used in the experiments.

Group	Total UV-experimental dose ($J/m^2/s$)	Time exposed per day (min)	Total time exposure after 5 days (h)	Lamp irradiance (mW/cm^2)	Lamp contamination (mW/cm^2)	Visible light contamiation (mW/cm^2)	Environmental dose ($J/m^2/s$)
CTR	0	30	2.5	96.0	<0.1	96.0	–
UVA	118	26	2.2	1.39	0.006 (UVB)	0.928	120
UVB	2	23	1.92	1195	0.000493 (UVA)	0.000113	10
SIM[a]	96.5 (UVA) 1.2 (UVB)	25	2.08	3575	–	–	–

[a] 780 W potency.