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Journal of Photochemistry and Photobiology B: Biology 56 (2000) 154–162

Journal of Photochemistry and Photobiology B:Biology

www.elsevier.nl/locate/jphotobiol

Comparative effects of UVA and UVB irradiation on the immune system of fish

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Received 21 March 2000; accepted 20 June 2000

Abstract

Aquatic organisms can be harmed by the current levels of solar ultraviolet radiation. We have recently shown that exposure of fish to UVB irradiation alters the functioning of the fish immune system, but the effects of UVA radiation are unknown. The present study continues this work by characterizing UVA irradiation-induced immunological changes in fish. Roach, a cyprinid fish, were exposed to a single dose of either UVA (3.6 J/cm^2) or UVB (0.5 J/cm^2) irradiation. Both irradiations suppressed transiently mitogen-stimulated proliferation of blood lymphocytes. UVA, but not UVB, decreased hematocrit, plasma protein, and plasma immunoglobulin levels and increased the proportions of blood cells classified as unidentified leukocytes, possibly consisting of UVA-damaged lymphocytes. UVB, but not UVA, altered the functioning of head kidney and blood phagocytes, induced granulocytosis and lymphocytopenia in the blood and increased plasma cortisol concentration. These results imply that both UVA and UVB are potent modulators of the immune defence of fish.

Keywords: Ultraviolet radiation; Photoimmunology; Fish immunology; Hematology

1. Introduction

Reduction in stratospheric ozone allows more ultraviolet radiation to reach the earth's surface, the wavelengths most affected being in the ultraviolet B (UVB: 280–320 nm) region with some increase also in ultraviolet A (UVA: 320–400 nm) [1]. Plenty of evidence has been gathered concerning the harmful effects of exposure of fish even to current levels of UVB radiation [2,3]. Enhanced mortalities are the most detrimental consequence of overexposure of fish to UVB [3,4]. The role of UVA radiation as a noxious environmental agent has been much less studied, despite the fact that it comprises the main component of solar ultraviolet radiation and has greater penetration in water than UVB radiation [5–7]. However, some recent reports on the negative effects of UVA indicate its significance. For example, long-term exposure to UVA affects the metabolic performance and survivorship of the convict cichlid *Cichlasoma nigrofasciatum* [8]. The hatching success of medaka (*Oryzias lapites*) is also adversely affected by UVA exposure [9], and an in situ exposure to UVA induced elevated mortality in yellow perch eggs [10]. On the other hand, the survival of Atlantic cod eggs were not negatively affected by exposure to UVA [4].

In mammals, exposure to UVA radiation causes immunomodulation, although its effects are currently less well-defined and are more controversial than those of UVB radiation [11]. For example, mouse contact hypersensitivity reactions to topically applied haptens have been either suppressed [12,13] or unaffected [14–16] following exposure to UVA. However, it is well established that UVA induces the production of immunomodulatory cytokines in keratinocytes [17] and fibroblasts [18] and that UVA is efficient at the photoisomerization of *trans* urocanic acid to the immunosuppressive *cis* form [19]. Furthermore, Langerhans cells, T lymphocytes, NK cells, mast cells and dermal endothelial cells can be listed as immunologically important cells that are affected by UVA radiation [11]. In fish, the immune system is strongly modulated by UVB radiation [20–22], but nothing about the immunological

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1011-1344/00/$ – see front matter
PII: S1011-1344(00)00072-5

effects of UVA radiation is currently known. The present study assesses the modulation of the piscine immune system after exposure to UVA and confirms earlier observations on the immunological effects of UVB in fish.

2. Materials and methods

2.1. Fish

Roach (*Rutilus rutilus*), average weight 34±14 g (±S.D.), were caught by angling from an oligotrophic, uncontaminated lake (Lake Peurunka in Central Finland) in November. The protozoan ectoparasites of roach were killed by bathing the fish with malachite green (0.1 mg/l) and formaldehyde (10 mg/l) for 1 h on every second day during a 10-day period in the beginning of acclimation. The fish were kept in 300-l flow-through tanks filled with dechlorinated and aerated tap water at 17–18°C. The fish were fed daily with commercial dry pellets (FinnEwos Aquo Co., Finland) and kept at a 12/12 h light (300–500 lux, no UV radiation)/dark cycle.

