VETERINARSKI ARHIV 78 (4), 289-296, 2008

Effect of the lethal activity of ^{32}P upon alpha amylase activity and glucose concentration in chicken blood plasma

Marinko Vilić*, Petar Kraljević, and Miljenko Šimpraga

Department of Physiology and Radiobiology, Faculty of Veterinary Medicine, University of Zagreb, Zagreb, Croatia

VILIĆ, M., P. KRALJEVIĆ, M. ŠIMPRAGA: Effect of the lethal activity of ^{32}P upon alpha amylase activity and glucose concentration in chicken blood plasma. Vet. arhiv 78, 289-296, 2008.

ABSTRACT

An attempt was made to evaluate whether the activity of alpha-amylase and the concentration of glucose changes in blood plasma of chickens after ^{32}P administration, and whether it helps in the diagnosis of organic or functional liver damage in chicken caused by ionizing radiation before the appearance of clinical simptoms of radiation sickness. Fifty day old hybrid chickens of heavy Jata breeds of both sexes were treated with ^{32}P, administered intramuscularly in a single amount of 333 MBq per kilogram of body mass. Blood samples were taken from the wing vein on days 1, 3, 5, 7 and 10 after administration of ^{32}P. The activity of alpha amylase was determined spectrophotometrically by using the kits produced by «Herbos d.d Sisak» (Croatia). Concentration of glucose was determined photometrically with o-toluidinom, by also using the kits produced by «Herbos d.d. Sisak». Alpha amylase activity in the blood plasma od experimental chickens was decreased from the 3rd day after administartion of ^{32}P to the 10th day of the experiment. A statistically significant difference was only recorded on the 7th day of the experiment. Concentration of glucose in the blood plasma of ^{32}P treated chickens was signifacantlly increased on the 5th and 7th days of the experiment. The results obtained indicate that alpha amylase activity and concentration of glucose are changed in the blood plasma of ^{32}P treated chickens. It seems that the concentration of glucose alone may serve as 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: phosphorus-32, alpha-amylase, glucose, blood plasma, chickens

Introduction

In our previous paper (ŠIMPRAGA et al., 2006) we investigated the effects of lethal activity of radioactive phosphorus (^{32}P) upon the clinical picture, hematological parameters and pathomorphological changes of tissues and organs in fattening chickens at slaughter ages. The obtained results showed that clinical signs of radiation sickness appear on the

*Contact address:

Dr. sc. Marinko Vilić, Department of Physiology and Radiobiology, Faculty of Veterinary Medicine, University of Zagreb, Heinzelova 55, 10000 Zagreb, Croatia, Phone: +385 1 2390 179; Fax: +385 1 2390 174; E-mail: marinko.vilic@vef.hr

ISSN 0372-5480
Printed in Croatia

6th day post contamination, and death of all contaminated animals occurred on the 9th and 10th day post-contamination. Results of pathoanatomic examination of dead animals of contaminated chickens revealed marked changes on the parenchymal organs manifested in spleen atrophy, nephrosis, fatty liver degeneration and myocard degeneration.

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; FLUCKIGER et al., 1977; FORENBACHER, 1972; FREEDLAND and KRAMER, 1970; KRALJEVIĆ, 1977; TIMET et al., 1975). It is also well known that changes of enzyme activity in blood plasma are a very useful test for an early diagnosis of some diseases and metabolic disorders of many organs (WILKINSON, 1976), including liver too (ROSALSKI,1976).

In our previous paper (KRALJEVIĆ et al., 2007) we showed that aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activity in the blood plasma of chickens significantly changes after a single intramuscular injection of radioactive isotope ^{32}P. Then we concluded that changes of activity of the investigated enzymes are a sign of functional and morphopathological damage of parenchymal organs, primarily the liver, caused by ionizing radiation. Namely, KRALJEVIĆ (1977), in his investigation on laying hens and chickens, used AST and ALT as a clinical test for the diagnosis of liver diseases and found that they are useful parameters for the discovery of different pathological changes in the liver. Besides, SHIRLEY et al. (1954), investigating the rate of deposition and turnover of radioactive phosphorus ^{32}P in the tissues of laying hens, reported a maximum concentration in the 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 at a high level for a long time. Even from 10 to 21 days later, the livers still contained over 1% of the initial dose of ^{32}P. According to those 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 sexglands, which is different in mammals (BACQ and ALEXANDER,1966).

