



Natural and Induced UV Radiation Effects on Haematological and Genetic Characteristics of Albino Mice

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Abstract: This is the first study on ICR albino mice continuously exposed to natural UV radiation in comparison with a parallel laboratory experiment in order to verify the naturally obtained effects on the animal organism. The mice were exposed at the Moussala Basic Ecological Observatory (2925 m a.s.l.) in Rila Mountain and in the laboratory, simulating the same radiation intensity. Results regarding the genotoxic effect of the UV background on the experimental animals are presented. Different types of early mutagenic effects in blood cells such as micronuclei in erythrocytes and diverse chromosome aberrations such as breaks, fragments, centromeric and telomeric fusions in the bone marrow cells were observed. A gradual increase of micronuclei from the 15th to the 40th day of the experiment was observed. The percentage of metaphases with aberrations in the samples from both Peak Moussala and laboratory varied similarly from about 8.75 ± 0.75 % on the 15th day after the beginning of irradiation and increased slightly on the 30th day, reaching a maximum value of 10.75 ± 0.92 % on the 40th day of the experiments. These experiments lead to the conclusion that the environmental impact of natural UV radiation induces abnormal DNA changes, harmful for the animal organism, especially at higher altitudes.

Key words: Albino mice, UV irradiation, High Mountain, Genotoxic effects

Introduction

For the last twenty years, UV radiation has been extensively studied in laboratory or in field conditions (WHITMORE et al. 2008). The UV part of the electromagnetic spectrum may be considered a stress factor, especially when combined with other factors (temperature, altitude) or with environmental air pollutants, acid rain, etc. (METCHEVA et al. 2019). The effects

of natural UV exposure on the animal organisms in chronic experimental conditions are presented in the present study. Additionally, to verify the expected results and to eliminate confounding variables, a laboratory experiment was conducted under a strictly controlled temperature, humidity and light-dark regime.

Natural sunlight on Earth contains much more UV-A than UV-B radiation, generating a potential for UV-A to have a larger contribution to skin dam-

age than UV-B. UV radiation interacts with mammalian cells in a complex manner (MOAN & PEAK 1989). It is classified as a powerful carcinogen and its injury to living cells is, to some extent, due to oxidative stress. Additionally, pyrimidine dimers are formed in mammalian DNA, leading to mutagenic and oncogenic potential (FREEMAN et al. 1989). Pyrimidine dimers constitute approximately 80 % of the observed UV-induced DNA photoproducts and are thought to be the main cause of melanoma-type skin cancers (PFEIFER 1997, WHITMORE et al. 2008).

The biota at high elevations is generally exposed to more solar radiation and, along with it, to more UV-B, in comparison with organisms at low elevations. At high elevations, UV-B radiation penetrates through less atmospheric air before reaching the ground, and so it has a smaller statistical probability of encountering radiation-absorbing molecules such as ozone and sulphur dioxide than it does at lower elevations.

The purpose of this study was to examine haematological parameters of chronically exposed ICR albino mice and genetic damage resulting from the combined UV-A and UV-B radiation spectrum at two altitudes in Rila Mountain. In addition, we compare the obtained data with a laboratory-based experiment in order to prove the real degree of effects.

Materials and Methods

Animals

One hundred and five healthy male albino ICR mice with av. 26.7 ± 1.9 g body weight were randomly divided into 3 groups. The first group of 35 mice was transported to the chosen experimental points – Peak Moussala, the second – to the area of Beli Iskar and the third group was reared under laboratory conditions. In addition, ten control animals were examined. The animals were housed in plastic cages, kept in groups of 6 per cage and fed a standard diet and water *ad libitum*. In every cage, a bundle of hay was placed to provide shelter. The cages were deployed in specially designed packing-cases with Plexiglas walls, permeable for all types of UV radiation.

The temperature in the cages under natural conditions was maintained between 18 and 25°C by natural ventilation. The packing-cases were kept outdoors at their locations for 12 hours (every day from 8 a.m. to 8 p.m.) for a period of 40 days. The body weight of the animals was measured weekly. On the 15th, 30th and 40th day of each of the experiments peripheral blood samples were collected from the tail vein and smeared on glass slides, followed by preparation of bone marrow cells obtained from their femora for cytogenetic analysis.

