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AMINOTRANSFERASE ACTIVITY IN CHICKEN BLOOD PLASMA AFTER APPLICATION OF A LETHAL ACTIVITY OF ^{32}P

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An attempt was made to evaluate whether in chicken the activity of plasma aspartate aminotransferase and alanine aminotransferase changes after ^{32}P administration, and whether it helps in the diagnosis of morphological or functional liver damage caused by ionizing radiation before the appearance of clinical simptoms of radiation sickness.

Fifty day old hybrid chickens of heavy Jata provenience of both sexes were treated by ^{32}P administred i.m. as disodium hydrogen phosphate in a single dose of 333 MBq per kilogram of body weight. Blood samples were taken from the wing vein on days 1, 3, 5, 7 and 10 after administration of ^{32}P. The activities of aspartate aminotransferase and alanine aminotransferase were determined spectrophotometrically using optimized kits produced by Boehringer Mannheim GmbH.

The obtained results have shown that aspartate aminotransferase activity increased on the 3rd and 5th day and it decreased on the 7th and 10th day of the experiment. A statistically significant difference was recorded on the 3rd day of the experiment. Alanine aminotransferase activity increased during the first five days of the experiment, and on the 7th day it decreased. On the 10th day of the experimet the activity of alanine aminotransferase in the blood plasma of ^{32}P treated birds was not detectable; a statistically significant difference was recorded on the 5th day only.

The obtained results indicate that the activity of aspartate aminotransferase and alanine aminotransferase may serve as an indicator of functional and/or morphological liver damage in chickens caused by ionizing radiation before the appearance of clinical symptoms of radiation sickness.

Key words: alanine aminotransferase, aspartate aminotransferase, chicken, phosphorus-32

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. It is just because of such simple and rapid production that, under the circumstances of a larger-scale radioactive contamination, poultry meat might become the main source of protein of animal origin. We have already experienced such radioactive contamination, both in war and in peace, and in the light of recent global geopolitical trends this possibility is once more becoming more topical. In particular, people are becoming increasingly aware that terrorist organisations in the New Terrorism doctrine, in addition to chemical and biological terrorism, include radiological and nuclear terrorism, as well. There are three ways of possible terrorist nuclear action. First, they could use fissile material, i.e. plutonium (^{239}Pu) or highly enriched uranium (HEU) and attempt to fabricate the so-called "improvised nuclear devices" (IUD). Second, they could use radioactive material and disperse it in the environment in order to cause radioactive contamination, Third, they could attack a nuclear plant – for example, a nuclear power plant – either to cause radioactive contamination or to steal radioactive material for their nuclear terrorist actions (Čižmek, 2005; Augustine *et al.,* 2005).

Because of all this, as well as the fact that no investigation of the effects of fissionable elements, in particular radioactive phosphorus (^{32}P) on fattening chickens has been conduced so far, in our previous paper (Šimpraga *et al.,* 2006) we investigated the effects of a lethal quantity of radioactive phosphorus (^{32}P) upon clinical picture, hematological parameters and pathomorphological changes of tissues and organs in fattening chicken at the time of slaughter. The obtained results showed that clinical signs of radiation sickness appear on the 6^{th} day post contamination, and death of all contaminated animals occurred on the 9^{th} and 10^{th} days post-contamination. Results of pathoanatomic examination of dead contaminated broilers revealed marked changes on parenchymal organs which manifested as spleen atrophy, nephrosis, fatty liver degeneration and myocardial degeneration.

After having investigated the rate of deposition and turnover of radioactive phosphorus ^{32}P in the tissues of laying hens, Shirley *et al.* (1954) reported a maximum concentration in bones and liver. Three hours after its parenteral administration the mean concentration of ^{32}P in the liver was 17% of the initial dose and remained on a high level for a long time. Even up to 504 hours later, the livers still contained over 1% of the initial dose of ^{32}P. According to these data one can expect that radiation from ^{32}P shoud provide a high enough irradiation dose within the livers very early, which may provoke some metabolic changes during the first five days, i.e. in the time when clinical signs of radiation sickness do not occur. This expectation is supported by the fact that the liver in birds is equally affected by radiation as the intestine, spleen bone-marrow and gonades (Bacq and Alexander, 1966).

So far many authors have reported that organic lesions and metabolic disorders of many organs, especially the liver, are followed by changes of some enzyme activities in the blood plasma of domestic animals and poultry (Cornelius *et al.,* 1959; Freedland and Kramer, 1970; Forenbacher, 1972; Timet *et al*., 1975; Fluckiger *et al.,* 1977; Kraljević, 1977). It is also well known that changes of

enzyme activities in blood plasma are a very useful test for early diagnosis of some diseases and metabolic disorders (Wilkinson, 1976; Rosalski, 1976).

In this investigation we tried to determine if there is a change of aspartate aminotranferase (AST; EC 2.6.1.1) and alanine aminotransferase (ALT; EC 2.6.1.2) activity in the blood plasma of broilers after an intramuscular injection of a lethal quantity of radioactive isotope ^{32}P by attempting to establish its possible validity in the recognition of the injury in parenchymal organs, especially in the liver, before the appearance of clinical symptoms of radiation sickness.