In this investigation we tried to determine if there is a change of alpha amylase and glucose concentration in the blood plasma of chickens after intramuscular injection of lethal activity 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. Namely, although the origin of alpha-amylase in the blood plasma of animals has not been definitively determined, most, if not all, of the amylase present in normal blood plasma is probably of liver origin (COLES, 1980).

Materials and methods

Animals. The experiments were performed on healthy chickens, hybrids of the Jata heavy breed of both sexes, at the age of 50 days and mass ranging from 1500 to 2000 g. The chickens 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 the temperature and relative humidity were recorded in the hen 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 chickens were treated with radioactive phosphorus isotope ^{32}P (Amersham International plc., England) administered intramuscularly as $Na_2H^{32}PO_4$, in a single activity of 333 MBq per kilogram of body weight (n = 5). The specific activity of the solution was 333 MBq per milliliter. Along with the ^{32}P treated chickens, there was a control group treated with a physiological solution of sodium chloride 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.

Assays. The dynamics of activity changes of alpha amylase in the blood plasma was investigated spectrophotometrically by using kits produced by «Herbos d.d. Sisak» (Croatia). The glucose concentration in the blood plasma was investigated with photometry methods with o-toluidin, using also kits produced by «Herbos d.d. Sisak».

Clinical examination. The animals were subjected to clinical examination on a daily basis, in the morning and in the afternoon, for eight days before and ten days after the application of ^{32}P. The examination included general appearance and behaviour of the animals, 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 parts of the liver, lungs, cloacal bursa, duodenum, pancreas, heart, spleen, kidney and adrenal gland.

Statistical analysis. Results are expressed as mean ± standard error (SE) and were statistically analysed by using Student's t-test with a five percent level of significance (STATSOFT, 2005).

Results

The results of alpha-amylase activity in the blood plasma of chickens after intramuscular injection of radioactive isotope ^{32}P in a single activity of 333 MBq per kilogram of body weight, are presented in Fig. 1.

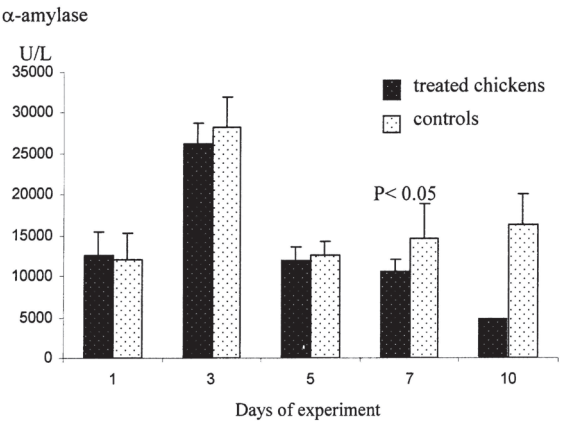

Fig. 1. Alpha-amylase activity in the blood plasma of chickens after intramuscular injection of radioactive isotope ^{32}P in a single activity of 333 MBq per kilogram of body weight. Results are expressed as mean ± standard error (SE) of five birds.

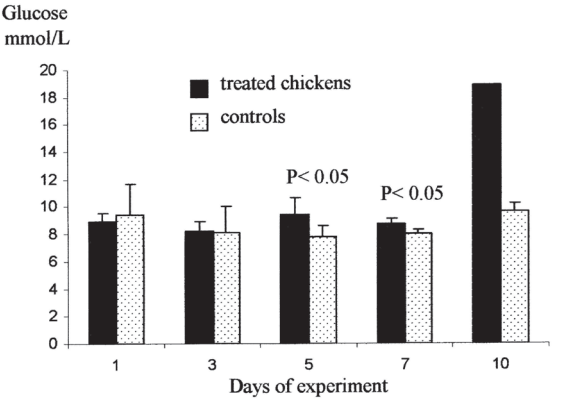

Fig. 2. Glucose concentration in the blood plasma of chickens after intramuscular injection of radioactive isotope ^{32}P in a single activity of 333 MBq per kilogram of body weight. Results are expressed as mean ± standard error (SE) of five birds.

The alpha amylase activity in the blood plasma of ^{32}P treated chickens was statistically significantly decreased only on the 7th day of the experiment ($P<0.05$). On the 10th day of the experiment a higher decrease was recorded in the blood plasma of one surviving ^{32}P treated chicken, but the statistical significance was not evaluated because of the small number of experimental animals.

