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Influence of absorbed radiation dose following computed tomography on the antioxidative status in rabbit testicles

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Abstract: In recent years, computed tomography (CT) has become very common in veterinary medicine. It is well known that testicles are organs with high radiosensitivity and their function can be impaired even after exposure to low radiation doses. In this work, we calculated the absorbed radiation doses after CT was performed with different voltage/current levels and correlated it with the activity of antioxidant enzymes in rabbit testicles. Two hours after CT, the activities of catalase (CAT) and glutathione peroxidase (GSH-Px) were increased in the testicles of animals that received an absorbed dose of 29.2 mGy. The same changes, along with elevated glutathione reductase (GR) activity, were observed after 7 days in animals that received the highest absorbed dose (46.3 mGy). It would appear that absorbed doses above 27.8 mGy provoked the antioxidant reaction but the time scale of the reaction was dose-dependent. Examination of the obtained results revealed that the main denominator of CT influence was a higher current. Our results suggest that CT influences the antioxidant status in rabbit testicles. The changes in antioxidant enzyme activities were dose- and time-dependent and influenced by the applied current.

Key words: computed tomography; oxidative stress; antioxidant enzymes; testicles; rabbit

INTRODUCTION

In sexually mature males, the testicles are metabolically very active, consuming significant amounts of energy for spermatogenesis. Spermatogenesis implies an extremely high rate of cells division, being capable of generating approximately 1000 sperm cells per second. High metabolic activity is accompanied by intensive oxygen consumption in the mitochondria of germinal epithelial cells that may consequently result in the production of substantial amounts of free radicals [1]. Male germ cells are more susceptible to oxidative stress than somatic cells, because their membranes contain more polyunsaturated fatty acids [2]. Thus, oxidative stress plays an important role in the etiology of sperm malformation, altered function, the sperm count profile and male infertility [3]. Testicles have a well-developed enzymatic antioxidant system [1].

In recent years, CT has become very common in veterinary medicine. Even though this diagnostic procedure benefits patients when used for appropriate indications, CT delivers considerably higher doses of radiation to the patient's body in comparison with conventional radiography. Previous results reported changes in the level of antioxidant enzyme activities in the testicles after exposure to radiation in the range from 0.5 to 3 Gy. These doses are high when compared

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to those received after CT [4]. Radiation doses from a single, routine CT examination belong to the category of low radiation doses defined as doses in the range of near zero up to about 100 mSv of low-LET radiation [5], which corresponds to an absorbed dose of 100 mGy. Therefore, it can exert only stochastic effects.

It is well known that testicles are organs with high radiosensitivity and their function can be impaired even after exposure to low radiation doses [6]. According to literature data, spermatogonia cells are particularly sensitive to radiation and can be damaged by exposure to doses lower than 0.1 Gy [7]. At the cellular level, the harmful effects of ionizing radiation are due to the production of free radicals from water radiolysis that causes direct damage to DNA molecules [8].

The main objective of this study was to calculate the absorbed radiation doses in rabbit testicles after CT performed by variation of voltage and current, and to correlate these values with the activity of antioxidant enzymes. Although it is well known that exposure to x-radiation results in oxidative stress, there are no literature data on the influence of diagnostic doses of x-radiation emitted during CT on the oxidative status in testicles.

MATERIALS AND METHODS

The study was conducted in agreement with existing ethical norms and was approved by the Permission of the Ministry of Agriculture and Environmental Protection – Veterinary Directorate, Republic of Serbia, No. 323-07-03455/2015-05/5. Experiments were performed on mature New Zealand white rabbit males in accordance with the ARRIVE (Animal Research: Reporting of In Vivo Experiments) guidelines for reporting experiments involving animals [9]. The rabbits were kept under standard laboratory conditions (12 h light, 12 h dark, 21±2°C ambient temperature). All animals were housed in individual cages and provided with standard diet and tap water *ad libitum*.