2.2. Irradiation

The fish were transferred from the maintenance tanks to 60-l flow-through aquaria 1 week prior to irradiation. In April, roach were exposed from above to UVA with two unfiltered Philips TL 40W/05 or to UVB with two unfiltered Philips TL 40W/12 lamps for 120 min (Fig. 1). The irradiance penetrating the water was measured with a UVX Digital Radiometer (Ultraviolet Products Inc., San Gabriel, CA, USA) equipped with interchangeable polyethylene plastic waterproofed sensors, UVX-36 (peak sensitivity at 360 nm) and UVX-31 (peak sensitivity at 310 nm). The waterproofed equipment was calibrated with an Optronic 750 spectroradiometer. The UVA dose received by the free-swimming fish was 3.6 J/cm^2 at a mean radiant intensity of 500 μW/cm^2. The UVB dose was 0.5 J/cm^2 at a mean radiant intensity of 74 μW/cm^2.

2.3. Sampling of fish

The fish were sampled on days 1, 7 and 14 following irradiation. Fish were anaesthetised (0.01% MS-222, Sigma Chemical Co., St. Louis, USA) and a blood sample was collected from the caudal vein using a heparinized 1-ml syringe with a 25-gauge needle. Aliquots of whole blood were taken for the assays and the remaining blood was centrifuged (400 *g*, 5 min). Plasma was stored frozen (−70°C) and blood cells were suspended in heparinized Hank's balanced salt solution modified for roach (rHBSS), diluted to 80% with water and supplemented with 0.5 mg/ml sodium pyruvate. Suspended cells were layered on the top of Percoll density gradient medium (1.080 g/ml, Pharmacia, Uppsala, Sweden). After centrifugation (400 *g*, 30 min) the lymphocytes from the interface were collected, resuspended in heparinized rHBSS, and run in an identical Percoll gradient for further removal of erythrocytes. Collected cells were washed twice (400 *g*, 10 min) with rHBSS and resuspended in roach incubation medium (rRPMI): RPMI-1640 diluted to 80% with water and supplemented with 3% Ultroser G serum substitute (Gibco), 0.5 mg/ml sodium pyruvate, 50 mM mercaptoethanol, 2 mM L-glutamine, 100 IU/ml penicillin, 100 mg/ml streptomycin and 10 mM Hepes, pH 7.4. Lymphocytes were counted by Trypan blue exclusion in a haemocytometer (viability >95%) and the number of cells was adjusted to 2×10^6/ml.

The head kidney, a major hematopoietic tissue in teleost

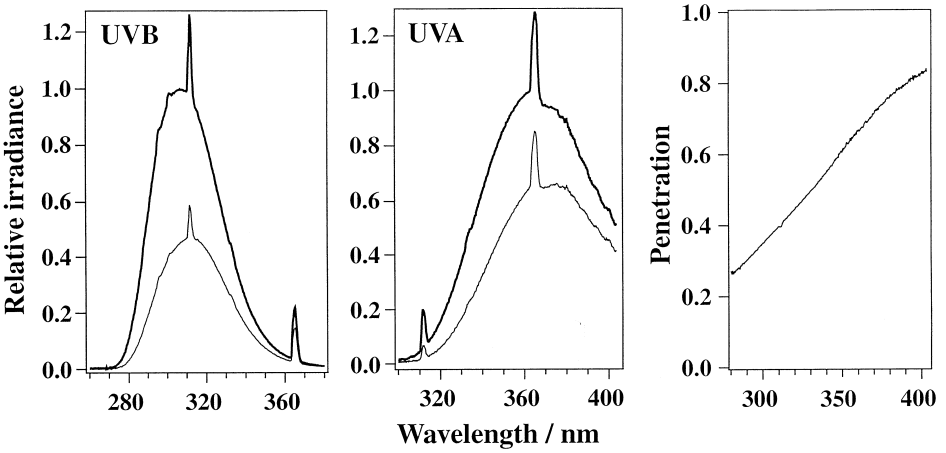

Fig. 1. UV spectra of Philips TL 40W/12 (UVB) and Philips TL 40W/05 (UVA) lamps were recorded using a Hamamatsu PMA-11 spectrograph equipped with a narrow (half width ~10°) entrance angle quartz fiber bundle as a light collector. Maximum irradiances of the continuous spectra in an empty aquarium (bold line) are set to 1. UV spectra in a water filled aquarium (collector 27 cm underwater, thin line) are expressed in the same relative units. The measured spectra represent the direct radiation from the lamp while reflected and scattered radiation remains mostly unobserved. The penetration of water by UV radiation is expressed as a ratio between underwater irradiance and irradiance in an empty aquarium.

fishes, was used as a source of leukocytes. The head kidney was removed and homogenised against a nylon net (80 mesh) in heparinized rRPMI. The homogenate was then layered on the top of a two-step Percoll gradient (Pharmacia LKB Biotechnology AB, Uppsala, Sweden) with densities of 1.070 and 1.090 g/ml. After centrifugation (400 *g*, 30 min), macrophages were collected from above a density of 1.070 g/ml and neutrophils from the interface between densities 1.070 and 1.090 g/ml according to a cell separation method for head kidney leukocytes of common carp [23]. The cells were washed twice and counted with Trypan blue exclusion in a haemocytometer (viability >95%).

