Effect of low dose gamma-radiation upon antioxidant enzymes in chick embryo liver

Einfluss einer geringen Dosis an Gamma-Strahlung auf die antioxidativen Enzyme in der Leber von Hühnerembryonen

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Introduction

It has been shown that low doses and low dose rates of ionizing radiation can stimulate induction of antioxidant defence systems, such as glutathione (GSH), glutathione peroxidase (GSH-Px), superoxide dismutase (SOD) and catalase (CAT) (Kojima et al., 1997; Yamaoka, 2006). Most of these studies of low dose radiation effects were carried out in various tissues of rodents. In the avian there are results of antioxidant status mostly in liver, brain, yolk and yolk sac membrane of bird embryo under physiological conditions (Gaal et al., 1995; Surai, 1999). Thus, chick embryo liver on the 19th day of incubation was found to be higher in SOD, CAT and GSH-Px activity as well as GSH level when compared with brain and yolk sac membrane. It is also known that the liver in birds comprises the major body storage of antioxidants (Surai, 2003).

In literature there is no investigation available which shows that low dose gamma radiation has effects upon antioxidant status in chick embryonic organs after chick embryo irradiation. Investigations, though a few, of low dose ionizing radiation in the range from 0.05 to more than 1 Gray (Gy) are primarily directed on fertility, hatchability and body weight of chickens (Mraz, 1971; Todorov et al., 1986; Zakaria, 1989; Jilo and Lohe, 1991; Zakaria, 1991; Gerrits and Dijk, 1992). Although these previous studies showed contradictory data, it is reported that dose of gamma radiation between 0.1 to 1 Gy, especially the dose between 0.1 and 0.5 Gy (Jilo and Lohe, 1991) or between 0.06 to 0.3 Gy, (Todorov et al., 1996), could increase chicken egg hatchability and chicken body weight after egg irradiation before incubation.

In a previous study (unpublished results) effects of doses of 0.15 Gy and 0.3 Gy of gamma irradiation on enzyme activity in chicken blood plasma and antibody titer of Newcastle disease virus in blood serum of chickens hatched from eggs irradiated on the 19th day of incubation were observed. The dose of 0.3 Gy showed a more significant effect when compared to the dose of 0.15 Gy. Therefore, if our investigations (this one and those in the future) show that low dose of gamma radiation stimulate antioxidant status in chickens, as is a case in mammals, our results could have real practical application. Consequently, the aim of this study was to investigate lipid peroxidation and antioxidant status in liver of chick embryo after acute low dose of 0.3 Gy gamma irradiation on the 19th day of incubation.

Materials and Methods

Experimental protocol

Fertilized eggs produced by a commercial flock of COBB 500 broiler breeds were irradiated with gamma rays (source ⁶⁰Co) on the 19th day of incubation. In the same experiment, there were included the same number of eggs unexposed to gamma-radiation and served as controls. Non-irradiated eggs were retained in the same place for a period of time equal to that required for irradiated eggs. Control eggs were subjected to a sham irradiation. All eggs were placed in the same commercial incubator Victoria (Pavia, Italy), capacity of 22100 eggs for 18 days. Incubator had automatic control of temperature (37.8°C), humidity (60–62% relative humidity), and incubation rack turning. On the 19th day of incubation the eggs were irradiated and transferred to hatching trays located in the same incubator. After the hatch, chicks were housed on the floor at a temperature of 33°C. Two-day-old chicks used in the studies did not have access to feed but had access to water ad libitum. Both groups of chicks hatched from irradiated eggs and chicks hatched from non-irradiated eggs were kept under the same conditions. The study was reviewed and approved by the Ethics Committee of the Faculty of Veterinary Medicine University of Zagreb. (Class: 640-01/06-17/30; Record Number: 61-01/139-06-60).

Irradiation and dosimetry

The eggs (chick embryos) were irradiated with the dose of 0.3 Gy gamma radiation from panoramic ⁶⁰Co source (activity about 3 PBq) of the Rudor Boškovic Institute, Zagreb, Croatia (Miljanic and Ranogajec-Komor, 1997). The dose rate was about 23.84 mGy/s, and a source axis-to-egg axis distance was 3.06 m. Dosimetric measurements were performed with an ionization chamber type 2581 and a Farmer Dosimeter type 2570 (NE Technology Limited). The dose is specified as absorbed dose to water (measured free in air).