Animals and grouping

Sixty-six experimental rabbits were divided into 11 groups consisting of 6 animals each. Three groups were not exposed to radiation and served as controls. Rabbits from the NT (not treated) group were killed without any treatment, while animals from groups A_1 and A_2 were anesthetized and served as the anesthetized controls. Rabbits from the remaining eight groups (I_1, I_2, II_1, II_2, III_1, III_2, IV_1 and IV_2) were anesthetized to ensure still positioning during examination and subjected to CT using different CT protocols (two different values of voltage and current-amperage in the x-ray tube were applied). For anesthesia, a ketamine hydrochloride (Ketamidor 10%, Richter Pharma, Austria) was used and administered intramuscularly (i.m.) (35 mg/kg body weight). Prior to anesthesia, a premedication by i.m. application of xylazine hydrochloride (Xylased, Bioveta, Czech Republic) was performed (5 mg/kg body weight). All animals were killed by decapitation. Rabbits from groups A_1, I_1, II_1, III_1 and IV_1 were killed after 2 h, while rabbits from groups A_2, I_2, II_2, III_2 and IV_2 were killed after 7 days. Immediately after sacrifice, testicle samples were collected and stored in liquid nitrogen before determination of the activities of antioxidant enzymes.

CT examination protocols

CT examinations of rabbits were performed using a CT SOMATOM AR STAR (Siemens Medical Systems, Erlangen, Germany). CT examinations were performed using the following examination protocols: Groups I_1 and I_2: tube voltage (U) 110 kV; tube current and exposure time product (It) 63 mAs; exposure time (t) 1 s; slice thickness 10 mm; Groups II_1 and II_2: tube voltage (U) 130 kV; tube current and exposure time product (It) 63 mAs; exposure time (t) 1 s; slice thickness 10 mm; Groups III_1 and III_2: tube voltage (U) 110 kV; tube current and exposure time product (It) 105 mAs; exposure time (t) 1 s; slice thickness 10 mm; Groups IV_1 and IV_2: tube voltage (U) 130 kV; tube current and exposure time product (It) 105 mAs; exposure time (t) 1 s; slice thickness 10 mm.

Dose quantities

The absorbed dose in the testicles was calculated by the computational dosimetry method. A Monte Carlo simulation of CT examination was performed using a three-dimensional mathematical model of the rabbit and the Monte Carlo general purpose simulation tool MCNP5/x – Monte Carlo Neutron Particle Transport Code [10] developed in ORNL Oak Ridge National Laboratory, USA. Input data form Monte Carlo simu-

lations included properties of the CT scanner and 110 kV and 130 kV x-ray spectra generated using the software tool SEPC78 (Institute of Physics and Engineering in Medicine, IPEM). A mathematical model of the rabbit was generated based on representative animal CT images. Each voxel of the testicles was assigned with relevant tissue parameters in terms of density and mass attenuation coefficient. As an outcome of the Monte Carlo simulations, the energy deposited in each voxel was registered, and was further used for organ dose calculation. A total of 10^7 photon histories were followed, using cut-off energy of 1 keV.

Determination of antioxidant enzyme activities

Thawed testicles were homogenized and sonicated in 0.25 M sucrose, 1 mM ethylenediaminetetraacetic acid and 0.05 M Tris-HCl buffer (pH 7.4) before centrifugation (90 min at 105000×*g*). The supernatant was used for enzyme activity measurements. Enzymatic essays were based on spectrophotometric measurements of absorbance changes. Measurements of absorbance were performed using a Shimadzu UV-160 spectrophotometer (Shimadzu Scientific Instruments, Shimadzu Corporation, Kyoto, Japan). Total superoxide dismutase (SOD) activity was determined by the adrenaline method [11]. One SOD unit was defined as the amount of the enzyme necessary to decrease the rate of adrenalin autooxidation by 50% at pH 10.2. For determination of SOD2 activity, the assay was performed after sample incubation with 8 mM KCN. SOD1 activity was calculated from the difference between total SOD and SOD2 activities. CAT activity was estimated by monitoring hydrogen peroxide consumption [12]. The activity of GSH-Px was determined using t-butyl hydroperoxide as a substrate and estimated by calculating NADPH consumption [13]. GR activity was measured as the rate of NADPH oxidation concomitant with glutathione disulfide (GSSG) reduction [14]. Specific activities were expressed per mg of tissue protein. The protein concentration was measured by the method of Lowry [15].