2.4. Hematological assays

Hematocrits were determined in heparinized 75-mm hematocrit tubes. Blood leukocytes and erythrocytes were counted after staining [24]. Briefly, 20 μl fresh blood samples were added to a tube containing 20 μl of solution A (0.85 mM Neutral red in 0.15 M NaCl) after which 400 μl of solution B (0.294 mM Crystal violet, 0.11 mM sodium citrate and 0.4% formaldehyde) were added. The unstained erythrocytes and blue-stained leukocytes were counted in a hemocytometer. Blood leukocytes were further identified by differential counting. Thin blood smears were prepared from fresh heparinized blood on microscope slides. The smears were air-dried and stained by a modification of the Wright–Giemsa hematological staining procedure (Diff-Quik, Baxter Diagnostic AG, Germany). A total of at least 200 leukocytes were counted and classified as lymphocytes, thrombocytes, granulocytes, monocytes or other, unidentified, cells under a light microscope using a 63× magnification oil immersion objective. The percentages of the various leukocyte types were calculated.

2.5. Determination of plasma protein, immunoglobulin and cortisol

Plasma samples were thawed and the protein concentration was measured by the modified Lowry method [25] using albumin as a standard. The amount of total IgM in roach plasma was determined by enzyme-linked immunosorbent assay (ELISA) as described by us earlier [26]. Briefly, diluted plasma samples were dispensed into anti-roach immunoglobulin M-coated and albumin-masked wells. The trapped roach IgM was detected with biotin-conjugated anti-roach IgM followed by alkaline phosphatase-conjugated avidin (Biomakor, Israel). *p*-Nitrophenylphosphate (Sigma) was used as a substrate and the optical density was read with a plate reader (Multiskan Plus, Labsystems, Finland) at 405 nm. The assay was standardised with known concentrations of purified roach IgM. The concentration of plasma cortisol was determined using a radioimmunoassay kit (GammaCoat™ Cortisol, INCSTAR Co., Minnesota, USA).

2.6. Lymphocyte proliferation

To determine blood lymphoproliferative responses, aliquots of 200 μl of suspension containing 4×10^5 lymphocytes were added to 96-well plates (Nunc Microwell 96U, Denmark) and the cultures were supplemented with autologous plasma (2%). Cells were activated with Concanavalin A (ConA, 50 μg/ml, Sigma) or lipopolysaccharide (LPS, from *Salmonella typhosa*, 150 μg/ml, Sigma) or were left inactivated. After incubation for 3 days at 26°C 10 μl methyl-[^{3}H]thymidine (0.5 μCi/culture, Amersham International, Buckingshamshire, UK) was added and the cultures were incubated for 18 h and harvested (Cell Harvester, Nunc, Denmark) by water lysis and adherence to glass fibre filters (Whatman Grade 934 AH, Maidstone, UK). The air-dried filters were transferred to vials and 1 ml scintillation liquid was added. Counts per minute (cpm) were recorded by a scintillation counter (Rackbeta 1217, LKB Wallac, Finland). The mean cpm of triplicate wells was calculated.

2.7. Respiratory burst by phagocytes

Phorbol 12-myristate 13-acetate (PMA)-stimulated respiratory burst by whole blood, head kidney granulocytes and head kidney macrophages were determined by the luminol-enhanced chemiluminescence (CL) method [27]. A model 1250 LKB-Wallac luminometer was used to monitor the whole blood CL at 25°C. The 1 ml reaction volume in the polypropylene vial contained 30 μl fresh blood, 10^{-4} M luminol (Sigma) and 2 μg/ml PMA (Sigma) diluted in phenol-red free rHBSS. A microplate luminometer (Victor^2, 1420 Multilabel Counter, Wallac, Finland) was used to monitor the CL by head kidney macrophages and neutrophils at 25°C. The 200-μl reaction volume in the microplate wells contained 2×10^5 cells, 10^{-4} M luminol and 2 μg/ml PMA diluted in phenol-red free rHBSS. The peak CL value was determined from each reaction.

