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Changes in serum enzyme activity as an indicator of injuries in irradiated chickens

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Abstract

Background and Purpose: *Organic lesions and metabolic disorders of many organs, especially the liver, are followed by changes in some enzyme activities in blood plasma of domestic animals and poultry. It is also known that the liver in birds is equally affected by radiation as the intestine, bone-marrow and sex glands, which is different in mammals. In this paper an attempt was made to investigate the influence of gamma ray irradiation of the whole body of chickens upon activity of several enzymes (enzymatic profile) in blood plasma. Our aim was also to evaluate whether investigation of the enzymatic profile in blood plasma can help in the diagnosis of organic or functional liver damages caused by gamma ray in chickens.*

Materials and Methods: *The experiments were performed on hybrid chickens of heavy »Jata« breeds of both sexes. Chickens were irradiated by gamma ray in the dose of 7.23±0.95 Gy. Blood samples were taken from the wing vein on days 1, 3, 5, 7, 9 and 15 after irradiation. The activity of aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyl transpeptidase (GGT), leucine aminopeptidase (LAP), lactate dehydrogenase (LDH) and alpha-hydroxy-butyrate dehydrogenase (HBDH) in blood plasma of irradiated chicken was determined spectrophotometrically by using Boehringer Mannheim GmbH optimized kits. At the end of the experiment all birds were sacrificed and the organs were pathomorphologically and histologically investigated.*

Results: *Throughout the experimental period, only GGT activity did not statistically significantly change. All other enzymes activity showed a decreasing tendency during the experiment; statistically significant decreases were recorded as follows: on the 1st day of the experiment – LAP and LDH activity; on the 3rd day – LAP activity; on the 5th day – ALT and LDH activity; on the 7th day – AST, LDH and HBDH activity, on the 9th day – LAP and HBDH activity, and on the 15th day – LAP activity.*

Conclusion: *The determination of a so-called enzyme profile in blood plasma which included measurement of AST, ALT, LAP, LDH and HBDH activities may serve as an additional test for functional liver damages in chickens caused by ionizing radiation before the appearance of clinical symptoms.*

INTRODUCTION

Due to its good quality and relatively low price as a result of a simple and short production cycle, poultry meat takes a very high position in the consumption of foodstuffs of animal origin. Apart from that, poultry are among the more radioresistant species of our domesticated

animals *(1);* semilethal dose ($LD_{50/30}$) after irradiation of the total body of a chicken with gamma or X-rays is 6,00–8,00 Gy, depending on dose rate *(2)*. Because of these two reasons, poultry might represent one of the more dependable sources of fresh food of animal origin that might be available after a nuclear attack. But, in case of nuclear attack, irradiated animals, in order to be used for human foodstaff, must be slaughtered before clinical signs of radiation sickness, or pathomorphological changes in organs.

Although chickens are more radioresistant species, the liver in birds (the chickens are also birds) is equally affected by radiation as the intestine, spleen, bone-marrow and sex glands, which is different in mammals *(3)*.

So far many authors have reported that organic lesions and metabolic disorders of many organs, especially the liver, are followed by changes in some enzyme activities in blood plasma of domestic animals and poultry *(4–9)*. It is also well known that changes in enzyme activities in blood plasma are a very useful test for an early diagnosis of some diseases of many organs and metabolic disorders *(10),* including also the liver *(11)*.

In this paper we presented all activities of the investigated enzymes in blood plasma of the gamma ray irradiated chickens. Thus, we attempted to use their changes in the diagnosis of organic or functional liver damages caused by ionizing radiation in chickens.

MATERIALS AND METHODS

Animals

The experiments were performed on 50-day-old hybrid chickens of »Jata« breed of both sexes, with 2.0 to 2.3 kg of body mass. The animals were divided into two groups, control and experimental, with five chickens in each group. They were kept separately in two wire-cages (75 cm high; 2000 cm^2/bird area) and each animal was also marked individually. Throughout the experimental period the microclimatic conditions were optimal for chickens of this age (18 °C temperature, 24-hour lighting, 7 m^3 of air h^{-1} kg^{-1} body mass with air velocity of 45 m min^{-1}). The microclimate was appropriate, since the concentrations of CO_2 and NH_3 did not exceed 0.20 and 0.003%, respectively *(12)*. The chickens were fed a commercial mash BRO-finisher for broilers produced by TSH Agroemona (Slovenia) from the 13th day of age which, as well as water, was given *ad libitum*.

Irradiation and dosimetry

The chickens in the experimental group were irradiated with gamma ray from panoramic ^{60}Co source (mean energy gamma ray of 1.25 MeV) of the Ruđer Bošković Institute (Zagreb, Croatia) *(13)*. Activity was about 666 TBq. The tissue equivalent absorbed dose in free space on the point of animal axis, measured with an ionization chamber type 2581 and a Farmer Dosimeter type 2570 (NE Technology Limited) was 8.02 Gy. Dose rate was about 746 mGy/s, and a source axis-to-animal axis distance was 3.06 m. The dose distribution in the body of irradiated animals was determined in the water phantom by personal dosimeter DL-M4 *(14),* and mean absorbed dose in animal's body was 7.23±0.95 Gy.