Sample preparation

Chick embryos and newly hatched chicks were sacrificed on 6, 24, 48, 72 and 96 h after irradiation. The liver was immediately removed, washed in cold saline, weighed,
quick frozen in liquid nitrogen and placed in deep-freezer at –80°C until analysis for antioxidant parameters. Seven chick embryos or chicks were used at each time intervals in both groups. For the measurement (three months liver storage ago) liver tissue was homogenized on ice in 0.14 mol/l KCl using a Teflon-glass Schütt homogenizer (Schütt Labortechnik, Germany) at 2,800 rpm during 0.5 min. Ration of tissue mass to volume of buffer was 1:5. Liver homogenate was centrifuged at 20,000 × g for 30 min. to prepare the supernatant using Sigma 3K15 centrifuge (Germany). The supernatant was used for lipid peroxide and GSH level, and activity of GSH-Px, SOD and CAT assay.

Assay of antioxidant parameters

Lipid peroxide concentration. The concentrations of thiobarbituric acid reactive substances (TBARS) were determined by the method of Oikawa et al. (1979). It detects malondialdehyde and other thiobarbituric acid-reactive substances generated by free radical-mediated peroxidation of unsaturated lipids (expecting cholesterol and monoenoic or dienoic phospholipids). Absorption peak was measured at 532 nm and concentration of lipid peroxide was expressed in terms of nanomoles of TBARS/g protein.

Gluthatione level. The concentration of GSH was determined by the method of Beutler et al. (1963). This method is based upon development of a relatively stable yellow colour when 5,5’-dithiobis-(2-nitrobenzoic acid) is added to sulfhydril compounds. The absorbance was read at 412 nm. GSH level was calculated as µmol/g protein.

Glutathione peroxidase activity. The GSH-Px activity was measured using commercial kit RANSEL (Randox Laboratories Ltd., Ardmore, Diamond Road, Crumlin, Co. Antrim, United Kingdom). The GSH-Px activity assay method is based on the ability of GSH-Px to catalyze the oxidation of glutathione by cumene hydroperoxide. In the presence of glutathione reductase and NADPH, oxidized glutathione is immediately converted to reduced form with concomitant oxidation of NADPH to NADP+ (PAGLIA and VALENTINE, 1967). The decrease in absorbance at 340 nm was measured. GSH-Px activity was expressed in terms of U/g protein.

Superoxide dismutase activity. The SOD activity was measured by the method of Flohe and Otting (1984). The SOD activity was determined on an SABA autoanalyzer with RANSOD reagent (Randox Laboratories Ltd., Ardmore, Diamond Road, Crumlin, Co. Antrim, United Kingdom). The method used on determination of SOD activity is based on the formation of superoxide radicals from xanthine by the action of xanthine oxidase, which react with 2-(4-iodophenyl)-3-(4-nitrophenoxy)-5-phenyltetrazolium chloride to produce formazan red stain. Activity of SOD was measured as the grade of inhibition of this reaction and expressed in terms of U/mg protein.

Catalase activity. The CAT activity was measured by the method of Johansson and Borg (1988). The method was based on the reaction of the enzyme with methanol in the presence of an optimal concentration of hydrogen peroxide. The produced formaldehyde was measured spectrophotometrically with 4-aminoo-3-hydrazino-5-mercapto,1,2,4-triazole (Purpald) as a chromogen at 470 nm. CAT activity was expressed in terms of U/g protein.

Estimation of protein. All parameters were expressed per g of protein. Protein concentration was measured by the method of Lowry et al. (1951) using bovine serum albumin as standard.

Statistical analysis

Obtained results of antioxidant parameters were expressed as mean ± standard deviation (SD) and the significance of differences between the control and irradiated groups was analysed in STATISTICA (StatSoft, 2005). After testing for normal distribution (Kolmogorov–Smirnov test of normality), the results were tested by Student’s t-test whereas a p value < 0.05 was selected to indicate significance.

Results

The results of TBARS and GSH level as well as antioxidant enzymes GSH-Px and SOD in the liver of chick embryo irradiated with 0.3 Gy gamma-rays on the 19th day of incubation are presented on Figure 1. The result of CAT activity in the liver of chick embryo irradiated with 0.3 Gy gamma-rays on the 19th day of incubation are presented on Figure 2.

