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Kinetics of the expressions of radiation-induced plasma proteins of the cardiac territory in electrophoresis

Cinética das expressões de proteínas plasmáticas radioinduzidas do território cardíaco em eletroforese

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ABSTRACT

Introduction: Radiation induces acute and late alterations in blood proteins. **Objective:** The present study aims at analyzing in time kinetics the electrophoretic profiles of expression bands of blood protein, in the molecular weight range greater than and equal to that of albumin, modulated by ionizing radiation in the cardiac territory, in animal model. **Material and methods:** Animals were exposed to a whole-body dose of 5 Gy ionizing radiation (Co-60). Serum samples were collected from isogenic Wistar rats (control and irradiated groups). At a time kinetics of 12, 24, 48, 72, 96 hours and 35 days post-irradiation, thoracolaparotomies were performed with anesthesia, and 0.3 ml of blood was collected between the left ventricle and the pulmonary artery. The samples were held in heparin and their components were separated by centrifugation. Protein bands with similar molecular weight were identified by vertical 10% electrophoresis, silver staining. **Results:** The findings indicate a systemic acute altered expression of proteins with molecular weight greater than or equal to that of albumin in acrylamide gel, presenting suppression and increased expression due to modulation of preexisting bands, identified in time kinetics. **Conclusion:** These findings point out to acute alterations of protein expression modulated in time, but also to a late modulation of gene expression.

Key words: radiation effects; serum albumin; blood; polyacrylamide gel electrophoresis; blood protein electrophoresis.

INTRODUCTION

All living beings, including the human being, are compulsorily subject to radiation, either by cosmic, cosmogenic, or terrestrial radiation; natural sources and radioisotopes in the body. Additionally, people have become more and more exposed to ionizing radiation from artificial sources. For example, control and treatment of neoplasms are still based on ionizing radiations for diagnostic imaging and radiation therapy. On the other hand, physiological and pathological conditions that limit quality of life, such as aging, cancer, hormonal and neural dysregulation, are associated with environment. Therefore, the correlation between these processes and exposure to low-dose ionizing radiation can be considered a hypothesis.

The human body exposure to a total body irradiation (TBI), on its turn, can occur in radiological accidents and in radiotherapy, by means of controlled exposures. The therapeutic modality of systemic high-dose TBI has been applied since 1950[1, 2]. TBI has used exposures with Co-60 keeping a large distance between patient's skin and the source, reducing the dose in lungs with physical blocks positioned near the irradiator gantry. Presently, megavoltage radiotherapy systems have been applied, such as intensity-modulated radiation therapy (IMRT), arc techniques and translational methods[3, 4]. TBI is frequently used to eliminate bone marrow cells for immunosuppression, necessary in bone marrow transplants; or eliminate leukemic cells, in treatments of leukemia and diverse lymphomas, high-risk Ewing sarcoma, advanced non-Hodgkin lymphoma, and lymphosarcoma. Total doses range from 3 to 10 Gy presented as a single dose, or 10 to

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14 Gy fractioned during the day for several days. TBI is a complex technique that depends on dose, fractioning method and dose rate, and presents high pulmonary toxicity[5].

There are no doubts about the beneficial action of radiotherapy, but the deleterious side effects must always be quantified and minimized, principally those on blood and tissues[6, 7]. Blood is a tissue with high metabolic rate, and extremely radiosensitive mononuclear cells. Its cell components are affected by exposure to ionizing radiation, although pathophysiologic evaluation after artificial exposures has not been a routine.

Human blood is known to be constituted by more than 500 different proteins, with a small group of utmost relevance for metabolic, osmotic, and maintenance functions. The main plasma proteins are albumin, antitrypsin, thyroxine-binding globulin (TBG), alpha-fetoprotein, alpha-1-acid glycoprotein, alpha 2-globulin, haptoglobin, macroglobulin, ceruloplasmin, immunoglobulins, transferrin, betalipoproteins, globulins, gammaglobulins and C3. All of them are made up by chains of aminoacids held together by peptide bonds[8].

