**Tolerance of an Albino Fish to Ultraviolet-B Radiation**

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**Abstract**

We exposed albino and pigmented medaka *Oryzias latipes* to simulated solar ultraviolet-B (UVB) radiation to determine if albino medaka were less tolerant of UVB radiation than medaka pigmented with melanin. There was no difference in the number of albino and pigmented medaka that died during the exposure period. Spectrophotometric analyses of the outer dorsal skin layers from albino and pigmented medaka indicated that, prior to exposure, both groups of fish had similar amounts of an apparent colorless non-melanin photoprotective substance that appears to protect other fish species from UVB radiation. Our results indicate that albino medaka were as tolerant of UVB radiation as pigmented medaka because they had similar amounts of this photoprotective substance in the outer layers of the skin.

**Keywords:** Fish; medaka; melanin; outer skin layers; photoprotection; photoprotective substance; pigment; skin pigmentation; tolerance; ultraviolet-B radiation; UVB radiation

1 Introduction

Fish that appear dark on the dorsal surface as a result of melanin pigmentation could be considered to be more tolerant of ultraviolet-B (UVB) radiation than fish that do not appear dark on the dorsal surface [1]. If skin melanin is the primary mechanism of photoprotection in fish, then albino fish should be considerably less tolerant of UVB radiation than fish pigmented with melanin that appear brown, gray, or black dorsally. Pigmented (wild-type) medaka have melanin in their skin and appear darker than albino medaka when viewed dorsally, while albino medaka are typical color mutants lacking melanin in their skin [2]. We exposed albino and pigmented medaka to simulated UVB radiation to determine if albino medaka were less tolerant of the radiation than pigmented medaka. We also examined the dorsal skin of unexposed albino and pigmented medaka for the presence of a non-melanin photoprotective substance.

2 Materials and Methods

2.1 Animals

Albino medaka were obtained from the Laboratory of Freshwater Fish Stocks, Bioscience Center, Nagoya University, Japan, and reared to adulthood. Pigmented (wild-type) medaka were obtained as eggs from the Gulf Coast Research Laboratory, Ocean Springs, MS, USA. Albino medaka had a mean total length [SE] of 3.0[0.03] cm and a mean wet weight [SE] of 0.35[0.02] g, while pigmented medaka had a mean total length [SE] of 3.2[0.1] cm and a mean wet weight [SE] of 0.36[0.04] g at the time of exposure. Prior to exposure to UVB radiation the fish were held in uncovered culture tanks under the same ambient conditions (3200 lux) provided by indirect sunlight from windows and supplemental cool white fluorescent lamps. During this time around one-fourth of the culture tank containing albino medaka was covered so the fish could seek shade from indirect and supplemental light. However, like pigmented medaka, albino medaka were observed to be dispersed throughout the culture tank and were not concentrated under the covered portion.

2.2 Exposure of fish

Fish were removed from the culture tanks and exposed in a solar simulator with a light cap fixture containing four 160 watt UVB lamps (peak emission at 313 nm), eight UVA lamps, ten cool white fluorescent lamps, and three halide lamps [3]. The simulator was suspended over a water bath and was enclosed with reflective specular aluminum. The UVB lamps were controlled by a timer to operate for 5 hours each day while the cool white and UVA lamps were controlled by separate timers to operate for 16 hours each day, simulating a midsummer photoperiod. Fish were stocked in open-top-glass 24 x 15 x 23 airlift chambers. The water depth in each chamber was around 13 cm. Groups of five albino medaka were stocked in each of eight uncovered chambers for a total of 40 fish and groups of five pigmented medaka...
were stocked in each of eight uncovered chambers for a total of 40 fish. The control conditions were created by covering the top of each of four additional chambers with two layers of 0.76 mm polycarbonate and then covering the sides of each of the four chambers with one layer of 0.76 mm polycarbonate and one layer of mylar. Replicate groups of five albino or five pigmented medaka were stocked in these chambers. The spectral characteristics of ultraviolet radiation that the fish received were measured with an Optronic Laboratories Model 754 spectroradiometer (→ Fig. 1). The appropriate spectral data were then converted to dosage of UVB radiation. Fish in uncovered chambers were exposed to an average UVB dose of 3.7 J/Joules)/cm²/day(d) for 10 days. Under the control conditions only 4.4 x 10⁻⁴ J/cm²/d of UVB radiation penetrated the polycarbonate layers.

2.3 Removal of tissue and preparation of methanol extracts

We also examined the dorsal skin of albino and pigmented medaka from the culture tanks for the presence of a photoprotective substance that appears to protect other fish species from UVB radiation. Six unexposed albino and six unexposed pigmented medaka from the culture tanks were killed by cold shock and the skin of each fish in an area behind the head, in front of the dorsal fin, and above the lateral lines was then scraped with a scalpel [4]. The skin scrapings (outer dorsal skin layers) were weighed and then homogenized in 2 ml of absolute methanol. The homogenate was centrifuged and the pellet was discarded, yielding a methanol extract.

2.4 Absorption spectroscopy and statistical analysis

Methanol extracts were scanned in a Beckman DU 640 spectrophotometer as described previously [4]. The absorption maximum and net absorbance were calculated for each extract by the software in the spectrophotometer. A semiquantitative estimate of the amount of each peak of absorbance was then calculated to yield absorbance units/mg wet weight of tissue. Statistical significance (p<0.05) was determined for each peak amount by a t-test (Corel® Quattro Pro 7, Farmingdale, NY).

3 Results and Discussion

If melanin in the skin of medaka was the primary means of photoprotection, we would have expected albino medaka to be affected by the UVB dose of 3.7 J/cm²/d soon after the onset of exposure and to be considerably less tolerant of UVB radiation than pigmented medaka. However, we found that neither albino nor pigmented medaka developed grossly observable effects after exposure to simulated solar UVB radiation for 10 days. We consider the dose of 3.7 J/cm²/d to be a threshold effective dose for these fish because this dose caused 10% mortality in both albino and pigmented medaka during the 10 day exposure period. None of the control medaka developed grossly observable effects or died during the exposure.

In prior studies we found that the tolerance to simulated solar UVB radiation among other species of pigmented freshwater fish differed and these differences appeared to be related to the amount of a non-melanin photoprotective substance that was found in methanol extracts of the skin [5,6]. This photoprotective substance was observed as a peak of absorption within the UVB wavelength range when methanol extracts were scanned in a spectrophotometer [5,6]. We also observed this peak of absorption at around 294 nm when methanol extracts of outer dorsal skin layers from albino and pigmented UVB-unexposed medaka were scanned in a spectrophotometer (→ Fig. 2). This photoprotective substance may be gadusol [3,5,6-trihydroxy-5-(hydroxymethyl)-2-methoxy-2-cyclohexane-1-one] or a compound similar to gadusol [4,7,8,9].

![Fig. 1: Spectral characteristics of the ultraviolet radiation that control and exposed medaka were treated with](image-url)

![Fig. 2: Example of spectrophotometric scans of outer dorsal skin methanol extracts from albino and pigmented medaka](image-url)