EFFECTS OF ARTIFICIAL ULTRAVIOLET C RADIATION ON SEVERAL BLOOD AND URINE PARAMETERS RELATED TO RENAL AND HEPATIC FUNCTIONS IN ALBINO MICE

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Abstract

The effects of artificial UVC radiation on some blood and urine parameters related to hepatic and renal functions in mice radiated with 30-watt UVC lamp (254 nm and 0.00014 J/cm²/s) were examined. Sixty female mice (Swiss Albino) were used as test animals. The animals were fed a mouse diet and water ad libitum and then exposed to artificial UVC for 8 h daily. Blood and urine samples were taken before radiation (control) and at the 7th, 15th, 30th, 45th, 60th, and 75th d after radiation exposure. The samples were collected in different pools. Urea, creatinine, cholesterol, ALT, AST, ALP, and GGT levels were determined in serum, and protein electrophoresis was made (SDS-PAGE) in urine samples. Significant increase (P<0.001) in serum urea values were observed on the 15th and 30th d of the exposure. Urea levels returned to the initial values. Increase in creatinine levels after 7th d of exposure and decrease until the 60th d (significant on the 15th and 45th d) were found. Creatinine levels increased again on the 75th d (P<0.001). Serum cholesterol and AST values were significantly increased (P<0.01) during the whole period of the exposure, except the 45th d. Serum ALT levels were not significantly affected during 30th d exposure. They decreased on the 45th d (P<0.01) and then increased on the 75th d (P<0.01). Serum GGT and ALP levels were decreased during the whole period of the exposure (P<0.01), and GGT activity was at immeasurable levels on the 30th, 45th, and 60th d. Proteinuria, determined by microscopic observation of urine sediment, and the increase in serum urea and creatinine, observed on the 7th and 75th d of the exposure, were considered a renal disorder, and according to the electrophoretical pattern of urine proteins (SDS-PAGE) were defined as mostly glomerulo-tubular type and glomerular type. This indicates that artificial UVC radiation significantly affected the renal function (P<0.01), while the hepatic functions were affected significantly (P<0.01) just on the 75th d of the exposure. Concerning the data, which coincide one on the top of the other, there was the opinion that the kidneys and liver showed different sensitivity to artificial UVC radiation.

Key words: mice, UVC–radiation, hepatic function, renal function, urine proteins.

Ultraviolet (UV) radiation (200–400 nm), an important part of the solar energy, is mainly divided into three sub-groups according to wavelengths. It is defined as UVA (400–320 nm), UVB (320–290 nm), and UVC (290–200 nm) (13). The most powerful and dangerous one is UVC (8). UVC and UVB are especially absorbed by nucleic acids, and UVA is less absorbed by nucleic acids and proteins but it causes oxidative events. As a result of these different properties, UVC and UVB radiations stimulate the release of stress proteins more effectively than UVA (30).

Sunburn, photosensitivity reactions, and immunosuppression are the acute effects of UV radiation, which are known in humans (17). Biochemical changes cover the release of histamine and the derivative products of arachidonic acid, such as cyclooxygenases and lipoxygenases, kinins, and cytokinins, probably in epidermal and dermal cell types (29). It was reported that UV radiation generated oxidative stress (9, 20, 28) and an increase in radiation dose caused functional changes in various physiological systems in animals (15).

UVA rays cause light brown tan (primary pigmentation) in a short time; the following darkening is due to melanin, which accumulates in the skin. Sunburns and erythema are milder than the UVB effects. UVB rays cause delayed but long-term tan (secondary pigmentation) mostly resulting in melanin synthesis in the skin. It causes serious sunburn, which is associated with intensifying erythema and oedema, ache, and blister formation in less than one day of exposure. UVC rays, which have sterilisation and biocidal properties, and are especially harmful for eyes. Usually, they cannot
reach the earth surface because of the absorption in the ozone layer of the atmosphere. But in Antarctica and in some regions of the north hemisphere, where the ozone layer has become thin and in some areas the ozone holes appeared as a result of human related effects, UV rays that are found at the hemisphere are more densely and perpendicularly, which increases skin cancer risk (5, 27).