Samples

Blood samples were drawn from the wing vein 1, 3, 5, 7, 9 and 15 days after gamma irradiation. The blood was heparinized and the cells were separated from the plasma by centrifugation at 2,000 g.

Enzymatic assays

The dynamics of activity changes of aspartate aminotransferase (AST; EC 2.6.1.1), alanine aminotransferase (ALT; EC 2.6.1.2)), gamma-glutamyl transpeptidase (GGT; EC 2.3.2.1), leucine aminopeptidase (LAP; EC 3.4.1.1), lactate dehydrogenase (LDH; EC 1.1.1. 27) and alpha-hydroxy-butyrate dehydrogenase (HBDH; corresponding to the isoenzyme LDH1 and partly LDH2) in blood plasma was investigated. AST and ALT activities were measured by the methods of Reitman and Frankel *(15),* LDH by the method of Wroblewski and La Due *(16),* HBDH by the method of Rosalski and Wilkinson *(10),* GGT by the method of Szasz *(17)* and LAP by the method of Nagel *et al. (18),* using Boehringer optimized kits (Boehringer Mannheim GmbH, Germany). The activities were measured at 365 nm (LDH and HBDH), 405 nm (GGT and LAP) and 546 nm (AST and ALT) on the Pye Unicam SP 600 UV spectrophotometer. The temperature of the reaction was kept at 25 °C using a water bath.

Pathomorphological investigation

At the end of the experiment all birds were sacrificed and pathomorphologically investigated. The liver, heart, lung, spleen, kidneys, duodenum and pancreas were also examined histologically.

Statistical analysis

Results of enzyme activities in the blood plasma were expressed as the percentage of the controls (controls=100%) and were statistically analyzed using Student's t-test with a 5% level of significance *(19)*.

RESULTS

The results of enzyme activity measurements in the blood plasma of chickens after gamma irradiation are presented in Figure 1.

Throughout the experimental period, only GGT activity did not statistically significantly change. On the 1st day after gamma irradiation, LAP and LDH activity was statistically lower ($P<0.01$) in the blood plasma of irradiated chickens than in the controls. On the 3rd day after gamma irradiation only the LAP activity was statisticaly lower in the experimental birds ($P<0.05$). On the 5th day of the experiment ALT and LDH activity was also statistically lower in the experimental birds ($P<0.05$). On the 7th day of the experiment AST activity ($P<0.05$)

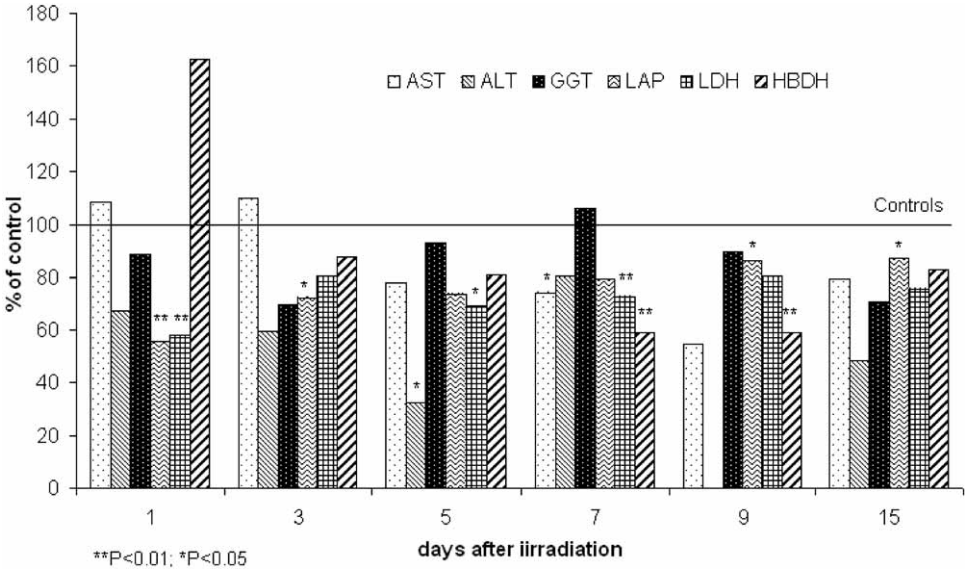

Figure 1. *Enzyme activities in blood plasma of chickens after irradiation with dose of 8.02 Gy gamma ray. AST = aspartate aminotransferase, ALT = alanine aminotransferase, GGT = gamma-glutamyl-transpeptidase, LAP = leucine aminopeptidase, LDH = lactate dehydrogenase, HBDH = alpha-hydroxy-butyrate dehydrogenase.*

and LDH and HBDH activity ($P<0.01$) in gamma irradiated chickens showed statistically significant decrease. The LAP and HBDH activity was also lower in the blood plasma of experimental chickens than in the control on the 9th day of the experiment ($P<0.05$ and $P<0.01$, respectively). Finally, on the 15th day of the experiment, the LAP activity was significantly lower ($P<0.05$) in experimental chickens than in controls.

