

Effects of Ultraviolet Radiation on Some Immunological Parameters in Rats

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Abstract: This study was carried out to investigate the effects of ultraviolet A (UVA) and ultraviolet B (UVB) radiation on some immunological parameters in rats. Twenty seven, 8-10 weeks old, male Wistar Albino rats were equally divided into three groups as control, UVA and UVB. UVA and UVB group rats were irradiated 6 h per day with UVA and UVB light during 60 days. Blood were taken from the rats in the control and experimental groups on days 0, 30, 45 and 60 and total leukocyte counts, differential leukocyte counts, T and B lymphocyte percentage and immunoglobulin G (Ig G) levels were determined. Total leukocyte number was significantly ($p<0.05$) higher in UVB group compared with control group on day 45. Lymphocyte percentage of the UVB group on days 30, 45, 60 were significantly ($p<0.05$) reduced compared with UVA and control groups. Neutrophil percentage was significantly lower ($p<0.05$) in the UVA group than control and UVB groups on days 30, 45 and 60. Eosinophil percentage of the UVB group on day 30 was significantly ($p<0.05$) higher compared with UVA and control group values on the same day. A significant increase ($p<0.05$) of T lymphocyte percentage on days 30, 45, 60 and a significant decrease ($p<0.05$) of B lymphocyte percentage on days 45 and 60 were observed in UVA and UVB groups compared with control group. IgG levels were decreased significantly ($p<0.05$) on days 30, 45 and 60 in UVA and UVB groups compared to control group. The results showed that both UVA and UVB radiation have suppressive effects on some humoral and cellular immune parameters in rats.

Key words: Ultraviolet radiation % leukocyte % lymphocyte % IgG % rat

INTRODUCTION

In recent years, Ultraviolet radiation (UV) reaching to the earth due to the reduction of the ozone layer has been noticed in terms of potential health hazard on humans, animals and environment [1-4]. Ultraviolet radiation has wavelengths in the range of 200-400 nm and is divided into three wavebands, UVA (320-400 nm), UVB (280-320 nm) and UVC (200-280 nm) [2, 5, 6].

Besides the beneficial effects of UV light exposure, such as vitamin D production, UV exposure can also have adverse consequences on animal and human health, notably sunburn, skin cancer and ocular damage. Over the last few decades, it has become evident that UV exposure also impairs specific and nonspecific immune responses [2, 4, 5, 7]. UVB induced immunomodulation plays at least a partial role in photocarcinogenesis. Experimental animal studies have also revealed that UVB

exposure can impair the immunological resistance to viral, fungal, bacterial infections, parasitic diseases and antigenic tumours [4, 8, 9].

In animals, exposure to UVA radiation causes immunomodulation, although its effects are currently less well defined and more controversial than those of UVB radiation [5, 10, 11]. UVA has longer wavelengths than UVB and thus penetrates deeper into skin. It is estimated that about 19 to 50% of the solar UVA can reach the depth of melanocytes, whereas only about 9 to 41% of solar UVB reaches the melanocytes [12]. Recently, some studies have shown that UVA is at least as mutagenic and carcinogenic as UVB, due to the deeper penetration of UVA as compared to UVB [13, 14].

Most of the UV studies have been intensified on the skin and eye that directly exposed to UV radiation. UVA and UVB exposure induce the production of immunomodulatory cytokines in keratinocytes and

Table 1: Total dose (j/cm²) of UVA and UVB irradiation received by the free-moving rats on days 1, 30, 45 and 60

Groups	Days			
	1	30	45	60
UVA	4.32	129.6	194.4	259.2
UVB	0.46	13.8	20.7	27.6

melanocytes of the skin, such as interleukin-1 (IL-1), IL-6, IL-8, IL-10, IL-12, IL-15, tumor necrosis factor alpha (TNF- α), granulocyte macrophage colony stimulating factor (GM-CSF); prostaglandins; growth factors; endothelins and neuropeptides [7, 15, 16]. It has been expected that some alterations might be occurred in the organs and systems except the skin and eye that directly exposed to UV radiation as a result of transforming these mediators via the blood. Therefore the objective of this study was to investigate and compare the effects of UVA and UVB radiation on IgG level and cellular immune parameters such as total leukocyte counts, differential leukocyte counts, T and B lymphocyte percentage.

MATERIALS AND METHODS

Animals: A total of 27, male, 8-10 weeks of age Wistar Albino rats obtained from Ministry of Health, Institute of H2fz2ss2hha, Ankara, Turkey were used in this study. The rats were housed in polycarbon cages (30x60x40 cm) with unlimited access to standard rat chow and top water. They were waited for the adaptation of their new environment for a month before UV irradiation. The rats were equally divided into three groups as control, UVA and UVB group. The study was approved by the local ethical committee.