Experimental design

Two experiments were conducted under different conditions:

1. Albino mice chronically exposed for 40 days to natural UV radiation at two altitudes in Rila Mountain Basic Ecological Observatory (BEO) station (Peak Moussala, 2925 m a. s. l.) and the area of the Beli Iskar dam (area “Skakavtsite”, 1470 m a.s.l.). This selection of altitudes is due to the fact that UV radiation increases by 1 % every 100 meters. For the purposes of standardising the experimental conditions, a period around the longest day of the year, with longest daylight exposure (June and July) was selected.

2. A laboratory experiment, which closely mimics the conditions in the high mountains. In order to eliminate the side effects for albino mice in nature, the investigation was conducted at standard laboratory temperature, humidity and under a pre-set 24-hour light–dark regime. The intensity of combined UV-A and UV-B radiation was the same as the one measured at the higher altitude.

UV sensor system

To register the daily dose of ultraviolet radiation, a special UV sensor instrumentation and portable data logger were constructed and installed on every packing-case (TYUTYUNDZHIEV et al. 2018). The UV irradiation at the Peak Moussala was measured by a 3-channel UV sensor system (UV-A, UV-B, UV-C). Every sensor is the latest generation of GaN, AlGaIn semiconductor selective photodiode, calibrated in microWatts per square centimetre by the manufacturer, on a chip connected to 16-bit ADC and communicating with a separate data logger by an I2C protocol. Raw sensor data from all channels were collected every 2 min. The collected sensor data were further processed by a Cortex M0 ARM processor to calculate the UV index according to the erythral function definition. The UV daily dose was calculated by the integration of UV power over the real daily hours when the UV irradiation reaches the sensor surface (TYUTYUNDZHIEV et al. 2018).

Haematological indices

To track changes in main haematological parameters in investigated mice, blood collection was provided from each animal at 15th, 30th and 40th day for all three experimental designs. Haematological investigations were conducted by placing a drop of peripheral mouse blood on clean glass slides, followed by Giemsa staining and differential counting. Specifically, the amounts of eosinophils (Eo) and basophils (Ba) in peripheral blood were examined.

Micronucleus test

The micronucleus test was performed as described in other studies (RIBEIRO et al. 2003, IARMARCOVAI et al. 2008). Two slides of each animal were prepared by placing a drop of blood on a clean slide, which is then air-dried, fixed in methanol and stained with Acridine Orange. The stained smears were scanned using a microscope at $\times 1000$ magnification (Olympus CX 41), 1000 polychromatic erythrocytes were counted on each slide to register the number of observed micronuclei and then expressed as a mean of two slides from each animal.

Cytogenetic analysis

Chromosomal aberration analysis was conducted as described by PRESTON et al. (1981). The bone marrow chromosome aberration assay was performed on 8 groups of 8 male animals. The experimental animals were injected intraperitoneally with colchicine at a dose of 0.4 mg/kg, 1 h prior to isolation of the bone marrow cells. Bone marrow cells were flushed from the femur and hypotonised in 0.075 M KCl at 37°C for 20 min. Subsequently, the cells were fixed in methanol-acetic acid (3:1), dropped on cold slides, air dried and stained with a 5 % Giemsa solution (Sigma Diagnostic). At least 50 well-spread metaphases were analysed per experimental animal at random. The frequencies of chromosomal aberrations were determined for each animal and then the mean \pm standard error of the mean for each group was calculated.

Statistical analysis

The data was standardised and one-way analysis of variance (ANOVA) was used to determine any significant differences between two or more independent groups of parameters. The data are expressed as mean \pm standard deviation. P-values less than 0.05 were considered to be statistically significant. Student's t-test was applied for statistical analysis of chromosome aberrations.

Results

The experimental work focused on natural and laboratory-based exposure to UV-A and UV-B radiation, with exposure patterns as presented below. While natural UV irradiation can vary from high to low depending on the location and altitude, the total received doses of UV in the two experiments with different altitude, temperature and humidity conditions were closely similar. On the Peak Moussala, the Beli Iskar area and in laboratory the accumulated dose of UV radiation was determined. The accumulated dose was calculated based on every 2-min

Table 1. Total received doses of UVA+UVB (mean \pm SD) by animal's organism for the experimental period from the two experiments.

Period	Localities		
	Peak Moussala	Beli Iskar	Laboratory
	Exposure (W.h/m ²)	Exposure (W.h/m ²)	Exposure (W.h/m ²)
0-15 th day	9.769 \pm 0.651	8.490 \pm 0.343	9.651 \pm 0.347
0-30 th day	17.781 \pm 0.659	15.451 \pm 0.326	17.475 \pm 0.490
0-40 th day	21.871 \pm 0.608	18.932 \pm 0.650	21.549 \pm 0.324

UV measurements. The daily doses were estimated for the whole period of the experiments (Table 1).