The results of glucose concentration 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 Fig. 2.

The glucose concentration in the blood plasma of ^{32}P treated chickens did not change during the first three days of the experiment. In the second half of the experiment, i.e. from the 5th to the 10th day of the experiment, the glucose concentration was higher in ^{32}P treated chickens than in the controls. A statistically significant difference was recorded on the 5th and 7th days of experiment ($P<0.05$). A statistical evaluation was not made on the 10th day of the experiment because of the small number of surviving experimental birds.

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

Pathoanatomic examination of the dead animals from the ^{32}P treated chickens revealed a general anaemic condition and dotted bleeding sites on the heart and mucous membrane of the intestines and stomach. Also, the contaminated chickens presented marked changes on the parenchymal organs, manifested in spleen atrophy, nephrosis, fatty liver degeneration and myocard degeneration. Pathohistological examination of tissues and organs confirmed the findings of pathoanatomic examinations, which indicated the changes caused by radioactive radiation.

Discussion

The results obtained indicate that alpha amylase activities in the blood plasma of ^{32}P treated chickens were lower during the experiment, than in the controls, and glucose concentration was higher in the blood plasma of contaminated chickens than in the controls during last five days of the experiment.

At the moment we do not know the actual reason for the decrease of enzyme activities in the 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 prefer 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 the mechanisms responsible for it. This hypothesis is primarily based on the fact that some radionuclide, including ^{32}P,

may cause lethal effects in cells via transmutation if this event takes place inside the DNA molecule (APELGOT, 1983). It is very likely that ^{32}P, used in our experiment, will be incorporated into the 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, especially for that of enzymes, the above mentioned transmutation effect of ^{32}P upon cells can result in a decrease of enzyme synthesis in cells, which is reflected in a decrease of enzyme activity in blood plasma.

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

We also do not know the actual reason for the increased concentration of glucose in the blood plasma of ^{32}P treated chickens. We suppose that it could be caused by hypoxia. Namely, it is known that hypoxia may result in hyperglycemia, since liver glycogen is relatively unstable in the presence of a deficient oxygen supply (COLES, 1980). The hypoxia can be caused by reduction number of erythrocytes in the blood plasma. In our previous paper (ŠIMPRAGA et al., 2006) we showed that number of erythrocytes in the blood plasma of ^{32}P treated chickens was statistically lower than in the controls on the 5th and 7th day of the experiment.

In conclusion, the results obtained indicate that the activity of alpha amylase and glucose concentration change in the blood plasma of ^{32}P treated chickens. At this moment it seems that glucose concentration alone 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.

Acknowledgements

This investigations was financed by the Ministry of the Science, Education and Sport of the Republic of Croatia. The authors wish to thank the Ministry for their financial assistance.

References

APELGOT, S. (1983): Some considerations of the transmutation effect due to radioactive isotopes incorporated in cells and on the irradiation effect. Inter. J. Radiat. Biol. 43, 95-101.

BACQ, E. M., P. ALEXANDER (1966): Fundamentals of Radiobiology, 2nd ed., Pergamon Press. Oxford, London, Edinburgh, New York, Toronto, Paris, Frankfurt. p. 300.

COLES, H. E. (1980): Veterinary Clinical Pathology, 3rd ed., Saunders Company. Philadelphia, London, Toronto. pp. 259 and 268.

CORNELIUS, C. E., J. BISHOP, J. SWITZER, E. A. RHODE (1959): Serum and tissue transaminase activities in domestic animals. Cornell Vet. 49, 116-126.

FLUCKIGER, M., U. ALTHAUS, H. M. STREBEL (1977): Enzymaktivitäten in Serum und Organen des jungen Schweines. 2. Mitteillung: Enzymaktivitaten im Serum nach experimentallen Organläsionen. Zentralbl. Veterinärmed. A 24, 496-502.

FORENBACHER, S. (1972): Eksperimentalni i klinički prilozi dijagnostičkom značenju serumskih transaminaza kod domaćih životinja (I). Vet. arhiv 42, 171-208.

FREEDLAND, B. A., J. W. KRAMER (1970): Use of serum enzymes as aids to diagnosis. Adv. Vet. Sci. Comp. Med. 14, 61-105.