Pathomorphological and histological investigation of the carcasses and organs of sacrificed birds did not show any morphological changes.

DISCUSSION

The results obtained indicate that AST, ALT, LAP, LDH and HBDH activities in the blood plasma of gamma irradiated chickens were significantly lower at a certain period of the experiment than in controls, while GGT activity remained unchanged throughout the experimental period. Although we did not find any morphological changes in the organs of experimental chickens, we suppose that the changes in activity of the investigated enzymes are an indication of biochemical lesion in the liver caused by ionizing radiation. This hypothesis is based on a discovery by Bogin and Israeli *(20),* Bogin *et al. (21)* and Rivetz *et al. (22)* who discovered that ALT and HBDH are specific for the chicken liver, as well as on the discovery by Kraljević *(23)* who found that LAP value is a useful parameter for the discovery of different pathological changes in the liver.

These results can be, therefore, compared with those obtained by Jones *(24),* Kraljević *et al. (25, 26)*, and Emanović *et al. (27),* but they differ from those obtained by Miller and Gates *(28),* Milch and Albaum *(29),* Hughes *(30)* and Miholjčić *et al. (31)*. Jones *(24)* investigated serum alkaline phosphatase activity in goats after a single oral intake of near-in (or local) fallout-simulating particles emitting β- and γ-radiation, ranging from 6.48 MBq to 14.8 GBq. He found that goats ingesting more than 3.7 GBq showed a decrease in the activity of the investigated enzyme. Kraljević *et al. (25, 26)* and Emanović *et al. (27)* investigated the activity of enzymes in the blood plasma of chickens after the injection of ^{32}P and found a decrease in alanin aminotransferase and hydroxy-butyrate dehydrogenase activity. The second group of authors investigated the activity of enzymes in the blood plasma or blood serum of an animal after X-radiation exposure. Miller and Gates *(28),* investigating the activities of peptidases in dog blood plasma, found an increase in peptidase activity after X-irradiation exposure. Milch and Albaum *(29),* Hughes *(30)* and Miholjčić *et al. (31)* found an increase in serum transaminase activity in rabbits and the rat after X-ray exposure.

At the moment we do not know the actual reason for the decrease in enzyme activity in the blood plasma of gamma irradiated chickens. This decrease might be due to: a) the destruction or inactivation of enzymes; b) the failure of their synthesis due to the destruction of the mechanisms responsible for it, or c) the release of some enzymes inhibitors or the disappearance of some enzyme activators.

It is also not clear why, for example, LAP activity decreased in the blood plasma on the first day after gamma irradiation, and AST and HBDH activities did not decrease earlier than the seventh day. Supposedly, this appearance could be caused by one of two causes: perhaps these three enzymes have different mean half-life in the chicken blood, and /or b) the mechanisms which are responsible for their synthesis have different

resistance to ionizing radiation. The first hypothesis is based on some data concerning the half-life of enzymes in human blood *(32)* and of some enzymes in animals *(5, 33)*. Different enzymes have a different half-life in the blood plasma of some animals *(33)* and mean half life of AST was 17 hours, ALT 47 hours and alkaline phosphatase about 5 days *(5, 32)*. Even the different isoenzymes of LDH have a different mean half-life *(32)*. Unfortunately, we do not have data concerning the half-life of investigated enzymes in chicken blood plasma but we can assume that they are different. Therefore, it is possible that all the mechanisms responsible for the synthesis of investigated enzymes were damaged at the same time, soon after gamma irradiation, but a significant decrease in activity was first noted for the enzyme whose rate of disappearance from the blood stream is much higher than that of others. The second hypothesis is based on the very well known fact that some cells in a given tissue are much more radiosensitive than other *(34),* and that some enzymes are also much more radiosensitive than others *(35)*. In this case, a decrease in activity of one enzyme occurs before a decrease in activity of the other.

In conclusion, the determination of a so-called enzyme profile in blood plasma which includes measurement of AST, ALT, LAP, LDH and HBDH activities, may serve as additional test for functional liver damage in chickens caused by ionizing radiation before the appearance of clinical symptoms. This enzyme profile also seems to be an indicator of radiation damage in the liver before any morphological changes in this organ of gamma irradiated chickens can be detected. And finally, this enzyme profile can also serve for determination of a so-called degree of urgency of the slaughter of gamma irradiated chickens.

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