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RADIATION-INDUCED CHANGES IN THE ELECTROPHORETIC PROFILE OF SERUM ALBUMIN

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ABSTRACT

The albumen profiles had been investigated in electrophoresis' system in function of the exposition to the radiation. Two groups of rats Watchman were set up, the control and an irradiated one, with equivalent age and body weight. The radiated group was exposed to Co-60 at dose of 5 Gy, entire body. At 72 h after-radiation, under deep anesthesia, thoracic and abdominal laparotomies had been carried through, and 600 mL of blood was collected in the lower veins, renal, splenic, and pulmonary jugular. The samples had been treating with heparin and its components separated by spinning. The proteins of the plasma had been identified by vertical electrophoresis in acrylamide, 10%. Alterations induced by radiation of the electrophoresis' profile of the albumen can be observed. Significant differences on in serum albumen concentration were found relating to physiological and radiation induced factors, analyzed on various vascular veins. These data not only point out the sanguineous alteration of the protein concentrations, but possibly a modulation of the gene expression.

Keywords: serum protein, rats, radiation.

INTRODUCTION

Human life limit factors, such as aging and cancer, are subject of research in order to mitigate or remediate its effects. The basis of the cancer control, resectable or not, remains radiotherapy and chemotherapy. Radiation induces molecular, cellular and tissue effects (Hawk et. al, 2014; Toledo et al, 2013). There are no doubt on the effectiveness of radiotherapy but quantify and minimize the side effects should always be addressed, especially on blood (Solter et. al. 1991) and in normal tissues (Gruys et. al. 1994).

Human blood is composed of over 500 different proteins and close of 12 of them are of the paramount importance for maintenance the metabolic functions of the blood. So the main plasmatic proteins are albumin, antitrypsin, TBG, alpha-fetoprotein, alpha1-acid glycoprotein, alpha2-globulin, haptoglobin, macroglobulin, ceruplasmin, immunoglobulins, transferrin, C3 and betalipoproteíns. All these proteins are formed by chains of amino acids joined together by peptide bonds, globulins and immunoglobulins (Smith and Paula et. al. 2008).

The most abundant plasma protein is albumin representing 60% of the total protein content, accounting for 80% of blood oncotic pressure and transport of substances, such as bilirubin, calcium, hormones, drugs, and also for controlling the plasma pH, among other functions. It is synthesized exclusively in the liver, being released as preproalbumina. After removal the initial sequences, the action of the proalbumin enzymes which will be modified by the removal of six residues from the new N-terminus propeptide Albumin appears shaped and sent to the blood with a half-life of 19 days. Recent studies indicate that serum albumin is approaching an ellipsoid of 140 X 40 Angstroms with three homologous domains of 67 kDa and the plasma concentration around 5.0 $mg.dL^{-1}$. Albumin is filtered in the nephrons, being reabsorbed by the distal peritubular capillaries. Albumin can be released in the urine when there is the presence of renal glomeruli demaged.

The relative concentration of albumin in serum can be analyzed by electrophoresis. Protein electrophoresis is a very simple technique that uses electrophoretic forces and electro-endosmotics present in the system. (Gordon, 1995) The separated fractions are visible after treatment with the proteins sensitive dyes. The present study is to characterize and analyze electrophoretic profiles of the protein albumin in control and irradiated animal model.

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 lineage, family, weight and age.
Irradiation. The animals were exposed together to a dose of 5 Gy of Co-60, Laboratory of Gamma Irradiation – LIG, Centro de Desenvolvimento de Tecnologia Nuclear - CDTN.
Sample collection and separation of components. Under deep anesthesia, thoracic and abdominal laparotomy was performed, and subsequently collected 600 mL of blood from the inferior vena cava, kidney, spleen, jugular, hepatic, and pulmonary vein. The samples were heparinized and their separated by centrifugation at 2500 rpm for 30 min components.
Sampling time. The samples were obtained from irradiated animals and control after 72 h of exposure to radiation.
Electrophoresis. Serum samples from the different vascular sites and animalgroups were submitted to vertical electrophoresis of 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 Commassi blue R-250, and fixed.
Standardization of albumin. A standard sample with albumin concentration of 4.0 $ng.mL^{-1}$ was employed and a high range protein weight pattern. SDS-PAGE was run with framework eluted concentrations, and stained with Commassi 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 of proteins was photographed, identified and analyzed using the digital imaging system ImageJ.
Semi-quantitative analysis. Aliquots of albumin obtained from a pattern of albumin were quantified, in which a scale of gray-intensity were created relating to the band intensity. The relative intensitiesfrom the standard albumin bands were analyzed and converted into concentrations. The relative intensities, compared with the standard, set up in equivalent conditions of electrophoresis and staining, provided the relative percentage of variation of the studied protein..
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 test ($p < 0.05$). The data and analysis of variance were evaluated.