UV irradiation: Rats were shaved dorsally with electrical clippers under mild ether anesthesia. The rats per cage were allowed to move freely and were irradiated within their cages. The rats' back were at a distance of 25 cm from the UV lamps. At this distance, UVA irradiation was 0.2 mW/cm² and UVB irradiation was 21 μ W/cm², measured with a Newport 1830-C Silicon Radiometer. UVA and UVB group rats were irradiated 6 h per day with UVA (Universal F8T5BLB) and UVB (Ultra-Lum, F8T5E) light during 60 days. UV light was given at 10.00-16.00 h. The total doses of the UVA and UVB irradiation received by the free-moving rats on days 1, 30, 45 and 60 were given in Table 1. Control group rats were shaved similarly and were not exposed to UV irradiation.

Sample collection and analyses: Blood were taken from the rats in UVA and UVB groups before UV irradiation

(on day 0) and on days 30, 45 and 60 of the irradiation period from the tail vein. Blood were also taken from the control rats on the same days by the same method.

Total leukocyte counts were determined using Türk's solution. Leukocyte differential counts were obtained in blood smear stained with May-Grunwald-Giemsa stain and calculated the percentage of each of the five basic leukocytes (lymphocytes, neutrophils, monocytes, eosinophils and basophils) [17]. Peripheral blood T lymphocyte percentages were determined according to Mueller *et al.*, [18]. Briefly, for "naphthyl acetate esterase (ANAE) demonstration, blood smears were prepared from blood samples and dried in air. They were then fixed in a mix of glutaraldehyde and acetone solution for 3 min at -10°C, rinsed in distilled water and dried in air. They were then incubated in the incubation solution (pH 5.8) for 3 h, rinsed with distilled water and counterstained with methylene blue for 10 min. After application of ANAE enzyme staining, they were examined under a light microscope. The lymphocytes in every smear were determined by counting 300 lymphocytes totally under a light microscope.

For serum isolation, blood samples in non-heparinized tubes were centrifuged at 1300 g for 15 min. Isolated sera were stored at -20°C. Total IgG concentrations in individual serum samples were measured automatically by the BNII Nephelometric Immunoassay of Dade Behring.

Statistical analysis: Data were analysed using the SPSS for Windows software, Version 11.0 (SPSS Inc., Chicago, IL, USA). Statistically significant differences between groups were determined by analysis of variance (ANOVA). When the differences were significant, Duncan's multiple range test was performed. Means were considered significantly different at $p < 0.05$.

RESULTS

The effects of UVA and UVB radiation on total and differential leukocyte counts, T and B lymphocyte percentages and IgG levels were presented in Table 2.

Total leukocyte number was significantly higher ($p < 0.05$) in UVB group according to control group on day 45. Lymphocyte percentage of the UVB group on days 30, 45 and 60 were significantly ($p < 0.05$) reduced compared with UVA and control groups. Neutrophil percentage was significantly lower ($p < 0.05$) in UVA group than control and UVB groups on days 30, 45 and 60. Neutrophil percentage of UVB group on day 60 was significantly higher ($p < 0.05$) than control and UVA groups. Eosinophil

Table 2: Immunological findings from control and exposed animals to UVA and UVB light according to exposure time (30, 45 and 60 days)