The biological effects of UV radiation were examined in calves (10), humans (11, 14, 16), mice (9, 21), pigs (24), and sheep (15). It was determined that UV radiation causes important physiological and biochemical changes in the body’s systems. These studies were mostly carried out with UVA and UVB, and this one, which concerns UVC radiation in mice (26), must be supported by new studies. Therefore, we intended to study the effects on selected urine and blood parameters related to renal and hepatic functions in albino mice exposed in different periods to artificially produced UVC radiation.

### Material and Methods

Sixty female 3-months-old Swiss Albino mice, weighing 30–35 g, were used. The animals were put into 6 cages, ten in each. A commercial feed (Table 1) and water were given ad libitum to all of them.

**Table 1**

<table>
<thead>
<tr>
<th>Contents and caloricity of mouse feed</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude protein</td>
<td>Min. 24%</td>
</tr>
<tr>
<td>Crude cellulose</td>
<td>Max. 7%</td>
</tr>
<tr>
<td>Water</td>
<td>Max. 12%</td>
</tr>
<tr>
<td>Crude ash</td>
<td>Max. 8%</td>
</tr>
<tr>
<td>Ash dissolved in HCl</td>
<td>Max. 2%</td>
</tr>
<tr>
<td>NaCl</td>
<td>Max. % 1%</td>
</tr>
<tr>
<td>Ca</td>
<td>Min. 1.0-1.8%</td>
</tr>
<tr>
<td>P</td>
<td>Min. 0.9%</td>
</tr>
<tr>
<td>Methionin</td>
<td>Min. 0.6%</td>
</tr>
<tr>
<td>Metabolic energy (kcal/kg)</td>
<td>Min. 2650</td>
</tr>
</tbody>
</table>

Before the experiment, the mice underwent the 10 d period of adaptation to the environment where the study was going to be performed. A 90 cm in length and 30-watt UVC lamp (Mazda TG) was used for exposure to radiation, and was installed in the lid of the compartment where the mice cages were put into (Fig. 1). The wavelength of UVC light spread out from the lamp was determined as 254 nm and its energy as 0.00014 joul/cm² per second. The animals were exposed to UVC for 8 h daily taking the sunlight as base (4 h between 8:00 and 12:00, and 4th h between 13:30 and 17:30). Between 12:00 and 13:30, there was a pause in radiation, and feed and water were supplied.

Before the radiation (control group) and on the 7th, 15th, 30th, 45th, 60th, and 75th d after the radiation started, blood samples were taken from the heart and urine samples were taken into clean test tubes by means of hand massage. Depending on situation, blood or urine samples collected from 5 or 6 animals were pooled. The serum was separated by centrifugation at 2 500 rpm for 10 min. Urine samples were centrifuged at 1 500 rpm for 10 min, the sediment was taken on a microslide, and after one drop of Lugol’s solution was added, the slide was covered with a cover glass and used for microscopic observation.

**Fig. 1.** The special compartment, where the mice were exposed to UVC radiation.

The level of serum urea was determined by a modified Gentzkow’s method (3) in colorimeter, the level of creatinine by Jaffe reaction (31), the level of total cholesterol and the activities of ALT, AST, ALP, and GGT were determined by an autoanalyser using commercial test kits (1). The electrophoretical research of urine proteins by SDS–PAGE were made in 10% polyacrylamid gel after the ultrafiltration by Millipore 10.000 NMWL, and the obtained protein bands were stained by Coomassie blue (4). The bands of urine proteins were evaluated densitometrically (Fig. 2). For this purpose, a Junior 24 model of Helena densitometer was used. The urine sediments, obtained after centrifugation, were examined under light microscope. The differences between average results of control and test groups were evaluated by “One-way analysis of variance” and for the significance; the "Duncan test" was applied (22).