2.8. Spontaneous cytotoxicity by natural cytotoxic cells (NCC)

The NCC activity of whole blood leukocytes against K562 target cells was determined with a ^{51}Cr release assay. In order to obtain heparin-free effectors, the whole blood cells were washed three times. The blood cells were then suspended with rRPMI to yield a whole blood dilution ratio of 1:3. The cultures of K562 targets were maintained in RPMI-1640 containing 10% fetal bovine serum, penicillin (100 U/ml) and streptomycin (100 μg/ml). On the day of the assay K562 ($2–3\times10^6$ cells in 250 μl) were labelled with 150 μCi sodium ^{51}chromate (Amersham Internation-

al) at 37°C for 2 h, washed three times and resuspended at 10^5 cells/ml in RPMI-1640 containing 10% FBS. Labelled K562 cells were pipetted in 100-μl aliquots to round-bottomed 96-well microtiter plates (Nunc). Thereafter 100 μl of effector cell suspension were added to each well. After gentle centrifugation (50 *g*, 2 min) the plates were incubated at 26°C in an atmosphere of 5% CO_2 for 18 h. Supernatants (100 μl) from each well were harvested after centrifuging the plate (400 *g*, 5 min) and counted in a gamma counter (LKB-Wallac RackGamma II 1270, Finland). Percentage cytotoxicity was calculated from the equation:

$$\%\ \text{Cytotoxicity} = 100 \times \frac{\text{experimental cpm} - \text{spontaneous cpm}}{\text{maximal cpm} - \text{spontaneous cpm}}.$$

Spontaneous-release counts were obtained from targets incubated in the absence of effector cells. To obtain maximal release-counts, 100 μl of 5% SDS were added to the wells containing the targets. Each value for ^{51}Cr-release is the mean of triplicate wells.

2.9. Statistics

The Mann–Whitney *U*-test was used to determine the differences between the UV exposed and non-exposed groups of fish. Probabilities of less than 0.05 were considered to indicate significance ($^*P<0.05$, $^{**}P<0.01$ and $^{***}P<0.001$). Data are expressed as arithmetic means of the indicated number of fish ±S.E. of the mean.

3. Results

3.1. Hematology and plasma chemistry

Exposure of roach to UVA radiation decreased hematocrit by 12% ($P<0.05$) and the number of white blood cells by 10% (non-significant) on day 1 post-irradiation (Table 1). No significant changes were observed in hematocrit values or in blood cell counts on days 7 or 14 post-UVA irradiation or in UVB-exposed fish. Both UVA and UVB irradiation markedly changed the proportions of blood leukocytes (Table 1). On day 1 following the UVA exposure the proportion of unidentified cells among the blood leukocytes increased twofold ($P<0.001$). On day 1 following the UVB exposure the proportion of lymphocytes in fish blood decreased by 40% ($P<0.001$) and the proportion of granulocytes increased sixfold ($P<0.001$) when compared to control values. The recovery was fast, however, and on days 7 and 14 following both the UVA and UVB irradiations no marked changes in leukocyte percentages were observed.

Plasma protein levels were not markedly changed by either types of UV exposure (Fig. 2a). However, plasma IgM concentrations decreased on day 1 by 35% ($P<0.05$) and on day 7 by 25% (non-significant), but increased slightly on day 14 following UVA irradiation (Fig. 2b). Exposure of fish to UVB had no marked effects on plasma IgM levels. Cortisol concentrations in the fish plasma were significantly increased on days 1 and 7 after UVB irradiation, but exposure to UVA had no effect on plasma cortisol level (Fig. 2c).

3.2. Lymphocyte proliferation

Proliferation of blood lymphocytes was suppressed transiently after both the UVA and UVB irradiations (Table 2). Depending on the mitogenic agent used, UVA caused a 73–89% decrease and UVB a 44–67% decrease in lymphocyte proliferation on day 1 post-irradiation, but the decrease did not reach statistical significance due to large individual variation in proliferative responses. ConA- and non-stimulated proliferative responses returned to control levels by day 7 post-UVA irradiation and by day 14 post-UVB irradiation. LPS-stimulated proliferation remained suppressed for a longer time, returning to control

Table 1
Hematological parameters of roach on days 1–14 after exposure to UVA or UVB irradiation