Irradiation resulted in significantly higher GSH, TBARS and GSH-Px levels 6 h after irradiation and in significantly lower GSH, TBARS and GSH-Px levels at 48 h after irradiation. Thereafter, no significant differences could be observed. SOD was significantly lower at 48 h, but no effects could be observed at other times. CAT was not significantly different during the experiment.

Discussion

The results of our study indicate that chick egg irradiation on the 19th day of incubation with low dose gamma radiation induces alteration of antioxidant status in the embryo liver during the first 48 h after egg exposure. The level of TBARS and GSH-Px activity increased significantly on the 6th hour after egg irradiation while the level of GSH increased on the 6th and on the 24th hour after egg irradiation. On the other side all investigated antioxidant parameters, except CAT activity, significantly decreased on the 48th hour after egg irradiation. After that all antioxidant parameters tended to increase and they were almost on the same level as control parameters.

At the moment we do not know the actual reason of changes of these parameters in chick embryo liver. However, we suppose that the increased level of TBARS on the 6th h after egg irradiation could be correlated with the lipid content of the chick embryo liver. Namely, it is known that the lipid metabolism in chick embryo is predominating energy source during the last week of the development (Noble, 1986) and that chick embryo accumulated a large amount of lipids in the liver and extrahepatic tissues, during which the total lipid content increased toward hatching (Moore and Doran, 1962). In addition, chick embryo liver on the 19th day of incubation contains very high content of unsaturated fatty acids (Noble, 1986; Noble and Cocchi, 1990; Speake et al., 1998). Furthermore, Sató et al. (2006) showed that the hepatic function may already differ between broilers and layers at the embryonic development where the lipid content in liver of broiler chick on the 18th day of incubation and newly hatched chicks was higher than the lipid content in liver of layer chick embryo. Consequently, this described lipid contents especially in broiler chick embryo liver at the end of embryonic development could be probably explained by the increase level of TBARS in our study.
Our results concerning this increase TBARS level in broiler chick embryo liver after egg exposure to low dose gamma radiation could hardly be compared with results in literature. Namely, in literature the contradictory results have been published on the level of lipid peroxidation in different organs of mammals after low dose irradiation; the TBARS level in different organs of mammals is mostly decreased after low dose gamma irradiation (Yamaoka et al., 1991; Yamaoka, 2006), while Pathak et al. (2007) reported the increase of TBARS level in kidney of mice after whole body irradiation.

The increase of GSH level and GSH-Px activity in the chick embryo liver on the 6th h after egg irradiation could be possible due to its function in cell defence from oxidative stress in earlier hours after irradiation. Namely, GSH-Px plays a primary role in antioxidant defence in chick embryo liver from hydrogen peroxide and lipid hydroperoxide particularly at the end of embryonic development (Surai, 1999). On the other side GSH, the most abundant non-enzymatic antioxidant present in the cell, directly reacts with reactive oxygen species (Meister and Anderson, 1983) thus playing an important role in protecting tissues from oxidative damage.

Our results are consistent with the reports of Kojima et al. (1997) and Pathak et al. (2007) who have demonstrated that low dose gamma-radiation enhances GSH content in mice liver and kidney after whole body exposure. Further, Pathak et al. (2007) also showed increase of TBARS level in kidney of mice after whole body irradiation and reported that this increase in the lipid peroxidation might cause an in-
crease in the antioxidant defence status to regulate the cellular homeostasis. Therefore, it can not be excluded that the increase of TBARS in our study is also responsible for increased GSH level and GSH-Px activity in the chick embryo liver.