The most abundant protein in plasma is albumin, with around 60% of total. It is responsible for 80% of blood oncotic pressure; transportation of substances, such as bilirubin, calcium, hormones and drugs; control over plasma pH, blood viscosity, and consequently, the biophysical nature of blood flow, among other functions[9-11]. It is exclusively synthesized in the liver as pre-proalbumin; after signal peptide is cleaved, proalbumin appears, and will be modified with removal of the new N-terminal six-residue propeptides[12, 13]. Once formed, albumin is sent to blood, and has a half-life of 19 days. However, there are other subgroups of molecular weight greater than that of albumin, of diverse forms and functions, constituted by a wide variety of substances, which are the focus of this study[13, 14].

In analysis and identification of blood proteins, agarose or vertical (acrylamide) gel electrophoresis[14-16] can be employed. This technique uses the electrophoretic and electro-endosmotic forces present in the system for protein separation according to molecular weight. The separated fractions are visible after treatment with protein-sensitive stains[17, 18].

The present study aims at characterizing and analyzing time kinetics of the electrophoretic profile of expression bands of blood proteins, in the band of molecular weight greater than that of albumin, in animal model, modulated by ionizing radiation in the cardiac territory. It is not the scope of this work to quantify, in an absolute manner, volumetric concentrations, or identify, in a specific form, proteins in the electrophoretic profile, but to point the radiation-induced kinetic modulation of bands of the group of blood proteins.

MATERIAL AND METHODS

Grouping

Eight isogenic healthy Wistar rats were used, with a rigid age-group criterion of 12 weeks and body weight of 320 ± 15 g. They were given free access to water and food, with a photoperiod of 12 hours. The animals were divided into two groups: a control group ($n = 2$), called C; and a group undergoing TBI ($n = 6$), named IR. In order to linearize the variables, animals of the same lineage, family, weight and age were used, with isogenic characteristics, provided by the animal facility of Universidade Federal de Minas Gerais (UFMG).

Irradiation protocol

The animals of the IR group were whole-body irradiated by a Co-60 source with a 5-Gy dose at Laboratório de Irradiação Gama (LIG) of Centro de Desenvolvimento da Tecnologia Nuclear (CDTN). The selected dose represents a single dose, possible to be delivered in TBI, but lower than the 30-day median lethal radiation dose (LD50/30) for rats, because of the interest in collecting samples after a month.

Sample collection and separation of blood components

On collection day, the animals were administered a general anesthetic agent. Thoracolaparotomy was performed, to gain access to the cardiovascular system, between the left ventricle and the pulmonary vein. A total of 0.3 ml blood was collected. The samples were heparinized, and their components were separated by centrifugation at 2,500 rpm for 30 minutes.

Sampling time

Samples were taken from animals of the C group on hour 0 and day 30; from the IR group, on hours 12, 24, 48, 72, 96 and day 35 for each irradiated animal.

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE)

Serum samples, in the aforesaid collection times, of IR and C groups, were run on a vertical electrophoresis system. The running gels were prepared with 10% resolving gel and 4% stacking gel, at 120 V and 15 mA. An amount of 15 ml of the sample was loaded in

the wells. The same amount of sample of high-molecular-weight standard (LGC Biotecnologia high range) was included in the run.

Silver staining

After the run, the gels were stained with silver, according to the protocol established by Kang *et al*. (2002)[19].

Image processing

The protein electrophoretic profiles were scanned, treated, and analyzed, using the image processing program *Image J*®. Gray intensities of bands of the same molecular weight were analyzed, and the values were converted into range percentages contrasted to the control. The relative data, put in equivalent conditions of electrophoresis and staining, provided the range percentage value of these protein bands of similar molecular weight in relation to control.

Division into subgroups

The electrophoretic profiles were divided into subgroups for better analysis: albumin (SGA), subgroup 1 (SG1), subgroup 2 (SG2), subgroup 3 (SG3), and subgroup 4 (SG4). These protein subgroups represent groups of proteins of similar molecular weight. Each subgroup refers to an attempt to assemble proteins, as in SGA, albumin; SG1, alpha 1-globulins; SG2, alpha 2-globulins; SG3, beta 1- and beta 2-globulins; and SG4, gammaglobulins.