Statistical analysis

All values were expressed as the mean±SEM. Statistical evaluation was calculated by two-way ANOVA, with factors: anesthesia (A) and the time of death (T), the absorbed dose (D) and the time of death (T) and *post hoc* compared using Tukey's HSD t-test. For all comparisons, $p<0.05$ was considered as significant.

RESULTS

The absorbed doses in testicles were calculated by computational dosimetry and are listed in Table 1. Since the rabbits were anesthetized prior to CT, the effect of anesthesia on antioxidant enzyme activities was checked. Our results showed that anesthesia had no effect on the activities of antioxidant enzymes in testicles, except for SOD2. There was a statistically significant increase in SOD2 activity after 7 days in comparison to non-anaesthetized rabbits (Fig. 1).

Table 1. Parameters of different CT examination protocols and absorbed doses in rabbit testicles.

Groups	U (kV)	It (mAs)	t (s)	D (mGy)
I1 and I2	110	63	1	17.5
II1 and II2	130	63	1	27.8
III1 and III2	110	105	1	29.2
IV1 and IV2	130	105	1	46.3

U – voltage; It – current; t – time; D – absorbed doses

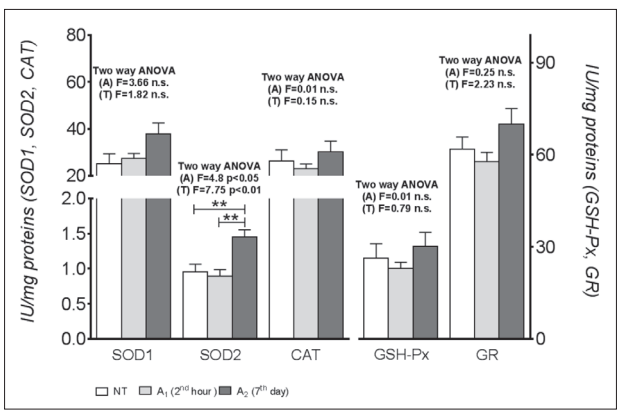

Fig. 1. The effect of anesthesia on antioxidant enzyme activities in rabbit testicles measured 2 h and 7 days after application. Statistical analyses were performed by two-way ANOVA (factors (F) are given, *p<0.05, **p<0.01, ***p<0.001), and *post hoc* compared by Tukey's HSD t-test (*p<0.05, **p<0.01, ***p<0.001).

Seven days after CT, the activity of SOD1 was lower in rabbits that had received the lowest absorbed dose (17.5 mGy) when compared to the anesthetized control group of animals ($p<0.01$) (Fig. 2A). Seven days after CT, SOD2 had significantly lower activity in all animals, regardless of the absorbed dose, when