On the other side the decreased levels of GSH and investigated antioxidant enzymes (GSH-Px and SOD) in chick embryo liver on the 48 h after egg irradiation are not consistent with the literature data on the same antioxidant parameters which were increased in different organs of mammals after exposure to low dose of X or gamma radiation (Kolima et al., 1998; Kolima et al., 1999; Yamoka, 2006). One of the reasons of these differences could be explained by specific biochemical processes such as lipid metabolism at the end of chick embryo development previously described in our discussion. The other reason of decreased level of GSH, GSH-Px and SOD could be physiologic processes such as lung ventilation which differs from those in mammals. Namely during the first 18 days of the embryonic development the oxygen transfer and carbon dioxide occurs at the chorioallantoic membrane. After that, on the 19th day of incubation the embryo internally pips the air cell inner membrane and begins lung ventilation on air cell gas with an increase in oxygen consumption between day 20 and post-hatching day 1 (Menna and Mortola, 2002; Mortola and Labbe, 2005). Therefore, it could be possible that the decrease of the antioxidant parameters recorded in our experiment on the 48th h after irradiation i.e. on the 21st day of incubation is probably due to a higher susceptibility of the chick embryo on oxidative stress which is supposedly caused by low dose irradiation on the 19th day of the embryonic development.

Conclusions

In conclusion, the obtained results show that the chicken eggs exposure to the dose of 0.3 Gy gamma-radiation with dose-rate of 23.8 mGy/s on the 19th day of incubation causes an oxidative stress in early hours after irradiation as well as a decrease in non-enzymatic and enzymatic antioxidant defence system in the hatching chicks i.e. 48 hours after low dose irradiation. Furthermore, it also seems, as is the case in mammals, that GSH and GSH-Px could be the first choice in antioxidant defence in chicken embryo liver at an early time after low dose irradiation. Irradiation of the chicken eggs on the 19th day of incubation with low dose of 0.3 Gy gammaraadiation and dose-rate of 23.84 mGy/s do not have an effect on the antioxidant status in liver of chickens hatched from irradiated eggs.

Our study on the effect of low dose gamma radiation on the antioxidant status in chick embryo liver is, actually, one of the basic scientific investigations, and we hope that other low doses and dose rates of gamma irradiation could increase antioxidant status and resistance of chickens hatched from irradiated eggs against stress, which is very often the cause of many disturbances in the production of chicken meat or eggs, as well as the cause of some desease.

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Summary

Exposure of rodents to low-dose whole-body irradiation could cause various antioxidant mechanisms that defend cells from oxidative stress. However, there is a lack of results on antioxidant status in organs of chicken after low dose eggs or whole-body irradiation. This study was performed to investigate the effect of low-dose gamma-irradiation upon activity of glutathione peroxidase (GSH-Px), superoxide dismutase (SOD), catalase (CAT) and level of glutathione (GSH) and lipid peroxidation (TBARS) in liver of chick embryo and newly hatched chicks. Fertilized chicken eggs were irradiated with the dose of 0.3 Gy gamma radiation (source 60Co) on the 19th day of incubation. The liver samples were taken on 6, 24, 48, 72 and 96 h after egg irradiation. The antioxidant parameters were measured spectrophotometrically.

Level of GSH and lipid peroxidation as well GSH-Px activity increased in the liver of chick embryos on the 6th hour after irradiation. GSH-Px and SOD activity significantly decreased in the liver of chick embryo on the 48 h after egg exposure. Lipid peroxidation level and GSH level also significantly decreased in the liver of chick embryo on the 48 h after egg exposure. CAT activity during the experiment was not statistically different when it compared to controls. These results suggest that chick embryo exposure to dose of 0.3 Gy gamma-radiation on the 19th day of incubation causes alteration of antioxidant status in chick embryo liver. It also seems, as is the case in mammals, that GSH and GSH-Px could be the first choice in antioxidant defence in chicken embryo liver at early time after low dose irradiation.

Key words

Chick embryo, gamma radiation, antioxidative enzymes, liver

Zusammenfassung

Einfluss einer geringen Dosis an Gamma-Strahlung auf die antioxidativen Enzyme in der Leber von Hühnerembryonen


Die Gehalte an GSH und TBARS sowie die GSH-Px-Aktivität nahm in der Leber der Embryonen 6 h nach der Bestrahlung zu. Dagegen nahmen die Aktivitäten von GSH-Px und SOD sowie die Gehalte an TBARS und GSH in der Leber 48 h nach der Bestrahlung signifikant ab. Im Vergleich zur Kontrolle waren keine signifikanten Veränderungen der CAT-Aktivität fest zu stellen. Die Ergebnisse lassen den Schluss zu, dass die Bestrahlung von Hühner-

Stichworte
Hühnerembryo, Gamma-Strahlung, antioxidative Enzyme, Leber

References


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