Variables	Groups	n	Days			
			0	30	45	60
Leukocyte (x 10 ³ /mm ³)	Control	9	9.48±11.06	9.46±7.01	8.68±5.56 ^a	8.51±6.32
	UVA	9	9.10±7.50	9.65±6.35	9.91±6.77 ^{ab}	6.79±6.32
	UVB	9	9.24±10.08	10.67±10.07	10.81±6.63 ^b	7.13±8.48
Lymphocyte (%)	Control	9	71.44±2.81	72.01±2.15 ^a	70.00±2.41 ^a	71.33±1.81 ^a
	UVA	9	75.10±2.57	79.30±1.83 ^a	78.30±1.50 ^a	73.90±2.82 ^a
	UVB	9	70.44±1.58	63.00±3.24 ^b	62.56±4.17 ^b	54.44±2.77 ^b
Neutrophil (%)	Control	9	21.22±2.47	20.89±2.47 ^a	22.56±2.30 ^a	21.19±2.53 ^a
	UVA	9	16.60±2.47	13.80±1.68 ^b	14.10±0.60 ^b	15.90±2.37 ^b
	UVB	9	21.78±1.24	26.68±2.66 ^a	27.11±3.75 ^a	35.67±2.67 ^c
Monocyte (%)	Control	9	3.78±0.49	2.78±0.32	3.22±0.57	2.67±0.37
	UVA	9	3.20±0.58	2.90±0.64	3.20±0.70	2.90±0.57
	UVB	9	3.44±0.47	3.11±1.10	3.78±0.97	2.89±0.39
Eosinophil (%)	Control	9	3.56±0.60	4.11±0.59 ^a	3.89±0.70	4.78±0.60
	UVA	9	5.00±0.93	3.70±0.40 ^a	4.00±0.97	7.00±0.70
	UVB	9	4.11±0.84	7.00±1.25 ^b	6.44±1.19	6.67±1.01
Basophil (%)	Control	9	0.11±0.10	0.22±0.15	0.22±0.15	0.11±0.10
	UVA	9	0.10±0.13	0.10±0.13	0.30±0.15	0.20±0.13
	UVB	9	0.22±0.15	0.22±0.15	0.11±0.10	0.33±0.24
T lymphocyte (%)	Control	9	90.21±0.71	89.94±0.45 ^a	89.36±0.62 ^a	89.36±0.58 ^a
	UVA	9	87.88±0.83	92.39±0.52 ^b	93.74±0.56 ^b	95.00±0.54 ^b
	UVB	9	88.31±1.03	92.22±1.41 ^b	92.52±0.45 ^b	93.14±1.12 ^b
B lymphocyte (%)	Control	9	9.78±0.70	10.05±0.61	10.63±0.60 ^a	10.63±0.56 ^a
	UVA	9	12.12±0.81	7.61±0.50	6.26±0.53 ^b	5.00±0.53 ^b
	UVB	9	11.68±1.01	7.77±1.40	7.47±0.43 ^b	6.85±1.10 ^b
IgG (mg/mL)	Control	9	3.41±0.13	3.37±0.12 ^a	3.34±0.11 ^a	3.38±0.12 ^a
	UVA	9	3.50±0.07	2.90±0.05 ^b	2.29±0.08 ^b	1.97±0.06 ^b
	UVB	9	3.42±0.16	2.67±0.12 ^b	2.04±0.12 ^b	1.95±0.03 ^b

Data are expressed as mean ± SEM

a,b: Mean values with different superscripts in the column are significantly different (p<0.05)

Fig. 1: ANAE staining in a rat. Arrows: ANAE positive granules in a T lymphocyte, B: B lymphocyte, 600x

percentage of the UVB group on day 30 was significantly ($p < 0.05$) higher compared with UVA and control group values on the same day. No significant differences ($p > 0.05$) were observed between groups on the basis of monocyte and basophil percentages.

Examination of the stained slides revealed the ANAE activity in lymphocytes, characterized by the presence of single or multiple small reddish-brown granules in the positive lymphocytes (T lymphocytes) (Fig. 1, arrow). The lymphocytes that weren't characterized by these granules defined as B lymphocytes (Fig. 1, B).

A significant increase ($p < 0.05$) of T lymphocyte percentage on days 30, 45, 60 and a significant decrease ($p < 0.05$) of B lymphocyte percentage on days 45 and 60 were observed in UVA and UVB groups compared with control group. Immunoglobulin G levels were decreased significantly ($p < 0.05$) on days 30, 45 and 60 in UVA and UVB groups compared to control group.

DISCUSSION

In this study, we observed an increase of total leukocyte number in UVB group on day 45. This finding is similar to those of Hansen and Ebbesen [19] in mice, but is inconsistent with Falkenbach and Sedlmeyer [20] in humans and Salo *et al.*, [21, 22] in fish reported no changes in leukocyte number with UVB application. Our result is also not parallel with Özcan *et al.*, [23] in rats and Lundin *et al.*, [24] in humans finding a decrease with UVB exposure. Various results found in the literature are probably due to the differences in animal species, time of exposure and methods of UV applied. It has been reported that even strain differences are the cause of different immune responses to UV radiation [9, 25]. Total leukocyte number of UVB increased on day 45 due probably to the secretion of IL-1 from melanocytes and keratinocytes of skin via effects of UVB radiation. IL-1 activates bone marrow to release its leukocyte stores into the circulation [26]. Very little work has been performed about the effect of UVA on total leukocyte number. Our results are consistent with Mc Grath *et al.*, [27] in humans and Salo *et al.*, [22] in fish observing no changes in total leukocyte number with UVA exposure.