**Results**

Throughout the examination, excessive sensitivity to light (photosensitivity), unease, as well as erythema in some parts of the skin was observed.

**Blood analyses.** In control and test groups, the average levels of serum urea, creatinine, cholesterol, ALT, AST, GGT, and ALP, with the statistical significance of standard errors and the differences between the groups, are given in Table 2.
Fig. 2. Densitometric urine protein patterns of mice at different times of UVC exposure.
It was found that serum urea levels were elevated significantly as the effect of UVC radiation on the 15th d (P<0.01), on the 30th d trend in elevation was going on (P<0.05), and beginning from the 45th d it was decreased near to the control value (Table 2).

Serum creatinine levels were elevated significantly on the 7th d after the radiation (P<0.01); and sudden significant decrease was observed on the 15th d (P<0.01). The decrease was found to be not significant on the 30th d (P>0.05) and significant on the 45th d (P<0.01). The serum creatinine levels continued to decrease on the 60th d and on the 75th d they were again markedly (P<0.05) elevated.

On the 7th d after the radiation, serum cholesterol levels showed a significant rise (P<0.01); although this rise on the 15th and 30th d was less than that of the 7th d, and it did not lose its significance. On the 60th and 75th d, it continued to rise significantly (Table 2). The serum cholesterol average level (244.0±9.2 mg/dL) was approximately two times higher than the control level (113.4±2.61) on the 75th d of the exposure.

Serum ALT levels followed a descent and ascent movement and they showed a marked fall (P<0.01) on the 45th d of the exposure and a significant rise (P<0.01) on the 75th d.

Table 2

Mean values and the statistical significance of the blood parameters in mice exposed to UVC radiation

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control (n=5)</th>
<th>7. d (n=6)</th>
<th>15. d (n=8)</th>
<th>30. d (n=6)</th>
<th>45. d (n=9)</th>
<th>60. d (n=7)</th>
<th>75. d (n=6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urea (mg/dL)</td>
<td>52.8 ±1.87</td>
<td>59.9 ±3.41</td>
<td>114.1 ±9.07</td>
<td>75.1 ±3.86</td>
<td>55.4 ±2.28</td>
<td>45.0 ±2.77</td>
<td>60.0 ±2.76</td>
</tr>
<tr>
<td>Creatinine (mg/dL)</td>
<td>1.26 ±0.16</td>
<td>4.67 ±0.26</td>
<td>0.67 ±0.5</td>
<td>1.13 ±0.12</td>
<td>0.88 ±0.08</td>
<td>1.15 ±0.20</td>
<td>1.88 ±0.16</td>
</tr>
<tr>
<td>Cholesterol (mg/dL)</td>
<td>113.4 ±2.61</td>
<td>318.3 ±67.5</td>
<td>172.9 ±41.2</td>
<td>204.8 ±64.6</td>
<td>122.4 ±8.4</td>
<td>199.4 ±8.8</td>
<td>244.0 ±9.2</td>
</tr>
<tr>
<td>ALT (IU/L)</td>
<td>60.6 ±5.04</td>
<td>54.3 ±8.61</td>
<td>65.0 ±7.5</td>
<td>46.6 ±4.4</td>
<td>33.1 ±1.98</td>
<td>46.3 ±1.97</td>
<td>80.5 ±7.69</td>
</tr>
<tr>
<td>AST (IU/L)</td>
<td>94.8 ±5.06</td>
<td>183.1 ±44.7</td>
<td>141.0 ±10.1</td>
<td>124.8 ±13.3</td>
<td>103.0 ±7.6</td>
<td>230.4 ±11.1</td>
<td>177.7 ±6.7</td>
</tr>
<tr>
<td>GGT (IU/L)</td>
<td>4.60 ±0.92</td>
<td>2.00 ±0.86</td>
<td>3.88 ±0.39</td>
<td>0.00 ±0.00</td>
<td>0.00 ±0.00</td>
<td>0.00 ±0.00</td>
<td>1.40 ±0.30</td>
</tr>
<tr>
<td>ALP (IU/L)</td>
<td>133.4±6.23</td>
<td>66.1±4.05</td>
<td>71.3±3.46</td>
<td>44.3±2.76</td>
<td>47.2±1.56</td>
<td>91.5±4.97</td>
<td>79.6±5.57</td>
</tr>
</tbody>
</table>

The different letters in the same row indicate the significant difference (P<0.001).