Ethical treatment

The project was previously approved by the ethics commission on animal use of UFMG 339/2014. The norms of Conselho Nacional de Controle de Experimentação Animal (CONCEA) and Colégio Brasileiro de Experimentação Animal (COBEA), as well as those of the Guide for the Care and Use of Laboratory Animals (Institute for Laboratory Animal Research/National Academy of Science, USA) were respected.

RESULTS

According to analysis of the electrophoretic gel presented in **Figure 1**, we can observe four distinct behaviors for the expression of protein bands, in the region of high molecular weight and albumin:

1) expression of protein bands that were not expressed in control and were induced after exposure to radiation (new expression-B_n);

2) reduction of protein expression after irradiation (down-regulation – B_d);

3) modulated reduction in the time of some proteins, such as albumin up to 24 h, with this effect being reverted in 48 h with the increased albumin expression. The increase was kept higher over the time analyzed, in relation to control (up-regulation – B_u);

4) unaltered behavior after irradiation in comparison with control (stable – B_s);

5) initial control (C 0 h) and control on day 30 (C 30 d) show slight differences between band intensities, non-significant in comparison with the altered expressions in post-irradiation time kinetics, possibly due to the accelerated metabolism of the animal, collected after 30 days.

Analyses can also be detailed in relation to subgroups SGA, SG1, SG2, SG3, and SG4.

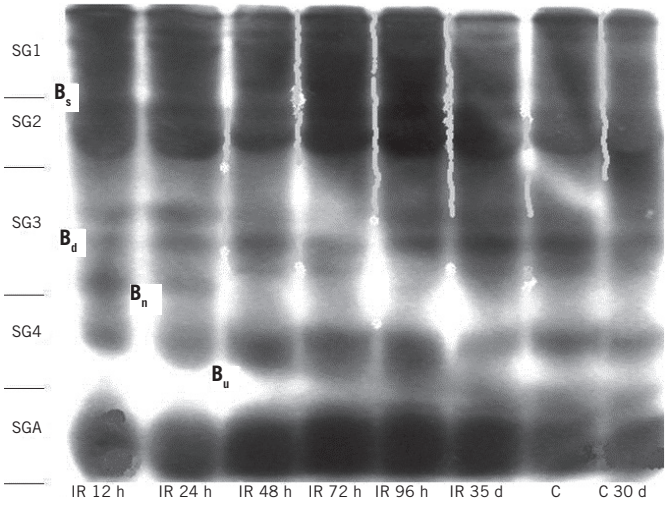

FIGURE 1 – *Electrophoretic profile at 10%, blood serum in cardiac territory of IR and C on days 0 and 30, in kinetics of 12, 24, 48, 72, and 96 h, and 35 days, identified in SG1, SG2, SG3, SG4, and SGA*

IR: irradiated animals; C: control group; SG1: subgroup 1; SG2: subgroup 2; SG3: subgroup 3; SG4: subgroup 4; SGA: subgroup albumin.

SGA

Figure 2 illustrates SGA response to radiation, in a profile developed on a 10% SDS-PAGE, with samples collected from animals of the IR group on hours 12, 24, 48, 72, and 96, and day 35 after irradiation; the latest two profiles belong to group C on hour 0 and day 30. A quantitative analysis can show a kinetic dynamics

of albumin response to radiation, because a reduced expression is initially observed after irradiation at 5 Gy (12 h-24 h), what goes on up to 24 h, but the behavior reversed in 48 h with the increased expression of this protein in comparison with the control. This increase was more intense between hours 72 and 96, still with a higher expression than the control, as observed in controls on hour 0 and day 30, indicating a possible modulation and/or reprogramming of protein expression.

SG4

Figure 3 illustrates SG4 protein response to radiation. A behavior similar to that of albumin was observed: within 12 h and 24 h after irradiation there was reduction of protein expression in comparison with the control. However, after hour 48, a marked expression of this protein occurred, increasing even more its expression on hours 72 and 96, with a decrease on day 35, returning to levels similar to those of the control on hour 0 and day 30.

