RADIATION-INDUCED CHANGES IN THE ELECTROPHORETIC PROFILE OF SERUM ALBUMIN

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RESULTS

Standardization of Albumin in electrophoresis. Figure 1 illustrates the response of the experiment to standardize the albumin protein in SDS-PAGE, under operating conditions. The percentage area of 64.78; 28.76; 5.06 and 1.46% was found at concentrations of 4; 2; 1; 0.5; 0.1 nmL. After imaging, one can observe the change in area of the band and the intensity level of the gray pattern in the albumin concentration.

Figure 1 - Standardization of albumin concentration.

Analysis at the control group. After running the gel, as well as coloring and final digital preparation, the profile of control and irradiated samples, in which the albumin bands were recorded and identified (Fig. 2).

Figure 2 - Illustration of electrophoretic profile of the control group: standard albumin ~ 1 mL; 15 mL plasma samples sites in vascular control obtained from the jugular vein, renal vein, hepatic vein, inferior vena cava and the pulmonary vein. As the blood was hemolized, pure heparin was assessed by SDS-PAGE verifying the absence of bands in the range of interest, the conditions of the experiment control. Thus, it was shown that heparin does not interfere with the electrophoresis experiment.

The physiological albumin serum concentrations in the control taken in vascular sites of jugular, renal, hepatic, inferior vena cava and pulmonary veins, respectively, showed 9.5 %, 52.1 %, 52.9 % and 58.1 % and 40.0 % higher than the standard albumin concentration recorded by electrophoresis.

Values were adjusted by the different concentrations of applied samples. The percentages obtained were analyzed in terms of the variation in response to the intensity of staining of the bands at electrophoresis. Analysis of the whole band, justified by the intersection of the areas of the bands was discontinued, limited to a linear rectangular section across the gels.

Table 1 - Concentrations, in mg/dL, and relative percentage changes in relation to control.

<table>
<thead>
<tr>
<th>Vascular site</th>
<th>Concentration</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Venous</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heparin</td>
<td>0.25</td>
<td>0.39</td>
</tr>
<tr>
<td>Liver</td>
<td>1.52</td>
<td>0.41</td>
</tr>
<tr>
<td>Expectorin</td>
<td>1.58</td>
<td>0.42</td>
</tr>
<tr>
<td>Lung</td>
<td>1.40</td>
<td>0.27</td>
</tr>
</tbody>
</table>

Table 1 shows a variability of albumin in the vascular sites, pointing out that this changes are related to the animal’s physiological control.

Analysis at the irradiated group. A decrease in performance was observed in electrophoresis albumin bands in almost all sites surveyed from the irradiated group, with the exception of the kidney. This analysis was made comparing to albumin bands from the control group not treated with radiation and the irradiated one. (Fig. 2)

Figure 3 – Serum albumin bands at SDS-PAGE from samples at vascular sites: renal, hepatic, pulmonary, jugular veins from control and irradiated groups, respectively.

Table 2 - protein concentration and the normalized values in the investigated sites.

<table>
<thead>
<tr>
<th>Vascular site</th>
<th>Concentration in groups [mg/dL]</th>
<th>Reduction(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heparin</td>
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</tr>
<tr>
<td>Liver</td>
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<td>0.28</td>
</tr>
<tr>
<td>Lung</td>
<td>0.37</td>
<td>0.51</td>
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</table>

Table 2 summarizes the results, in which the quantitative values representing the albumin concentration reduction are presented in the vascular sites under study.

MATERIALS AND METHODS

Grouping: Two groups of animals. Watchman rats were used, with the age of 12 weeks and weighing 300 g, with free access to water and food with a photoperiod of 12 h. The animals were divided into control group (n = 2) and one subjected to irradiation (n = 2). To linear the variables were given the same image, family, weight and age.

Irradiation. The animals were exposed together to a dose of 5 Gy of Cs-137. Laboratory of Gamma Irradiation - UFMG, Centro de Desenvolvimento de Tecnologia Nuclear - CTN.

Sample collection and separation of components. Under deep anesthesia, thoracic and abdominal laparotomy was performed, and subsequent processing of tissues from the inferior vena cava, kidney, spleen, jugular, hepatic, and pulmonary veins. The samples were heat-inactivated and separated by centrifugation at 2000 rpm for 30 min components.

Sampling time. The samples were obtained from irradiated animals and control after 72 hours of exposure to radiation.

Electrophoresis. Serum samples from the different vascular sites and amigridroups were submitted to vertical electrophoresis in acrylamide, SDS-PAGE. Running the gels were prepared at 10%, with comb 4% gel and subjected to 120 V and 15 mA. An amount of 15 mL sample was used in the wells. After the run, the gels were stained with Commassie Blue R-250, and subsequently heat-denatured.

Standardization of albumin. A standard sample with albumin concentration of 4 ng mL was employed and a high range protein weight pattern. SDS-PAGE was run with formaldehyde eluted concentrations, and stained with Commassie Blue R-250. The intensity of the gray level of the sample after processed in software ImageJ was obtained and generated a pattern of intensity and degree of concentration for semi analytical analysis.

Scanning and processing information. The electrophoretic profile was photographed, digitized, and analyzed using the digital imaging system ImageJ.

Semiquantitative analysis. Analysis of albumin obtained from a pattern of albumin concentration, in which a scale of gray-intensity were created relating to the band intensity. The relative intensification of the standard albumin bands were analyzed and converted into concentrations. The relative intensities, compensated with the respective bands standard, set up in equivalent conditions of electrophoresis and staining, provided the relative percentage of variation of the studied samples.

Statistical analysis. The experimental data were separated into two groups, control and irradiated. The analysis of variance (ANOVA) was applied to the data set and presented significant contrast table (p = 0.05). The data and analysis of variance were evaluated.

CONCLUSION

Our findings from electrophoresis analysis suggest that the expression and the serum albumin concentrations may be modulated by radiation. This finding could be of importance in increasing the immunity and severity of the side effects of the whole-body radiation, since the albumin serums are responsible for drugs and active substances transports like hormones. Also, it is the most abundant proteins in the blood and it is responsible for the control of systemic toxicity.

ACKNOWLEDGEMENTS

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REFERENCES