The measurements of daily temperatures on the Peak Moussala varied greatly – from 5 to 27°C and an average humidity of 61–63 % was established during the experimental period. The respective UV radiation level varied daily, depending on the sun position during the day as well as changes in cloud cover. The temperature variations of the Beli Iskar area were from 13 to 28 °C. In contrast, mice in the laboratory were under standard temperature (22°C) and humidity conditions. Both results of the end of the experiments showed that the received simulated UV radiation in laboratory conditions increased more uniformly than in nature but no statistically significant differences were obtained between the total received doses.

A different degree of changes in some haematological indicators such as basophiles and eosinophils of the investigated animals in all three experiments was observed. The reason for these three parameters to be preferred is the fact that their increase is one of the first signs of allergic reactions, inflammatory processes and the presence of tumours in the organism. In addition, micronucleus counts followed similar patterns under natural conditions and in the laboratory (Table 2, Fig. 3).

The data showed statistically significant increases in Eo values in the experimental animals from the Peak Moussala compared to those from the Beli Iskar area and the control group (Table 2). Their values ranged from 0.05 % on the 30th day of Beli Iskar to 1.3 % on the Peak Moussala for the 40th day. Significant differences were obtained on the 30th day at both altitudes compared to the 15th and 40th day (Fig. 1). It is important to note that they were not found in the animals under UV irradiation in laboratory conditions.

Basophilic granulations in white blood cells followed a similar trend (Fig. 2), indicating that mouse haematopoiesis was affected in the early days of UV irradiation and exhibited an adaptive response over the course of the experiment.

Table 2. Comparisons among the amounts of eosinophils (Eo), basophils (Ba) and micronuclei in erythrocytes (MN) in the blood samples of experimental mice from the two localities in Rila Mountain, laboratory and control groups.

Indices	Period	n	Moussala Mean ± SD	Beli Iskar Mean ± SD	n	Laboratory Mean ± SD	n	Control Mean ± SD
Eo%	0-15 th	24	0.50 ± 1.06	0.13 ± 0.33	25	0.09 ± 0.01	10	0.1 ± 0.03
	0-30 th	20	0.80 ± 1.60	0.25 ± 0.22	25	0.11 ± 0.01		
	0-40 th	24	0.33 ± 0.56	0.13 ± 0.33	25	0.08 ± 0.02		
Ba%	0-15 th	24	6.54 ± 2.73	3.92 ± 3.90	25	7.75 ± 3.75	10	2.55 ± 0.46
	0-30 th	20	11.15 ± 3.81	8.60 ± 5.61	25	13.23 ± 4.32		
	0-40 th	24	10.54 ± 4.74	8.04 ± 2.91	25	12.76 ± 4.45		
MN‰	0-15 th	8	9.01 ± 1.34	6.43 ± 1.23	25	8.16 ± 2.75	10	3.94 ± 1.10
	0-30 th	10	10.92 ± 2.04	7.21 ± 1.45	25	10.13 ± 2.04		
	0-40 th	12	8.96 ± 1.83	6.99 ± 1.56	25	8.40 ± 1.36		

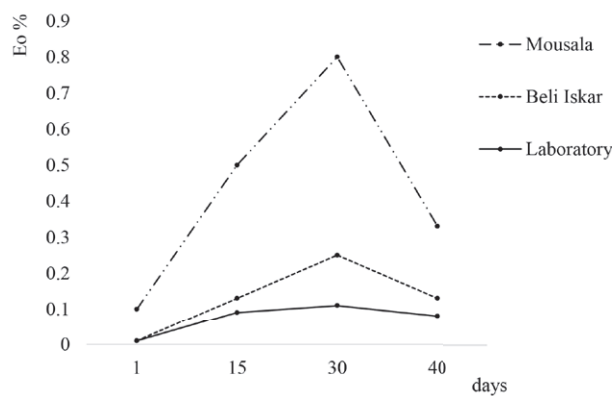


Fig. 1. Dynamics of eosinophil counts during the investigated period..