MATERIALS AND METHODS

Animals

The experiment was performed on healthy broiler, hybrids of the Jata heavy breed of both sexes, at an age of 50 days and mass ranging from 1500 to 2000 g. The birds were kept in wire–cages and fed a commercial mash produced by Agroemona-Domžale, Slovenia, which, as well as water, was given *ad libitum.* Throughout the experimental period both temperature and relative humidity were recorded in the pen house and their values were adjusted to optimal limits for chickens of this age (Ivoš, 1966). The microclimate was appropriate, since the concentrations of CO_2 and NH_3 did not exceed 0.20% and 0.003%, respectively.

Isotope administration

The broilers (n=5) were treated by radioactive phosphorus isotope ^{32}P (Amersham International plc., England) administered intramuscularly as $Na_2H^{32}PO_4$, in a single dose of 333 MBq per kilogram of body weight. The specific activity of the solution was 333 MBq per milliliter. Along with ^{32}P treated chickens, there was a control group treated with saline in a dose of 1 milliliter per kilogram of body weight (n=5). All other conditions were the same for both groups.

Samples

Blood samples were drawn from the wing vein 1, 3, 5, 7 and 10 days after ^{32}P injection. 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 AST and ALT in the blood plasma was investigated by the methods of Reitman and Frankel (1959), using Boehringer optimized kits (Boehringer Mannheim GmbH, Germany). The activities were measured at 546 nm on the Pye Unucam SP600 UV spectrophotometer. The temperature of the reaction was kept at 25 ^oC using a water bath.

Clinical examination

The animals were subjected to clinical examination on a daily basis, in the morning and in the afternoon, eight days before and ten days after the application of ^{32}P. The examination included: general appearance and behaviour of the birds, respiration, response to extraneous stimuli (hand-clapping), eating and drinking, as well as colour and consistency of faeces.

Pathomorphological investigation

Immediately after death, ^{32}P treated animals were dissected and subjected to pathohistological examination, which included the liver, lungs, cloacal bursa, duodenum, pancreas, heart, spleen, kidney and adrenal gland.

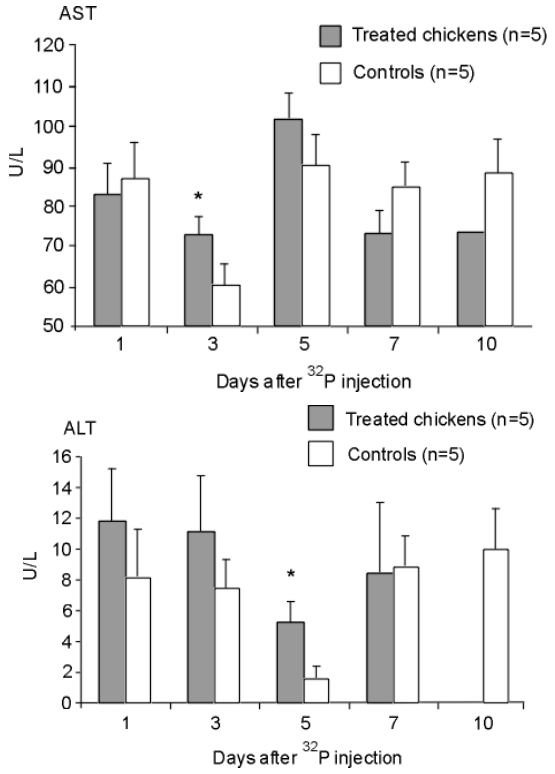
Statistical analysis

Results are expressed as mean ± standard error (SE) and statistically analysed by Student's t-test with a five percent level of significance (Renner, 1970).

RESULTS

AST and ALT activity

The results of AST and ALT measurements in the blood plasma of chickens after intramuscular injection of radioactive isotope ^{32}P in a single dose of 333 MBq per kilogram of body weight, are presented in Figure 1.



Values are given as mean±SE (n=5). *statistically significant with P<0.05

Figure 1. Aspartat aminotransferase (AST) and alanin aminotrasferase (ALT) activity (U/l) in the blood plasma of chickens after intramuscular injection ^{32}P in a single dose of 333 MBq/kg of body weight

AST activity in the blood plasma of ^{32}P treated chickens increased on the 3rd and 5th days after ^{32}P administration, and decreased on the 7th and 10th day of the experiment. Only AST activity recorded in the blood plasma of the experimental group on the 3rd day was significantly higher ($P<0.05$).

ALT activity in the blood plasma of ^{32}P treated chickens was higher than in the controls during the first five days. The greatest difference ($P<0.05$) was recorded on the 5th day after ^{32}P administration. On the 7th day of the experiment ALT activity in the blood plasma of ^{32}P treated chickens was lower than in the controls. At the end of the experiment ALP activity in the blood plasma of the only survived bird could not be determined.

Clinical examination

Clinical examination showed that clinical signs of radiation sickness appear on the 6th day after ^{32}P administration, and death of all contaminated animals occurred on the 9th and 10th days post-contamination.