	Control	UVA			UVB		
	Days 1–14[a] ($n=40$)	Day 1 ($n=13$)	Day 7 ($n=12$)	Day 14 ($n=11$)	Day 1 ($n=14$)	Day 7 ($n=13$)	Day 14 ($n=10$)
Hematocrit (%)	43±1	38±2*	40±1	41±2	42±3	46±2	43±3
RBC ($\times10^6$/mm^3)	1.5±0.1	1.4±0.1	1.6±0.1	1.5±0.1	1.5±0.1	1.5±0.1	1.6±0.1
WBC ($\times10^3$/mm^3)	49±3	44±5	46±7	49±7	47±5	53±6	49±4
Differential counts							
Lymphocytes (%)	56±2	51±3	60±2	59±3	33±4***	57±3	61±2
Thrombocytes (%)	32±2	27±5	29±3	31±3	27±4	31±2	28±2
Granulocytes (%)	6±1	9±3	6±1	4±1	35±4***	8±1	6±1
Monocytes (%)	2±1	4±1	2±1	1±1	2±1	2±1	2±1
Others (%)	4±1	9±1***	3±1	4±1	3±1	2±1	4±1

[a] Data from control fish on days 1–14 are pooled.
* $P<0.05$ vs. control.
*** $P<0.001$ vs. control.

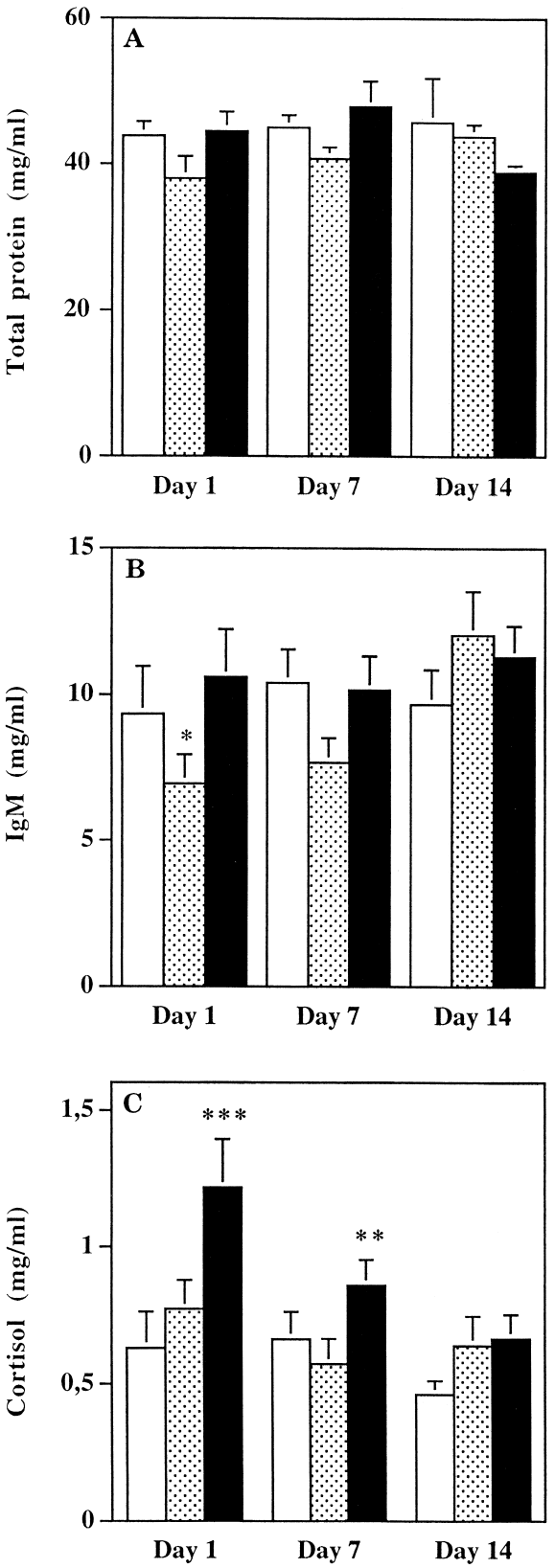

Fig. 2. Concentrations of plasma protein (A), plasma immunoglobulin (B) and plasma cortisol (C) in non-exposed (open bars), UVA-exposed (shaded bars) and UVB-exposed (black bars) fish on days indicated. Each bar represents mean + S.E., $n = 11–14$.

levels by day 14 post-UVA irradiation but remaining reduced throughout the whole 14-day follow-up in UVB-exposed fish. In contrast, ConA-stimulated proliferation was enhanced on day 14 after both UV irradiations.