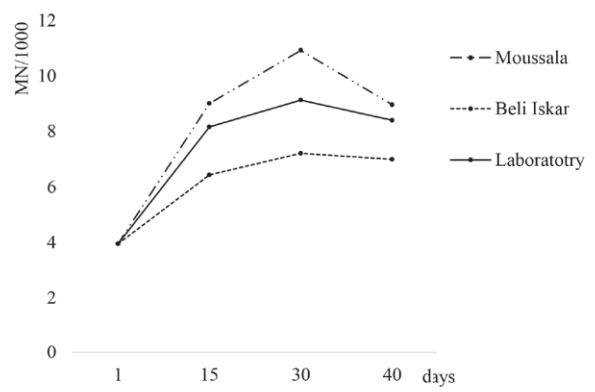


Fig. 3. Dynamics of micronuclei in erythrocytes during the investigation period.

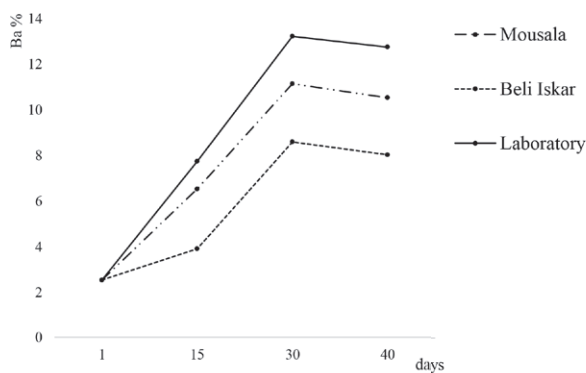


Fig. 2. Dynamics of the basophil counts during the investigated period.

Different types of intrachromosomal recombination like breaks, fragments, centromeric and telomeric attachments were observed. The most frequent were the breaks that lead to the formation of axial fragments, chromatid and isochromatid breaks. Taking into account the clastogenic effect of the combined effect of UV radiation and the natural background radiation on bone marrow cells, espe-

cially in the experiments in natural conditions, the most representative indicator was the sum of the chromatid breaks and fragments (Table 3).

The obtained results showed statistically significant differences between the sampling periods and between the experimental groups. The observed chromosomal aberrations in investigated mice from the Peak Moussala and those under laboratory UV irradiation exceeded about twice those of Beli Iskar and three times those of the control group. These results suggested considering the effects as moderate. The analysis of metaphases with aberrations in the bone marrow in mice from Moussala showed that the rate of aberrations varied from $8.75 \pm 0.75\%$ on the 15th day of early impact, slightly increased on 30th day ($9.5 \pm 0.82\%$) and reached a maximum ($10.75 \pm 0.92\%$) on the 40th day of the experiment. Very close to them, from 8.50 ± 0.062 on the 15th day to 10.50 ± 0.76 on the 30th day, were the results obtained under laboratory conditions. No significant differences were found between them. The lowest percentage of cells with damaged chromosomes was recorded on the 15th day (5.50 ± 0.63) and the highest on the 40th

Table 3. Clastogenic effect of bone marrow cells in ICR line laboratory mice after UV treatment.

Locality	Type of chromosome aberrations								% of aberrant cells Mean \pm SD
	Period	N of meta- phases	Breaks	Fragments	Breaks + fragments	Rearrangements			
						c/c	t/t	c/t	
Peak Moussala	0-15 d	400	16	12	28	6	0	0	8.75 \pm 0.75
	0-30 d	400	7	8	15	22	0	0	9.5 \pm 0.82
	0-40 d	400	11	21	33	9	0	0	10.75 \pm 0.92
Beli Iskar	0-15 d	400	2	10	12	15	0	0	5.50 \pm 0.63
	0-30 d	400	6	5	11	8	0	0	6.00 \pm 0.75
	0-40 d	400	10	10	20	9	0	0	7.25 \pm 0.99
Laboratory	0-15 d	400	9	21	30	4	0	0	8.50 \pm 0.62
	0-30 d	400	9	26	35	8	0	0	10.50 \pm 0.76
	0-40 d	400	8	20	28	10	0	0	10.50 \pm 0.73
Mit. C 3.5 mg/ kg	24 h	200	17	30	47	7	1	1	30.5 \pm 2.36
	48 h	400	17	24	41	20	0	0	15.8 \pm 0.81
Control 0.9% NaCl	24 h	700	4	0	4	4	0	0	1.14 \pm 0.34
	48 h	500	0	0	0	3	0	0	0.6 \pm 0.3

day (7.25 \pm 0.99) from the Beli Iskar region. A tendency of slightly increasing damaging effect during the experiments was established and the difference is statistically significant between low altitude and the other two experiments – on high altitude and the laboratory simulation. There are no significant differences between the second two cases. There is also no significant difference between the types of aberrations of chromatid-type (chromatid breaks and fragments) and chromosomal type (centromeric/centric fusions), which was understandable as the factor causing changes in the structure of the chromosomes was the same for the three variants of the experiments.