Pathoanatomical examination

Pathoanatomical examination of dead animals of ^{32}P treated chickens reveald a general anaemic condition and petechial bleeding on the heart and mucous membranes of the intestines and stomach. Also, contaminated chickens presented marked changes on parenchymal organs manifested by spleen atrophy, nephrosis, fatty liver degeneration and myocardial degeneration.

Pathohistological examination

Pathohistological examination of tissues and organs confirmed the findings of pathoanatomic observations, which indicated the changes caused by radioactive radiation.

DISCUSSION

The obtained results indicate that AST and ALT activities in the blood plasma of ^{32}P treated chickens are higer during the first five days of the experiment, compared to the controls. After that time the activities of both enzymes in the plasma of contaminated chickens are lower than in the controls. We suppose that the increase of activity of the investigated enzymes is an indication of biochemical and morphological lesions in the liver caused by ionizing radiation. This hypothesis is based on the discovery of Bogin and Istraeli (1976), Bogin *et al.* (1976) and Rivetz *et al.* (1977) who discovered that AST and ALT are specific for the chicken liver, as well as on the discovery of Kraljević (1977), who found that AST and ALT-values are useful parameters for the discovery of different liver pathological changes in hens and chicken.

At the moment we do not know the actual reason for the decrease of enzyme activities in blood plasma of ^{32}P treated chickens. This decrease might be due to: a) the destruction or inactivation of enzymes; b) the failure of its synthesis due to the destruction of the mechanisms responsible for it, or c) the release of some inhibitors or the disappearance of some activators of the enzyme. We opt for the second assumption, i.e. that the decrease of enzyme activity in the blood

plasma of treated chickens is caused by the failure of its synthesis, due to the destruction of mechanisms responsible for it. This hypothesis is primarily based on the fact that some radionuclides, including ^{32}P, may cause lethal effects in cells via transmutation in the case this event takes place inside the DNA molecule (Apelgot, 1983). It is very likely tha ^{32}P, used in our experiment, will be incorporated into DNA molecules. Phosphorus is one of the most important elements for the synthesis of DNA, and it can cause damage of the DNA molecule and /or the death of cells. Since the DNA molecule serves as a "matrix" for the synthesis of mRNA, which is responsible for protein synthesis, the above mentioned transmutation effect of ^{32}P upon cells can result in the decrease of enzyme synthesis in cells, which is reflected in the decrease of enzyme activity in the blood.

The second reason for the decrease of enzyme activities in the blood of ^{32}P treated chickens could be the degeneration of the liver in the chickens, with occasional focal hepatic lesions, which was confirmed by pathohistological examination of organs and tissues. Enzyme synthesis in degenerating organs, i.e. in degenerative liver, is decreased or it completely disappears.

In conclusion, the obtained results indicate that the activity of AST and ALT changes in the blood plasma of ^{32}P treated chickens. At this moment it seems that both enzymes may serve as indicators of functional and/or morphological liver damage in chickens caused by ionizing radiation before the appearance of clinical symptoms of radiation sickness.

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AKTIVNOST AMINOTRANSFERAZA U SERUMU PILADI TRETIRANE LETALNOM DOZOM ^{32}P

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SADRŽAJ

U ovom radu se željelo istražiti da li se aktivnost aspartat aminotransferaze i alanin aminotransferaze u krvnoj plazmi pilića mijenja nakon aplikacije fosfora-32 (^{32}P), i da li te promjene mogu služiti u dijagnozi organskih ili funkcionalnih oštećenja jetre u pilića uzrokovanih jonizacijskim zračenjem prije pojave kliničkih znakova radijacijske bolesti.

Pilićima hibridima teške pasmine Jata oba spola, starim pedeset dana apliciran je i. m. ^{32}P kao natrijev monobazini fosfat (NaH_2PO_4) u jednokratnoj dozi od 333 MBq po kilogramu tjelesne mase. Uzorci krvi vađeni su iz krilne vene 1, 3, 5, 7. i 10. dana nakon aplikacije ^{32}P. Aktivnosti aspartat aminotransferaze i alanin aminotransferaze određivane su spektrofotometrijski koristeći gotove komplete reagencija proizvođača Boehringer Mannheim GmbH.

Dobiveni rezultati su ukazali da je aktivnost aspartat aminotransferaze bila povećana 3. i 5. dana, a smanjena 7. i 10. dana pokusa. Statistički značajna razlika bila je zabilježena 3. dana pokusa. Aktivnost alanin aminotransferaze bila je povećana tijekom prvih pet dana pokusa, dok je 7. dana pokusa bila smanjena. Desetoga dana pokusa aktivnost alanin aminotransferaze u krvnoj plazmi pilića tretiranih sa ^{32}P se nije mogla odrediti; statistički značajna razlika zabilježena je samo 5. dana pokusa.

Aktivnost aspartat aminotransferaze i alanin aminotransferaze u krvnoj plazmi može služiti kao indikator funkcionalnih i/ili morfoloških oštećenja jetre u pilića uzrokovanih jonizacijskim zračenjem prije pojave kliničkih simptoma radijacijske bolesti.