3.3. Respiratory burst by phagocytes

Exposure of fish to UVA radiation had no significant effects on the respiratory burst responses by fish phagocytes (Fig. 3). On day 1 following UVB exposure, the PMA-stimulated respiratory burst by whole blood increased 10-fold ($P < 0.001$) returning to the control levels by day 7 post-irradiation (Fig. 3a). The respiratory burst by head kidney granulocytes and macrophages fell to one fourth of control values ($P < 0.001$ for both) on day 1 after UVB irradiation (Fig. 3b and c). The respiratory burst by head kidney granulocytes returned to control levels by day 7 post-UVB irradiation. On the other hand, the respiratory burst by macrophages was almost twofold on day 7 ($P < 0.05$) returning to the level of controls by day 14.

3.4. Spontaneous cytotoxicity

Spontaneous cytotoxicity by blood leukocytes was 15–35% lower in UVA-irradiated and 5–60% lower in UVB-irradiated fish than in non-irradiated controls, but the decreases did not reach statistical significance (Fig. 4).

4. Discussion

This is the first study investigating the effects of UVA irradiation on the immune system of fish. UVA, as well as UVB, irradiation of roach led to transient impairment in blood lymphocyte proliferation. Exposure of roach to UVA also decreased hematocrit and plasma IgM levels, neither of which occurred following UVB exposure. In contrast, UVB-exposure induced strong granulocytosis and lymphocytopenia in the blood, suppression of head kidney phagocyte function and increase in plasma cortisol levels, whereas none of these effects were noted in UVA-exposed fish.

Under natural conditions fish may try to avoid exposure to solar radiation by escaping to deeper waters or to shade. In clear and shallow waters and in fish farming, however, fish are subject to exposure to solar radiation. In the present study fish received a dose of either 3.6 J/cm^2 UVA or 0.5 J/cm^2 UVB during a 2-h irradiation period. In Finland's latitudes (65° N) a corresponding UVB dose can be received outdoors in about 5 h in a cloudless summer day (calculated from Ref. [28]). A corresponding UVA dose can be achieved in an even shorter time period, since generally more than 95% of the solar ultraviolet radiation reaching the earth's surface is UVA [29]. However, the comparison between UV doses produced artificially by lamps and UV doses in the wild is not easy, because of the

Table 2
The mitogen-stimulated proliferation[a] of blood lymphocytes on days 1–14 following the indicated exposure

Mitogen	Exposure	Day 1 (n=4–7)	Day 7 (n=4–6)	Day 14 (n=4–6)
ConA	Non-exposed	3020±1070	11 550±4780	6960±2710
	UVA	830±430	18 990±10 610	24 940±12 970
	UVB	1700±1440	5390±3050	27 530±16 600
LPS	Non-exposed	1320±530	2850±1080	2300±830
	UVA	150±70	1460±320	2480±1270
	UVB	430±160	1520±480	1470±330
None	Non-exposed	180±70	160±60	110±50
	UVA	40±10	210±20	180±100
	UVB	60±10	100±20	270±110

[a] Proliferation is expressed as incorporated radioactivity (cpm, mean±S.E.).

differences in the spectral radiances of lamps and the sun and in the penetration of UV radiation into different waters [6,7].

Fish possess lymphocytes functionally equivalent, in many aspects, to mammalian B and T cells [30]. In the present study a T cell-specific mitogen, ConA, and a B cell-specific mitogen, LPS, were used to activate roach blood lymphocytes to proliferate in vitro. The lymphoproliferative responses varied greatly between individual roach, a phenomenon observed in other fish species as well [31]. Exposure of fish to both UVA and UVB irradiation transiently suppressed both ConA- and LPS-stimulated as well as non-stimulated proliferation. Suppressed lymphoproliferation returned to normal during the 14-day follow-up, and the recovery was faster in UVA-exposed than in UVB-exposed fish. Suppressed lymphoproliferative responses have similarly been noted in humans and in mice exposed to a single dose of UVA or UVB [13,32,33]. However, exposure of rats for 3–7 successive days to suberythemal doses of UVB resulted in enhanced lymphoproliferative responses to T cell-specific mitogens [34]. In the present study ConA-stimulated proliferation returned to normal in a shorter time than LPS-stimulated proliferation. Indeed, T-like cell proliferation by ConA was even enhanced on day 14 after both UV irradiations. Taken together, these results indicate that exposure both to UVA and to UVB radiation suppresses proliferation of piscine blood lymphocytes and that lymphoproliferation recovers more rapidly from UVA-induced suppression than UVB-induced suppression, and that T-like lymphocytes recover from the UV-induced suppressed stage sooner than B-like lymphocytes.