Table 3

The classification and distribution of urine protein bands according to their molecular weight

<table>
<thead>
<tr>
<th>Protein bands</th>
<th>Control</th>
<th>7. d</th>
<th>15. d</th>
<th>30. d</th>
<th>45. d</th>
<th>60. d</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt;45 kDa</td>
<td>17.3</td>
<td>27.0</td>
<td>73.9</td>
<td>58.0</td>
<td>41.7</td>
<td>88.7</td>
</tr>
<tr>
<td>45-25 kDa</td>
<td>35.1</td>
<td>39.0</td>
<td>12.3</td>
<td>31.4</td>
<td>49.6</td>
<td>30.5</td>
</tr>
<tr>
<td>25-10 kDa</td>
<td>47.6</td>
<td>34.0</td>
<td>13.8</td>
<td>10.6</td>
<td>08.7</td>
<td>07.8</td>
</tr>
</tbody>
</table>

Serum AST activities were elevated at all time-points after the radiation, but it was found that statistically significant rise was on the 7th, 15th, 30th, 45th, and 75th d (P<0.01); only on the 45th d it was not significant (P>0.05) (Table 2).

There was such a decrease in serum GGT activities on the 30th, 45th, and 60th d after the radiation that the activities could not be measured (Table 2).

Serum ALP levels were also decreased markedly at all time-points (P<0.01) and the lowest level (44.3±2.7 IU) was found on the 30th d (Table 2).

Urine analysis. Proteinuria resulting from the artificial UVC radiation was observed in all urine samples collected.

The microscopic observation of the urine. In the microscopic observation of some urine samples collected before radiation, common findings were: 3–5 leukocytes, a few renal epithelial cells, and squamous epithelial cells, which number was slightly increased in each area. In the urine samples taken 7th d after the radiation, the leukocyte rise (7-8) took place. This rise was more evident on the 15th and 30th d. The rise was also found in the renal epithelial cells (4–5 and 5–6 in each area, respectively). In the urine samples taken on the 45th and 60th d after the radiation, the leukocyte number decreased to the initial levels (3–5 in each area). Additionally, 1–2 and 3–4 renal epithelial cells were
observed in each area on the 45th on the 60th d respectively. Like in the urine samples taken before radiation, there was also noted a rise in the number of the squamous epithelial cells of the urine samples collected throughout the study period. On the 30th and 60th d after radiation, ammonium–magnesium–phosphate (struvite) and leucine crystals were observed. On the 75th d of the examination, urine samples could not be collected from the animals.

The electrophoretical observation of urine proteins (SDS–PAGE). After the finding of proteinuria, to evaluate the effects of artificial UVC radiation, the urine proteins were separated according to their molecular weights. In order to differentiate the origin of the renal disorder, urine samples were subject to 10% acrylamide gel SDS–PAGE after they were made denser by ultracentrifugation.

Urine protein bands were also obtained when the urine samples taken after artificial radiation were processed directly, without necessity of ultracentrifugation, because the proteinuria was evident after testing of a few samples.

The rise in the serum urea and creatinine with proteinuria were noticed one week after the radiation, because there was evident renal disorder and so it was interpreted. It can be concluded that on the 15th d of the radiation, renal disorder was glomerulo-tubular; on the 30th and 45th d, it was tubulo-interstitial, and on the 60th d, it was near to the glomerular type, when the percentage distribution of protein bands of urine proteins was observed.

