CHANGES IN THE ACTIVITY OF SELECTED ADAPTIVE ENZYMES IN CHICKEN LIVER AFTER SINGLE GAMMA IRRADIATION

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Abstract

Chickens were used as experimental animals to investigate the influence of gamma radiation on changes in the activity of selected liver adaptive enzymes and serum corticosterone. Analyses were carried out on days 1, 5, 14, and 30 after irradiation. The activity of tyrosine aminotransferase increased significantly on day 5 after irradiation. The activity of tryptophane-2,3-dioxygenase increased significantly on days 1 and 5 after irradiation. Serum corticosterone levels showed an increase on days 1 and 5 and decrease on days 14 and 30 after irradiation. Total liver proteins decreased significantly on day 1 and increased insignificantly on days 5, 14, and 30 after irradiation.

Key words: chickens, corticosterone, gamma irradiation, tryptophane-2,3-dioxygenase, liver proteins, tyrosine aminotransferase.

In the history, organisms have been always exposed to the effect of ionizing radiation of various intensity. During evolution adaptive mechanisms have developed in several animal species and helped them to eliminate harmful influence of ionizing radiation. The sensitivity to the radiation depends on the level of phylogenesis. Therefore, as the object of our experiments we chose Gallus domesticus that belongs to the most resistant domestic animals. The response of the organism to radiation depends not only on the species of animals but also on their age, sex, nutrition and health status. In the present study we observed the reaction of 21-day-old chickens at the beginning of irradiation. Irradiation with lethal or sublethal doses causes significant metabolic changes in the exposed organisms. Ionizing radiation causes changes in the metabolism of lipids, saccharides, proteins and enzymes and induces acute phase mechanisms. Corticosterone plays an important role in this process.

The aim of the study was to observe the changes in corticosterone level in relation to the changes in the activity of “adaptive enzymes” in the liver of fattening chickens after single whole-body irradiation.

Material and Methods

Animals. The experiment was carried out on 21-day-old broiler crossbreds. From hatching up to day 21 they were reared in previously disinfected (11) experimental facilities (12) and were supplied with feed and water ad libitum. The rations consisted of BR I and BR II commercial granulated feed. The broilers were irradiated at the Faculty of Natural Sciences of UPJS in Košice, directly in adjusted plexiglas cages, using an apparatus CHISOSTAT Co-CHIRANA. 

Experimental procedure. The experimental broilers were exposed to a single-dose whole-body gamma radiation of 4.5 Gy (output 0.295 Gy/min). Analyses were carried out on days 1, 5, 14, and 30 after the irradiation. Control broilers were exposed to sham radiation, i.e. they were handled in the same way except for irradiation with gamma rays. The birds were killed by decapitation (2, 3). The pooled blood samples were collected in Petri dishes which were kept on ice and the blood serum, obtained after centrifugation, was analysed. Liver samples were also taken and were stored frozen in liquid nitrogen. The corticosterone concentration was determined by fluorimetric analysis (17). Total protein concentration in the liver was determined by Bio-Las sets (Lachema-Brno). The activity of liver tryptophane-2,3-dioxygenase (TO) was determined by the method described by Knox and Auerbach (9), and the activity of liver tyrosine aminotransferase (TAT) by the method of Diamondstone (4). Measurements were done using a spectrophotometer Spekol 11 and fluorimeter (Spektrofluorimeter FP-550, JASCO). Six birds from each group were analysed on average. The significance of differences between experimental broilers and the controls was evaluated by the unpaired t-test (Prism 3.0, Software). The experiment was conducted in summer.
Results

The serum concentration of corticosterone (Fig. 1) increased insignificantly on days 1 and 5 and decreased on days 14 and 30 after irradiation. When compared to the control, the concentration of total proteins in the liver (Fig. 2) was decreased significantly on day 1, increased insignificantly on days 5 and 14 and did not differ on day 30 of the experiment.