IVOŠ, J. (1966): Higijena u peradarstvu. In: Peradarstvo I. (Ivoš, J., M. Kralj, Eds.). Veterinary Faculty. Zagreb. pp. 254 and 317.

KRALJEVIĆ, P. (1977): Istraživanje aktivnosti nekih fermenata u krvnoj plazmi kokošiju pod normalnim i patološkim okolnostima. Dissertation. Veterinary Faculty. University of Zagreb. Zagreb, Croatia.

KRALJEVIĆ, P., M. VILIĆ, M. ŠIMPRAGA (2007): Aminotransferase activity in chicken blood plasma after application lethal activity of ^{32}P. Acta Vet. Beograd 58, 203-210.

ROSALSKI, S. B. (1976): Enzyme tests in disease of the liver and hepatobiliary tract. In: The Principles and Practice of Diagnostic Enzymology (Wilkinson, J. H., Ed.), Edward Arnold. London. pp. 303-360.

SHIRLEY, R. L., J. C. DRIGGERS, J. T. MCCALL, M. NEINBERG, G. K. DAVIS (1954): The rate of deposition and turnover of ^{32}P and ^{45}Ca in the tissues of the laying hen. Poultry Sci. 33, 932-936.

ŠIMPRAGA, M., M. TIŠLJAR, Ž. GRABAREVIĆ, M. VILIĆ, P. KRALJEVIĆ (2006): Clinical picture, haematological parameters and pathomorphological findings in fattening chickens after application of a lethal quantity of ^{32}P. Vet. arhiv 76, 507-519.

TIMET, D., M. HERAK, D. EMANOVIĆ, M. TRANGER, P. KRALJEVIĆ, S. JUZBAŠIĆ, I. MAJDAK, M. JURKOVIĆ (1975): Activities of certain blood enzymes as indicators of liver fattening in forced-fed geese. Proceedings of the 20th World Veterinary Congress, 6-12 June. Thessaloniki, Greece. pp. 2363-2364.

WILKINSON, J. H. (1976): The principles and practice of diagnostic enzymology. Edward Arnold. London. pp. 241-493.

Received: 8 March 2007
Accepted: 2 July 2008

VILIĆ, M., P. KRALJEVIĆ, M. ŠIMPRAGA: Utjecaj letalne aktivnosti ^{32}P na aktivnost alfa-amilaze i koncentraciju glukoze u krvnoj plazmi pilića. Vet. arhiv 78, 289-296, 2008.

SAŽETAK

Istraživana je aktivnost alfa-amilaze i koncentracija glukoze u krvnoj plazmi pilića, nakon primjene letalne aktivnosti ^{32}P s ciljem da se utvrdi mogu li ti pokazatelji poslužiti za dijagnozu organskih ili funkcionalnih oštećenja jetre prije pojave kliničkih znakova radijacijske bolesti. Pokusi su načinjeni na pilićima, hibridima teške pasmine Jata, oba spola, starim 50 dana, nakon jednokratne, intramuskularne primjene radioaktivnog fosfora ^{32}P, letalne aktivnosti od 333 MBq po kilogramu tjelesne mase. Krv za analizu uzimana je iz krilne vene 1., 3., 5., 7. i 10. dana nakon primjene ^{32}P. Aktivnost alfa-amilaze određivana je spektrofotometrijski upotrebljavajući gotove komplete proizvođača «Herbos d.d., Sisak». Koncentracija glukoze određivana je fotometrijski o-toluidinom, rabeći također gotove komplete proizvođača «Herbos d.d., Sisak». Aktivnost alfa-amilaze u krvnoj plazmi pokusnih pilića bila je smanjena od 3. dana nakon primjene ^{32}P pa do kraja pokusa, ali statistički značajan pad zabilježen je samo 7. dana pokusa. Koncentracija glukoze u krvnoj plazmi pokusnih pilića bila je statistički značajno povećana 5. i 7. dana nakon primjene ^{32}P. Dobiveni su rezultati pokazali da se vrijednosti oba istraživana pokazatelja mijenjaju u krvnoj plazmi pilića nakon primjene letalne aktivnosti ^{32}P. No, čini se da samo koncentracija glukoze može poslužiti kao pokazatelj oštećenja u pilića uzrokovanih ionizacijskim zračenjem prije pojave kliničkih znakova radijacijske bolesti.

Ključne riječi: fosfor-32, alfa-amilaza, glukoza, krvna plazma, pilići