Discussion

It was demonstrated that the values observed in the mice of the control group were within the established reference limits (6, 7, 18, 23). Normal values for mice were different in particular investigations and were reported as 25.6–59.9 mg/dL for urea, 0.3–1.0 mg/dL for creatinine, 26–82 mg/dL for total cholesterol, and 65.0 IU/L for ALP (6), 97±11 mg/dL for total cholesterol (23), 37.0 IU/L and 19.0 IU/L for AST and ALP (18), respectively; and 12.5±0.65 IU/L, 51.80±1.53 IU/L, and 219.80±3.86 IU/L for GGT, ALT, and AST (7), respectively. It was determined that the effects of artificial UV radiation on serum urea and creatinine levels were especially expressed at the second week of the exposure (Table 2). These two parameters, which are evaluated together with clinical biochemistry and rise in blood levels, indicate at renal disorders. However, the rise alone in blood creatinine levels was also evaluated as an important criterion (19). In this point, the rise (4.67±0.26 mg/dL) in creatinine levels on the 7th d of the exposure showed that the kidneys were seriously affected. Because the highest rises were on the 7th and 15th d, the conclusion can be drawn that the kidneys were affected by UVC radiation mostly at the first and second weeks of the exposure. The leukocyte rise and proteinuria, which were observed at the microscopic examination of the urine, is a finding supporting the diagnosis of the renal disorder. It can be said that the percentage distribution of the bands, which were obtained in the electrophoretical separation of urine proteins (Table 3), showed the disorder, although not clear, is inclined to be of a glomerular type, according to the exposure time. With regard to the percentage distribution of the bands of urine proteins, it is assumed that when 25–10 kDa proteins are at the rate of ≥70%, the disorder is of a tubular type, when 45–25 kDa proteins are at the rate of ≥70%, the disorder is of tubulo-interstitial type, and when >45 kDa protein distribution is at the rate of ≥70%, the disorder is in glomerular type (2). It can be said that the UVC radiation causes a disorder in kidneys of a glomerular type, mostly by damaging the glomerular membrane permeability by oxidative stress (9, 20, 28).

Blood total cholesterol levels rose throughout the exposure of UVC and the most important rise occurred on the 7th and 75th d (Table 2). This finding can be related with the balance between hepatic cholesterol synthesis and cholesterol progressive mechanism. According to this, it can be thought that there can be a deficiency in cholesterol catabolism. The rise of serum ALT and AST activities (P<0.01) occurred on the 75th d of the exposure and was related to hepatic cell destruction. The important fall in serum GGT and ALP values could be related to the inhibitory effect of the radiation on enzyme activity, because it was reported that UV radiation decreased serum ALP level (25). These two enzymes are related to the endothelial cell membranes, which are arranged through hepatic gall channels and it can be thought that they are more easily affected by the cytoplasmic enzymes (AST and ALT). It is thought that endothelial cell death caused by radiation is connected with apoptosis, and these cells are too sensitive to radiation and the basal membrane that can protect the cell from the radiation (12).

It can be easily seen that one week of the exposure to UVC lights causes serious changes (Table 2). In this period, besides a marked rise in serum urea level, there occurred marked rises (P<0.01) in serum creatinine, cholesterol, and AST levels. On the other hand, the falls in serum GGT and ALP values (P<0.01) were noted.

It is reported that the effects of radiation can be different, depending on the animal species, dose, and way and time of application. It was demonstrated that the increase in doses of long-term UVB radiation causes functional alterations in different physiological systems of animals. These alterations observed in early stages of exposure in sheep are related to the adaptation to new environmental conditions. The functional alterations observed in late stage, are related to different sensitivity of various body systems to UVB radiation (15).

Concerning the enzyme values, small differences observed in the parameters can be related to the slight haemolysis in serum samples. According to the reports, UV radiation at different wavelengths (UVA, UVB, and UVC) causes haemolysis.
References