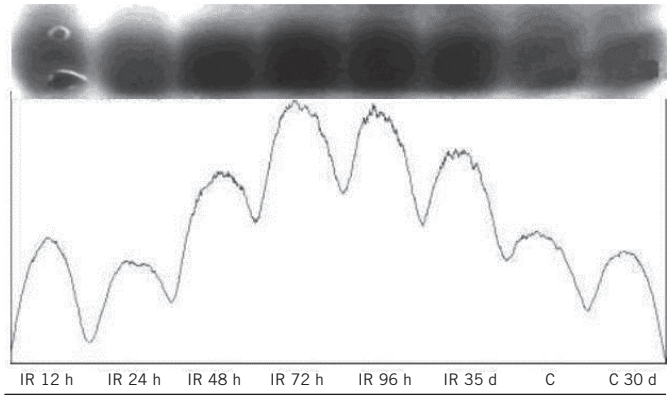

FIGURE 2 – *Electrophoretic profile of subgroup SGA after whole-body exposure to 5 Gy*

SGA: subgroup albumin.

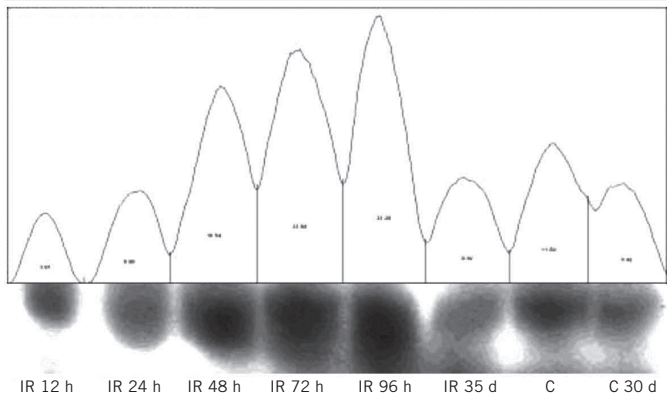

FIGURE 3 – *Electrophoretic profile of SG4 proteins after whole-body exposure to 5 Gy*

SG4: subgroup 4.

SG3

Figure 4 presents the response of SG3 to 5 Gy Co-60, in time kinetics on hours 12, 24, 48, 72, and 96 and day 35. Band incidence was maintained in controls 12, 24, and 48 hours post-irradiation; however, increased expression of this protein occurred within 72 h to 35 days. Thus, after 35 days there is a differentiated expression rate of these proteins in relation to the normal physiological rates of the control group.

SG2

It represents proteins of the group beta-globulins. It behaves in a different manner, as reported in SG1. According to **Figure 5**, a slight reduced expression was observed on hours 12, 24, 48, 72 (IR), and after this time, there was a slight increase on hour 96; however, a significant increase occurred on day 35 (IR) post-irradiation, when the protein presented higher expression than the control. This fact corroborates the possibility of late existence of radiation-induced modulation and reprogramming of expression.

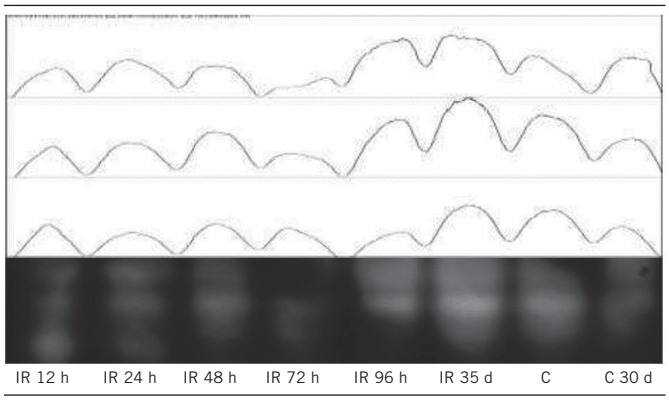

FIGURE 4 – *Electrophoretic profile of SG3 proteins after whole-body exposure to 5 Gy*

SG3: subgroup 3.