DISCUSSION

Calazans S. G. et. al. 2009 observed no variation of albumin in control group of dogs and those with lymphoma. In turn, these data show that the whole body radiation dose of 5 Gy could alter the expression of albumin in the blood. This fact may contribute to the worsening of side effects, since albumin is a major blood protein responsible for controlling blood osmolarity and much of the transport of substances such as hormones and drugs.
As noticed at the control group, each organ analyzed represented by a specific vascular site, showed a distinct albumin serum concentration itself, corresponding to a physiological type identity of the vascular site.
In the irradiated group, there was a significant variation in the concentration of albumin in the vascular sites at the time of 72 h after irradiation.
The increasing of plasma proteins is associated with increasing in protein synthesis by hypergamma globulinemia or paraproteinemia; by the hemoconcentration due to blood stasis during puncture or by decreasing the distributed volume due to dehydration. In turn lowering the concentration of plasma proteins is associated with the decreasing protein synthesis; the reduction of volume of distribution by hyper hydration and increasing capillary permeability; catabolism by increasing excretion of proteins and loss catabolic state. Thus, the amount of albumin in serum can for failure due to reduction of its synthesis in the liver, kidney loss, bowel or skin or conditions that increases the capillary permeability in states of bad absorption and bad nutrition. In turn, there are also many disease processes that cause changes in plasma concentrations. (GABAY and KUSHNER, 1999; MURATA et al, 2004) Our findings demonstrate radiation induced concentration changing of the albumin protein in serum. This phenomenon points to a genetic alteration in the liver, source organ of this protein, justified by a possible induction of genetic damage from radiation.

CONCLUSIONS

Our findings from electrophoresis analysis suggest that the expression and the serum albumin concentrations may be modulated by radiation. This fact may be adjuvant on the increasing of intensity and severity of the side effects of the whole-body radiation, since the serum albumin is 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 osolarity.

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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; 5.00 and 1.45% was found at concentrations of 4.0; 2.0; 1.0; 0.1 $ng.mL^{-1}$. After imaging, one can observe the change in area of the band and the intensity level of the gray pattern to the albumin concentration.

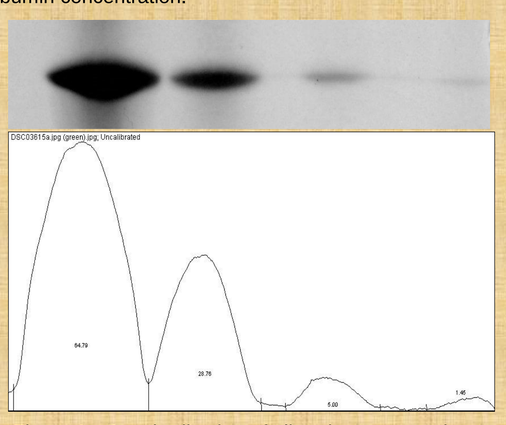


Figure 1 - Standardization of albumin concentration.

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

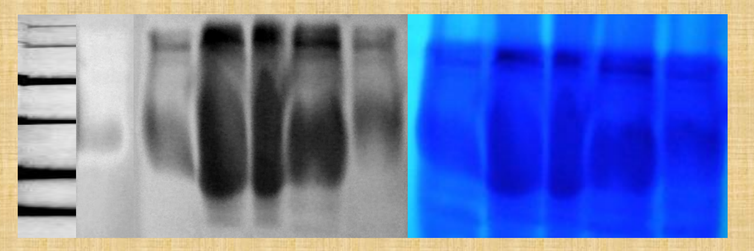


Figure 2 - Illustration of electrophoretic profile of the control group: standard albumin – 1 mL; 15 mL plasma samples sites in vascular control obtained from the jugular vein, renal vein, hepatic vein, inferior vena cava, and the pulmonary vein.

As the blood was heparinized, 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 discarded, limited to a linear rectangular section across the gels.

Table 1 – Concentrations, in $mg.dL^{-1}$, and relative percentage changes in relation to control.

Venous vascular site	Percentage	Concentration
Cava	1,09	0,29
Kidney	1,52	0,41
Liver	1,52	0,41
Esplenics	1,58	0,42
Lung	1,40	0,37

Table 1 showsthe variation 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 control group not treated with radiation and the irradiated one. (Fig.2)

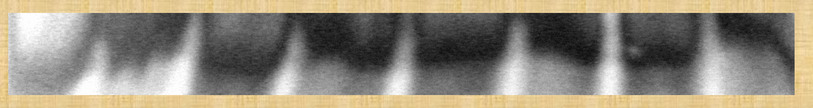
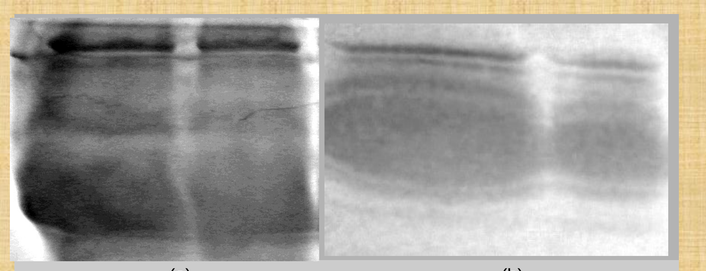


Figure 3 – Serum albumin bands at SDS-PAGE from samples at vascular sites: renal, hepatic, pulmonary, jugular veins from control and irradiated groups, respectively.



(a) (b)

Figure 4 - SDS-PAGE from samples a) of the control and the irradiated pulmonary vein, b) of the control and the irradiated jugular vein.

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

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

Vascular site	Concetration in groups [mg.dL-1]		Reduction (%)
	Controle	Irradiado	
Jugular	**0,29**	**0,20**	**31,3**
Kidney	**0,41**	**0,39**	**4,0**
Liver	**0,41**	**0,29**	**28,4**
Lung	**0,37**	**0,31**	**15,3**