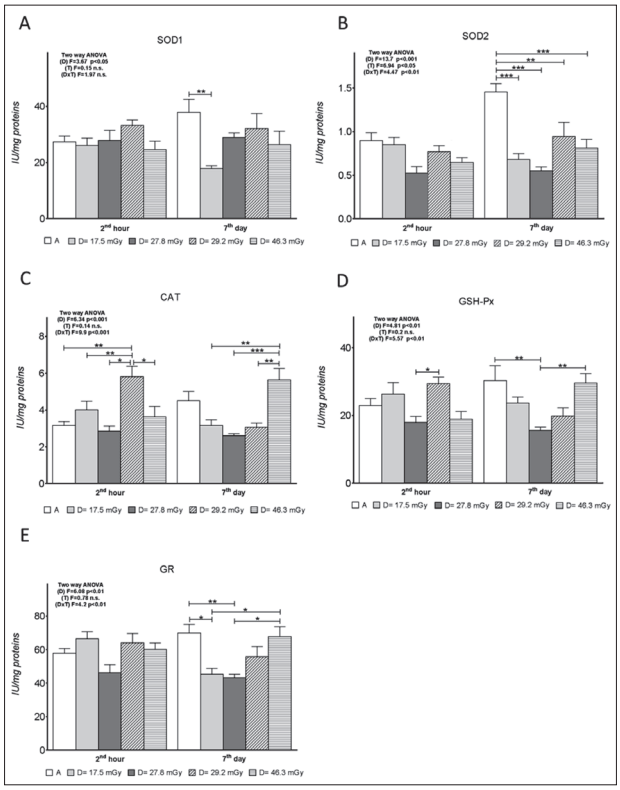

Fig. 2. The effect of different absorbed doses on: **A** – SOD1 activity; **B** – SOD2 activity; **C** – CAT activity; **D** – GSH-Px activity; **E** – GR activity in rabbit testicles, measured 2 h and 7 days following CT. Statistical analyses were performed by two-way ANOVA (factors (F) are given, *p<0.05, **p<0.01, ***p<0.001), and *post hoc* compared by Tukey's HSD t-test (*p<0.05, **p<0.01, ***p<0.001).

compared with the anesthetized control group. However, 7 days following CT, there were no differences in SOD2 activity in rabbit testicles between groups that received different absorbed doses (Fig. 2B).

CAT activity increased after 2 h in the group that received 29.2 mGy and was at the control level after 7 days (Fig. 2C). On the other hand, after 7 days, CAT activity was elevated in the group of rabbits that received the highest amount of radiation (46.3 mGy).

GSH-Px activity was lower in the group of animals that received an absorbed dose of 27.8 mGy when compared to a dose of 29.2 mGy after 2 h (Fig. 2D). Lower GSH-Px activity in the first group (27.8 mGy) persisted after 7 days as compared to the anesthetized control group and the group that received the maximal absorbed dose (46.3 mGy).

There were no significant differences in GR activity between experimental groups 2 h after CT when compared to the anesthetized control group. However, after 7 days, a significant decrease was noted in animals that received absorbed doses of 17.5 and 27.8 mGy when compared to the anesthetized control animals (Fig. 2E).

DISCUSSION

Our results suggest that CT influences the antioxidant status in rabbit testicles, with the changes in antioxidant enzymes activity being time- and dose-dependent and influenced by the applied anesthesia. There are no literature data about the influence of ketamine and xylazine on the antioxidant enzyme activities in rabbit testicles. In our experiments, there were no changes in SOD1, CAT, GSH-Px and GR activities in animals that were anesthetized using ketamine and xylazine in comparison to the non-anesthetized control group. However, there was a significant increase in SOD2 activity after 7 days. This implies that the applied anesthesia influenced mitochondrial ROS production, which should be considered in the interpretation of data. There are reports that many anesthetics have profound effects on mitochondrial membranes at concentrations as low as those known to produce general anesthesia, and they can destabilize lipid-protein interactions [16-18]. Zaugg et al. [19] showed that the intravenous anesthetics R-ketamine exert pronounced mitochondrial effects that are reflected on ROS production. Therefore, the elevation of SOD2 in testicles observed in our experiment can be considered as the effect of anesthesia on mitochondrial ROS homeostasis. Thus, the effects of CT on antioxidant enzyme activities were compared to the activities of antioxidant enzymes in testicles of anesthetized control animals.

Two hours after CT we did not observe a general effect of x-radiation on antioxidant enzyme activities in comparison to the anesthetized group of animals; however, subtle increases in CAT and GSH-Px activities were observed in the group of animals that received the absorbed dose of 29.2 mGy in comparison with the other groups. In our experiment, the same changes, along with elevated GR activity, were detected after 7 days in animals that received the highest absorbed dose (46.3 mGy). This means that the absorbed doses above 27.8 mGy provoked an antioxidant reaction, and for these doses, the time scale of the reaction was dose-dependent.