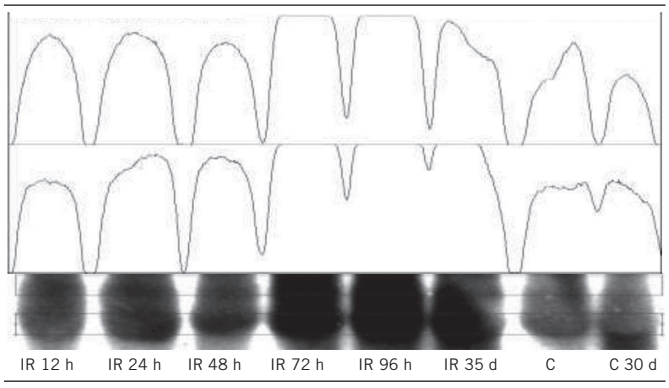

FIGURE 5 – *Electrophoretic profile of SG2 proteins after whole-body exposure to 5 Gy*

SG2: subgroup 2.

SG1

Figure 6 presents bands of pre-albumins of subgroup 1, possibly gammaglobulins. There was no apparent altered intensity of protein of higher molecular weight in this subgroup in time kinetics with radiation. There are alterations in bands with lower weight within the subgroup; however, there is low resolution, insufficient for further detailing.

Analysis of time kinetics

Figure 7 presents time kinetics of protein expression variation in electrophoresis of the main blood proteins of high molecular weight, in a period of up to 96 h. Two types of variation were observed: in subgroups SGA and SG1, there is a decrease followed by high expression, while in subgroups SG2, SG3, and SG4, there is high expression followed by reduction, and again high expression. These fluctuations point out to modulation and regulation of temporal protein expression induced by whole-body exposure to ionizing radiation.

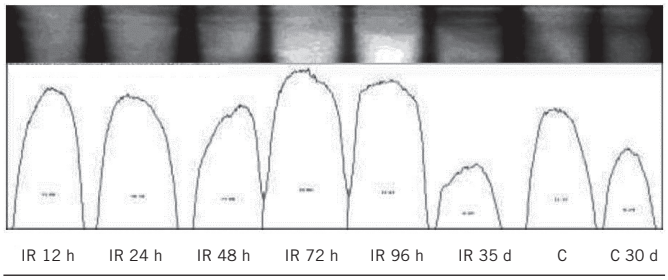

FIGURE 6 – *Electrophoretic profile of SG1 proteins after whole-body exposure to 5 Gy*

SG1: subgroup 1.

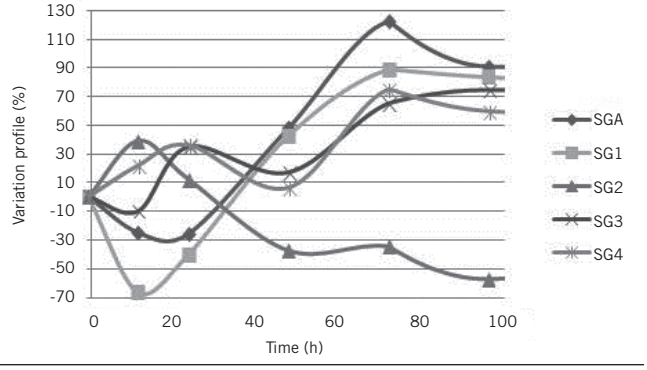

FIGURE 7 – *Time kinetics of protein expression variation in electrophoresis, contrasted to control, of the main blood proteins of molecular weight greater than and equal to that of albumin, in a period of 12 h to 96 h*

SGA: subgroup albumin; SG1: subgroup 1; SG2: subgroup 2; SG3: subgroup 3; SG4: subgroup 4.

DISCUSSION

According to Maratsubaki *et al*. (2002), serum is an extremely elaborate solution of proteins hardly found in an isolate form; they are often associated, as, for instance, albumin and antitrypsin[20]. Therefore, it is difficult to designate a control because the baseline comparison parameters are very complex. Many times the establishment of pure parameters is necessary, what is not always possible. The authors also report the existence of few studies about blood proteins heavier than albumin, 67 KDa, due to its low clinical interest. This keeps their study and understanding in a secondary plan, if compared with low-weight proteins, as cytokines, growth factors and tumor necrosis factor (TNF), interleukins, vasoactive peptides, as bradicinin, angiotensins, prostaglandins, and hormones, all with mass smaller than that of albumin[21, 22]. However, there is a reference point that allows for comparisons and analyses of the radiation effects tested here: albumin.