Whole blood natural cytotoxic cell (NCC) activity was at lower level after both UVA and UVB irradiation when compared to the non-irradiated control fish. In our previous studies NCC activity by roach whole blood was also slightly and NCC activity by head kidney neutrophils markedly suppressed following UVB exposure [20,21]. In human studies decreased NK-cell activity has been demonstrated after exposure to both UVA and UVB irradiation [35,36]. Since fish NCCs are important in defense against transformed and virus-infected cells, the results of our studies suggest that these defenses might be decreased by both short and long wavelength ultraviolet radiation.

Phagocytosis is an essential component of the innate defence against invading microorganisms. The successful destruction of pathogenic microbes during phagocytosis requires the production of reactive oxygen species (ROS) in a process known as respiratory burst. In the present study ROS production by macrophages and granulocytes, the professional phagocytes isolated from the head kidney, were strongly suppressed on day 1 following UVB radiation, but unaffected after UVA exposure. In cyprinid fish the head kidney is the major phagocytic organ [37], and the suppression of functions by head kidney phagocytes, observed in the present as well as in our previous study [20], suggests that exposure of fish to UVB, but not to UVA, may have negative effects on this first-line defense mechanism. The capacity of phagocytes to produce ROS, however, was restored during the first week after UVB irradiation and macrophage ROS production was even significantly elevated on day 7 post-irradiation, as reported in our previous study [20]. We have at the moment no explanation for this state of overreactivity by macrophages.

The whole blood respiratory burst responses were increased in UVB-exposed fish. This is probably due to the marked increase of granulocytes in the circulation following the UVB exposure. Granulocytosis and lymphocytopenia together with increased cortisol levels in the present and earlier study [21] support the consideration of UVB as a potential environmental stressor. In mammals certain aspects of immunity are depressed during stress, while those at the periphery are enhanced [38,39]. This may facilitate immune challenge at the site of entry of microorganisms into the organism. Our results with UVB-induced stress-related changes in the immune system of fish are well in line with these mammalian results. No similar stress-related changes were observed following UVA exposure.

In the differential cell counting blood leukocytes were distinguished on the basis of their morphological and staining properties [40,41]. According to these criteria,