Our results are consistent with those of Matsuoka *et al.*, [25] and Mc Grath *et al.*, [28] in humans, Jokinen *et al.*, [29] and Salo *et al.*, [21, 22] in fish in terms of decreases in lymphocytes ratios with UVB, but are inconsistent with those of Özcan *et al.*, [23] in rats, Falkenbach and Sedlmeyer [20] in humans finding no changes in lymphocyte percentage with UVB application. In the present study, there was no effect of UVA on

lymphocyte percentage. Similar results have been shown in humans [30] and in fish [29].

It has been thought that UVB radiation may be a source of stressor on animals increasing ratio of granulocytes but decreasing ratio of lymphocytes. Salo *et al.*, [22] and Jokinen *et al.*, [29] observed an increase in serum cortisol concentrations along with formation of granulocytosis and lymphocytopenia in fish being stressed via exposing UVB. The same authors, however, did not detect any changes in the same parameters with UVA. Release of IL-1 is increased by UVB. Interleukin 1 causes neutrophils pass into the blood from the bone marrow. Therefore, neutrophil percentage in UVB group can be increased on day 60 [26]. The reason for decreases of lymphocyte percentage in UVB group can be due to migration of lymphocytes from peripheral blood to lymph nodes under the effect of UVB radiation [31]. In case of UVA radiation, an *in vitro* PUVA study in rats showed no effect of UVA in ability of lymphocytes migration [32]. These results support the findings of the present study in terms of no changes of lymphocyte percentage in UVA group.

In the present study, increases in eosinophil percentage in UVB treatment on day 30 could have been due to formation of erythema by UVB and increases in eosinophil percentage in skin reactions. Also, release of IL-4, IL-6 and IL-10 cytokines increases with the effect of UV radiation [7, 15]. These cytokines activate eosinophils and play an important role in inflammatory reactions related to allergy [4, 33].

Our results are similar to researchers who found an increase in T lymphocyte percentage under UVA and UVB and a decrease in B lymphocyte percentage in mice [34] and in humans [35, 36]. Studies have showed that UV radiation changes the ratio of lymphocytes subgroups in peripheral blood [1, 37]. Response of lymphocyte associated with an increase in IL-2 reflects T lymphocyte percentage which is parallel with an increase in T lymphocyte subtypes responsible for formation of that cytokines [27, 33].

Antigenic cells include macrophages, Langerhans cells, fibroblasts, keratinocytes, lymphocytes and granulocytes in the skin [33]. Antigenic cells produce major histocompatibility complex (MHC) class II molecules similar to that of immune response linked antigens (Ia), play a role in presenting antigen into the immune system and identifying the antigen of the immune system and form a specific immune response. It has been reported that UV light can be used in treatment of certain diseases caused by allergic reactions [rheumatoid arthritis, allergic encephalomyelitis] via

inhibiting immune response through weakening formation of Ia in antigen producing cells [27, 38]. It has been also thought that UVA is more effective than that of UVB in treatment of depressing formation of Ia [38, 39].

In the present study, an important decrease in IgG level starting from day 30 in rats received UVA and UVB is consistent with the results obtained in humans [20], rats [23] and mice [40], but is inconsistent with the results of Kasahara *et al.*, [41] observed in mice. Different results could be due to a low dose and shorter exposure time of UVB in the work of Kasahara *et al.*, [41] compared with other studies.

A decrease in IgG level in groups receiving UVA and UVB treatments in the present study could be due to activation of UV radiation on suppressor T lymphocytes [35, 36]. Suppressor T lymphocytes inhibit formation of immunoglobulin via inhibiting functions of B lymphocytes producing immunoglobulin [33]. A study in mice exposed to UVB radiation showed that production of IgG_{2a} and IgG_{2b}, mediating agents of helper T lymphocytes are depressed [40]. B lymphocytes can not produce immunoglobulin without the help and mediation of T lymphocytes [33]. However, UV radiation depresses the immune response of helper T lymphocytes [4, 40, 41]. In addition, a decrease in B lymphocyte percentage was a cause of depressed IgG level released by B lymphocytes.

Although not significantly, IgG levels were lower in animals receiving UVB treatment compared with animals receiving UVA on days 30, 45 and 60. The reason why UVB decreased IgG concentration more than that of UVA could be due to stronger effect of UVB in suppressing immune system [4, 42].

Taken together, these findings suggest that some humoral and cellular immune parameters of rats are significantly affected by exposure to UVA and UVB. In addition, we can say that compared with UVA, UVB is more effective in rats for reducing lymphocyte count.

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