Calazans *et al*. (2009) observed there is no albumin variation in control dogs and lymphoma patients[23]. Our data show that TBI at a 5 Gy dose may affect the expression of high-molecular-weight proteins and albumin itself in blood, acutely, with identified albumin reduction up to 24 h after irradiation; with constitutive overexpression observed 48 h after exposure, with levels being kept high over time, differently from the control levels. This fact may contribute to worsen side effects, as albumin is one of the main blood proteins, responsible for blood osmolarity control and great part of transportation of substances as hormones and medicines.

The findings demonstrated the existence of distinct behaviors in the expression of proteins with molecular weight greater than that of albumin, and albumin itself, in the cardiac territory. Thus we observe a high dynamics for SG4 in favor of time. After 12 h exposure, decreased expression was observed, as an acute effect, with its expression gradually increasing after 24 h on hours 48, 72, and 96 after exposure. This behavior was reduced within 35 days, remaining apparently with a lower expression than control levels even after one month of radiation. However, late analyses need to be further investigated, since metabolic alterations are also influencing this variation.

SG3 proteins behave differently from SG4, because after irradiation three discriminated proteins reduce their expression when compared with the control. However there is a marked increase of expression on hour 96, kept 35 days after the assay, a fact that corroborates the hypothesis of a modulation and/or protein reprogramming, with the new baseline levels becoming higher than the presented controls.

SG2 presents distinct behaviors, because it initially exhibits a slight increasing expression up to hour 72, when a marked increase appears and continues up to day 35 after the assay. SG1 proteins are very stable; they do not demonstrate major variations in relation to the control. There is little resolution regarding the proteins of this subgroup.

CONCLUSION

The findings from the assays indicate that the expression and the serum concentrations of proteins of molecular weight greater than or equal to that of albumin are modulated and/or reprogrammed by TBI. It was demonstrated that there is still no stable behavior pattern of electrophoretic profile to differentiate a radiation-induced pathology, but a temporal dynamics pattern. We observed changes in the expressions of the aforementioned proteins, especially: 1) inhibition; 2) increase and modulated reduction in time, with the physiologic expression remaining altered in contrast with the control; 3) a stable behavior, not altered by radiation. Yet, it is worth noting that all the observed changes are distinct from those described in the literature for infectious, inflammatory and hypersensitivity processes caused by traumatic events.

Our data suggest the existence of a group of genes and proteins whose expression is radiation-induced. Its activation or inhibition promotes acute modulation in time kinetics, with signs of a systemic late reprogramming of plasma proteins of molecular weight greater than that of albumin in individuals subject to TBI.

RESUMO

Introdução: *A radiação de corpo inteiro induz alterações agudas e tardias no perfil proteico sanguíneo.* ***Objetivo:*** *O presente estudo tem como escopo analisar em cinética de tempo o perfil eletroforético de bandas de expressão das proteínas solúveis do sangue, na faixa de peso molecular superior e igual à albumina, moduladas por radiação ionizante no território cardíaco em modelo animal.* ***Materiais e métodos:*** *Animais foram expostos à radiação de Co-60, em dose de 5 Gy corpo inteiro. Foram coletadas amostras de soro sanguíneo em ratos Wistar isogênicos (no grupo-controle e no irradiado). Em uma cinética de tempo de 12, 24, 48, 72 e 96 horas e 35 dias pós-exposição, foram realizadas toracolaparotomias com anestesia profunda e, posteriormente, foi coletado 0,3 ml de sangue entre ventrículo esquerdo e artéria pulmonar. As amostras foram heparinizadas, sendo, em seguida, separados seus componentes por centrifugação. As bandas proteicas de peso molecular similar foram identificadas por eletroforese vertical a 10%, coradas com prata.* ***Resultados:*** *Os achados indicam alteração aguda sistêmica da expressão das proteínas de peso molecular superior ou igual à albumina em gel de acrilamida, representando supressão e aumento de expressões por meio da modulação de bandas preexistentes identificadas em cinética de tempo.* ***Conclusão:*** *Esses achados apontam tanto para uma alteração aguda modulada no tempo da expressão proteica quanto para uma alteração moderada tardia da expressão gênica.*

Unitermos: *efeitos de radiação; albumina sérica; sangue; eletroforese em gel de poliacrilamida; eletroforese das proteínas sanguíneas.*

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