Since there was no effect on the activities of antioxidant enzymes in rabbits that received the absorbed dose of 27.8 mGy, it would appear that the dose threshold range was very narrow. Our previous results obtained in erythrocytes indicated that the dose threshold was about 25 mGy [20]. In the present study, we also found that doses below that level did not produce any significant changes in antioxidant enzyme activities, but the threshold was a little higher (27.8 mGy). These results can be interpreted as a tissue-specific sensitivity. On the other hand, a radiation level just above 27.8 mGy had a significant impact on the antioxidant defense in testicles, but after a relatively short time (2 h after exposure), as compared to the higher dose that required a longer adaptive period. It has been documented that local radiation as low as 0.35 Gy induced a response in testicles, but mutagenic changes were detected at 0.5 Gy [21,22].

The elevation of CAT and GSH-Px activities suggests that the concentration of hydrogen peroxide was raised above a tolerable ROS level, and there was a need for its faster elimination. Both CAT and GSH-Px metabolize hydrogen peroxide, but with different enzyme constants, i.e. CAT removes H_2O_2 faster and is physiologically operative at higher concentrations of hydrogen peroxide [23,24]. At the same time, GSH-Px is efficient at lower cellular H_2O_2 levels, but it also metabolizes lipid peroxides. Since in our study GSH-Px was elevated along with CAT, it seems that the ROS attack involved lipid molecules as well. This was especially important for rabbits that received the highest absorbed dose, since an additional elevation of GR activity found in these rabbits suggested that increased glutathione mediated antioxidant activity and its faster turnover. If the received absorbed dose was about 29.2 mGy, the response was rapid and occurred after 2 h. If the dose was about 70% higher, an elevation in antioxidant enzymes was noted after 7 days. The antioxidant response after 7 days was stronger, but it required more time to be expressed. The time effect on antioxidant enzyme activity after irradiation is already known [4,25]. However, the precise dynamics and sequence of changes, particularly of individual antioxidant components, depend on the dose, time, tissue and antioxidant [26].

In our experiment, different CT protocols were performed by changing the voltage and amperage (current) parameters. We found that CAT activity in the testicles at 2 h post CT was increased when a low voltage/high current was applied, as compared to a high voltage/low current, or a low voltage/low current. Moreover, on the 7th day, the CT examination that was carried out with the use of high voltage/high current resulted in increased CAT activity, when compared to CAT activities recorded in animals where CT examination was performed with other combinations of applied voltage and current parameters. Additionally, our results showed that GSH-Px and GR activities were also increased in high-current CT conditions. It appears that the main element influencing radiation effects and subsequent changes in antioxidant activity is a high current. A higher current provides a higher density of radiation per surface unit and intensifies interactions between molecules, while voltage influences x-ray penetration and energy.

CONCLUSIONS

Our results show that CT produced changes in the antioxidant status of rabbit testicles, suggesting a ROS imbalance. The effects were prominent above the absorbed dose of 27.8 mGy and were expressed on different time scales. The effects of the dose slightly above the threshold dose of 29.2 mGy were noted after 2 h, but the effects of much higher doses (48.2 mGy) were noted after 7 days. Since the applied anesthetics, as an integral part of this procedure, also influenced antioxidant enzyme activities, it can be concluded that CT disturbs the ROS balance in rabbit testicles.

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Author contributions: Duško Blagojević, Mirjana Lazarević-Macanović conceptualized and defined the research idea and created the research design. Marko Mitrović, Zorana Oreščanin-Dušić, Aleksandra Nikolić-Kokić, Nikola Tatalović searched the literature. Duško Blagojević selected the statistical tests. Marko Mitrović, Zorana Oreščanin-Dušić, Aleksandra Nikolić-Kokić collected and prepared the experimental data. Nikola Tatalović and Duško Blagojević performed the statistical analyses. Marko Mitrović, wrote the first draft of the manuscript. Duško Blagojević,

Mirjana Lazarević-Macanović, Zorana Oreščanin-Dušić wrote the second draft of the manuscript. Mirjana Lazarević-Macanović, Duško Blagojević edited the manuscript. Nikola Krstić performed the CT procedure. Zoran Jovanović created the three-dimensional mathematical model of the rabbit. Dragana Krstić performed a Monte Carlo simulation of the CT examination. Olivera Ciraj-Bjelac calculated the absorbed dose.

Conflict of interest disclosure: The authors declare that there is no conflict of interests.

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