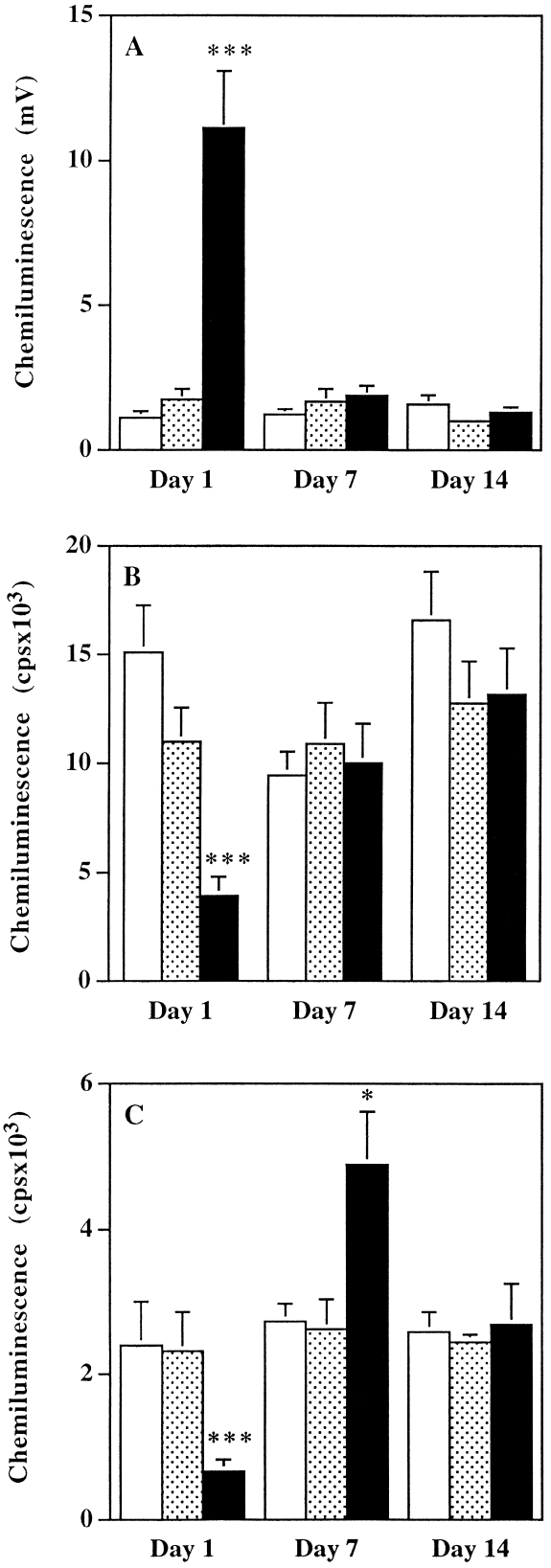

Fig. 3. Respiratory burst activity of whole blood (A), head kidney granulocytes (B) and head kidney macrophages (C) of non-exposed (open bar), UVA-exposed (shaded bar) and UVB-exposed (black bar) fish on days indicated. Each bar represents the mean+S.E. of chemiluminescence, $n=13$–14 in A and $n=6$–7 in B and C.

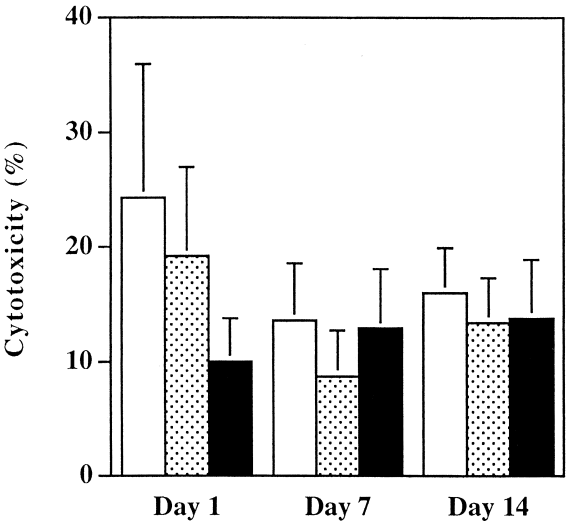

Fig. 4. Spontaneous cytotoxicity by whole blood of roach against K562 cells. Percentage cytotoxicity (mean±S.E., $n=5$–7) for non-exposed (open bar), UVA-exposed (shaded bar) and UVB-exposed fish (black bar) were determined by the ^{51}Cr-release method on days indicated.

there was an increase in the number of cells in the blood of UVA-exposed fish that fell in-between lymphocytes and monocytes and were classified as unidentified cells. The distinction between lymphocytes and monocytes is difficult in other fish species as well [40]. In the present study the proportion of lymphocytes in the blood decreased from 56% (controls) to 51% following UVA exposure, with simultaneous suppression in the functioning of blood lymphocytes (proliferation). Lymphocytes are very sensitive to the damaging effects of UV radiation [42] and it is possible that the unidentified cells may be UVA-damaged lymphocytes.

In fish, rapid alterations of serum protein levels are mainly caused by changes in blood volume [43]. Following UVA exposure, decreased plasma protein and IgM concentrations, decreased hematocrit as well as a decreased number of white blood cells were observed, suggesting that these UVA-specific changes in fish are linked to a UVA-induced increase in blood volume.

Roach can see in UVA region [44] and most probably sense the radiation from both broad band lamps used in the present study via eyes. However, visible light is not sufficient to induce immunological changes in roach [21] suggesting that the skin plays a pivotal role in UV-induced immunosuppression in fish, like in mammals. In the skin of platyfish (*Xiphophorus*) the more energetic UVB radiation induce DNA-damage more efficiently than UVA radiation [45]. On the other hand, in mammals and most probably also in fish, tissue transmission increases with wavelength allowing UVA to reach targets well below the surface of the skin, and it is possible that a small quantity of long wavelength UV can be absorbed by blood components [46]. Differences in the ability to induce DNA-damage and in penetration into the skin may be responsible for the

different effects following exposure to UVA and UVB irradiation.

Taken together, it is obvious that both UVA and UVB radiation have notable effects on the immune system of fish such as roach. However, it is not clear whether ultraviolet radiation-induced alterations of immune parameters lead to compromised disease resistance in fish. Further research is needed to elucidate this aspect.

Acknowledgements

This work was supported by the Academy of Finland and Finnish Graduate School in Environmental Science and Technology. Professor Aimo Oikari is acknowledged for critical reading of the manuscript.

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