Discussion

There are relatively few studies that demonstrate an important contribution of environmental factors such as UV radiation to harmful effects on the health of exposed animals and the cumulative effects of these factors on the increasing of these harmful effects (WANG et al. 2010). In itself, UV light has been studied mostly in the laboratory. Numerous publications have described the phototoxic effects of UV-A and UV-B light on skin cells *in vitro*. Several studies on different animal species have involved the long-term effects of UV-B radiation. However there are only limited reports on the acute effects of UV light and a general paucity of information on UV-A toxicity *in vivo*. Additionally, very little information exists on changes in non-skin tissues following combined UV (A+B) radiation except for some that evaluated photoprotection. For these reasons, the present study is focusing

on the effects of a natural UV (A+B) on male albino ICR mice, as well as the laboratory verification of the results obtained under natural conditions.

The functional penetration of light energy depends on the wavelength (0.1 to 0.6 mm) and the optical properties of the environment. Biologically active UV radiation interacts in a complicated way with mammalian cells. However, it must be kept in mind that not only skin directly exposed to solar light is affected by UV radiation but non-skin tissues can also be influenced through low-molecular weight mediators generated upon irradiation (KADHIM et al. 2013). This is known in radiation biology as non-targeted, or bystander effects (BELYAKOV et al. 2001).

When considering blood counts, it may be suggested that the increased amount of eosinophils of animals in natural conditions is probably due to the complex environmental stresses that is not typical for the laboratory mice. This corresponds to literature data. ÇAVUOĞLU et al. (2010) observed the number of erythrocytes in the blood of animals and described inclusion as an important indicator to detect genetic damage induced by different types of radiation. A similar trend is also seen when considering Ba granulations and micronuclei in erythrocytes. The results of the micronucleus test indicate chromosome instability. The presence of micronuclei in erythrocytes is evidence for structural and/or numerical chromosomal aberrations occurring during mitosis (KADHIM et al. 2013). When analysing the data for micronuclei detected in erythrocytes, it becomes apparent that values for this endpoint peak around day 30th of the laboratory experiment. Combined with the knowl-

edge that mouse erythrocytes have a biological life span of around 40 days (WANG et al. 2010), this may indicate an adaptive response of mouse hematopoietic tissues starting in the early days of irradiation.

Experimentally obtained results regarding the clastogenic effect of UV radiation on metaphase chromosomes of the experimental animals shows that this factor has a low-to-moderate clastogenic effect that does not change significantly by prolonging the time of exposure. At the same time, chromosome aberrations are significantly higher than the values of this indicator recorded in the control group. According to the results presented in Table 3, there is a good agreement that the investigated mice are sensitive enough to the induction of different kinds of chromosome aberrations and, in all probability, to the production of mutations. DNA is certainly one of the key targets for UV-induced damage in a variety of organisms. Ultraviolet radiation is a well-established causal agent for mutagenic and cytotoxic DNA lesions. However, cells have developed a number of repair and damage response mechanisms to counteract DNA damage (SANCAR et al. 2004).

In part, the observed result of external UV radiation exhibiting effects on the hematopoietic system can be explained by the concept of non-target (NT) effects of radiation. This means that organs outside the direct target area of mammalian organisms can be affected. In this case, the small but measurable increase in micronuclei and the increase in chromosomal aberrations can be attributed to NT effects, coming from UV skin exposure, a notion consistent with literature data for NT effects in mammals, obtained using ionizing radiation (KADHIM et al. 2013). In addition, the greater degree of haematological changes in mice, obtained during natural exposure on the Peak Moussala can be attributed to increased total stress, stemming from the higher altitude and accompanying factors (temperature, ozone, and the presence of a slightly higher radiation background on Rila Mountain).

Conclusions

The current project stands out as a novelty in its approach to study the effects of exposure to skin-penetrating UV radiation. Individual blood cells were counted and cytogenetics and micronuclei were analysed. The results showed a significant dose-dependent increase in micronuclei from 15th to the 40th day of the experiment in addition to significantly increased cytogenetic abnormalities compared to controls. At present, methods used to evaluate the consequences of UV irradiation and gamma background involve also the assessment of genotoxic damage

and other cell-detrimental effects on organisms. However, our publication opens up new venues of research involving the study of non-targeted and combined effects of UV radiation on living beings.

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