The activity of tryptophane-2,3-dioxygenasis (Fig. 3) was not significantly decreased on day 30 after irradiation. As for other periods of our observation, it showed a significant increase on day 5 after irradiation.

The liver activity of tyrosine aminotransferase (Fig. 4) decreased significantly on day 1. As for other periods, it was increased significantly on day 5 compared to the controls.

![Fig. 1. Changes in chicken serum corticosterone. The values are given as means ± S.E.M.](image1)

![Fig. 2. Changes in total liver proteins. The values are given as means ± S.E.M. ** P<0.01.](image2)

![Fig. 3. The changes of tryptophan-2,3-dioxygenase activity in the liver. The values are given as means ± S.E.M. *** P<0.001.](image3)
Discussion

Metabolic changes in animal organisms under physiological conditions as well as after the action of some negative factors (10), such as ionizing radiation, have been the subject of many studies.

It is well known that the activity of some adaptive enzymes, e.g. tyrosine aminotransferase and tryptophane-2,3-dioxygenase increases rapidly under the action of stress factors or hormones. Even non-lethal doses of ionizing radiation can activate the hypothalamo-hypophyseal-adrenal axis (15). This activation is accompanied by an increase in secretion of adrenal cortex glucocorticoids that may induce the activity of the studied enzymes. Changes in the serum corticosterone were also observed.

After enzyme activation by steroid hormones or other stress factors (e.g. ionizing radiation), the de novo synthesis of proteins occurs through increasing the amount of m-RNA available for translation.

The different sensitivity of inductive processes may also be explained by a specific way of irradiation damage to DNA, which may occur in some genes only while other genes remain undamaged, or by irradiation-interference with biosynthesis of certain proteins on the translation level (13). Similar to glucocorticoids, irradiation alone stimulates the transfer of amino acids into hepatocytes (7). The excessive amino acids, released from radiosensitive tissues after their accumulation in the liver, induce the activity of the enzymes which control the metabolism of amino acids in the liver. It is known that in vivo concentration of amino acids is a limiting factor in the process of their transformation to glucose, and glucagon or c.AMP are the main activators of amino acid transport to hepatocytes and the concentrations of these activators are increased after irradiation (8). Our results showed marked changes in the serum enzyme activities in the irradiated chickens. The initial increase in corticosterone concentrations observed after the irradiation indicated stressful action of ionizing radiation on the organism.

This change was observed in all groups tested. Corticosterone induces activity of many enzymes and therefore the changes in the activity of adaptive enzymes in relation to the changes in corticosterone concentrations were studied.

Neither decrease nor significant increase in the enzyme activities is observed after irradiation of pure isolated enzymes, contrary to irradiated live systems (14).

The enzymes in irradiated organisms may be damaged due to changes in the “active centre of proteins”, prosthetic compartments of enzymes, or damages to enzyme bonds. An increase in the activity of these enzymes indicates a prevalence of catabolic processes in irradiated bodies, associated not only with changes in cellular permeability (1), development of serious histological changes in hepatocytes during the first three days after irradiation, but also with stimulation of synthesis of de novo transamination enzymes induced by increased secretion of adrenal cortex after irradiation. In addition to direct damage caused by ionizing radiation and to other factors, the activity of adaptive enzymes may also be influenced by intake of food and water. Toropila et al. (1996) observed an increase in tryptophane pyrolase and alanine aminotransferase and a decrease in aspartate aminotransferase activity in the liver during 12-day starvation of rats.

According to the literature data, an organism in the stage of rapid growth is more sensitive to ionizing radiation than that in adulthood. During the postnatal growth, the irradiation causes growth retardation or growth disorders because of the damage to differentiation of cell processes in the growth zones (5).

References

3. Decree No. 231 of the Code, (1998) of the Ministry of Agriculture of the Slovak Republic about keeping pet animals, wild animals, and dangerous animals and about the protection of laboratory animals.