INFLUENCE OF RADIATION DOSE IN COMPUTED TOMOGRAPHY ON ANTIOXIDANT ENZYME ACTIVITY IN RABBIT ERYTHROCYTES

by


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INRODUCTION

Despite the universal consensus that computed tomography (CT) overwhelmingly benefits patients when used for appropriate indications, concerns have been raised regarding the potential risk due to the increased use of CT in medicine and the relative high radiation doses associated with this type of examination. When compared to conventional radiography, CT delivers a considerably larger dose to the patient. Although technological developments provide the opportunity to decrease individual CT doses, the attempt to obtain quality images and cover a larger area of the patient's anatomy can lead to the opposite result. It is increasingly being documented that patient doses are higher than necessary for the high quality image and in CT often exceeds the level needed for confident diagnosis [1]. Thus, keeping the radiation dose as low as reasonably achievable consistent with the diagnostic task, remains the most important strategy for decreasing this potential risk [2] in line with general radiation protection principles. Because of that, optimization of CT based on the balance of the radiation dose and image quality, is very important for avoiding excessive patient doses [3, 4]. Similarly to human medicine, X-rays are equally used in veterinary medicine. The reasons for using radiation in veterinary medicine are to either obtain optimum diagnostic information or to achieve a specific therapeutic effect, while maintaining the radiation dose to the radiological personnel and the general public as low as reasonably achievable. Similarly, it is also important to avoid all unnecessary irradiation of the animal patient [5].

In terms of radiation protection and associated radiation effects, radiation doses from a single, routine computed tomography examination belong to the category of low radiation doses and therefore can exert only stochastic effects. Low doses are defined as doses in the range of near zero up to about 100 mSv (0.1 Sv) of low-LET radiation [6]. Based on epidemiological data of radiation-induced cancer occurrences, various authors agree that low doses are below 200 mGy as below this level the statistical evaluation of data becomes more and more uncertain [7, 8]. However, certain cellular reactions like enzyme inductions, DNA-repair processes, adaptive responses, chromosome aberrations, etc. could already be observed between 10 and 100 mGy by various sensitive assay techniques [9].
Low dose significance is especially relevant in triggering oxidative stress in organs and tissues submitted to irradiation, resulting in elevated activity of the main antioxidant enzymes: superoxide dismutase – SOD, catalase – CAT, glutathione peroxidase – GSH-Px, and glutathione reductase – GR [10, 11]. Changes in the antioxidant enzymes activity were demonstrable when the applied dose is 50 mGy [12]. However, it was reported that oxidative stress can be induced with extremely low radiation doses from 0.1 mGy [13, 14] to 1 mGy [13]. In the rat spleen, an elevated activity of SOD and GSH-Px was registered following exposure to 250 mGy [15], and for the SOD this change lasted 8 weeks. In another study [16] CAT activity in chicken kidneys was elevated by 40 % at the dose of 500 mGy and doubled at the radiation load of 2 Gy. Same authors documented that in samples of chicken brains at the dose of 500 mGy, CAT activity was elevated only by 25 %, while at the dose of 2 Gy, the activity was 2.4 times higher when compared to the controls. It was not possible to find data regarding changes in the activity of GR following a low dose irradiation exposure. Apart from the fact that in staff, professionally exposed to irradiation (X-ray technicians), when mild elevation of CAT activity in red blood cells was noted [17], there is not much data regarding X-ray low dose influence in triggering oxidative stress in these cells.

In veterinary medicine changes in the activity of antioxidant enzymes following CT diagnostics must be justified with special care because all examinations in animals are conducted under general anaesthesia and there are data about the significant influence of anaesthetics in triggering oxidative stress [18]. In order to perform anaesthesia of rabbits, the best results are achieved by combining ketamine and xylazine [19]. There are no data regarding oxidative stress in rabbits during general anaesthesia but in horses, there is a significant elevation in SOD, CAT, and GSH-Px activity in blood, following premedication by xylasine (0.5 mgkg$^{-1}$ BW$^{+}$) [20]. An additional elevation was noted after application of ketamine in the dose of 2.2 mgkg$^{-1}$ BW. Decreased activity of SOD and CAT in rat brains following ketamine in subanaesthetic doses (4, 10 or 30 mgkg$^{-1}$ BW) was recorded [21].

The main objective of this study was to assess the radiation dose in computed tomography examinations of rabbits using different examination protocols and to correlate these values with the activity of antioxidant enzymes in the erythrocytes of animals submitted to CT examinations.

**MATeRIALS AND METHODS**

The study was conducted in line with existing ethical normatives and based on the Permission of the Ministry of Agriculture and Environmental Protection

$^{+}$ BW means body weight

CT examinations of rabbits were performed using a single slice CT units SOMATOM AR STAR (Siemens Medical Systems, Germany).

**Animals and grouping**

Experimental rabbits (66) were alienated in 11 equal groups consisting of 6 animals each. The first three groups (NT, $A_1$, and $A_2$) were not exposed to radiation and served as controls. Rabbits from the NT group were sacrificed without any treatment while animals from the $A_1$ and $A_2$ group were only anesthetized and served as anaesthetised controls. Animals from the A1 group were sacrificed 2 hours after anaesthesia and rabbits from the $A_2$ group 7 days after the anaesthetic procedure. Rabbits from the remaining eight groups were also anaesthetized to ensure still positioning during examination and submitted to CT examination with different CT protocols (different values of voltage and amperage in the X-ray tube). For anaesthesia, a ketamine hydrochloride (Ketamidor 10 %, Richter Pharma, Austria) was used and administered i. m. (35 mgkg$^{-1}$ BW). Prior to anaesthesia, a premedication by i. m. application of xylasine hydrochloride (Xylased, Bioveta, Czech Republic) was performed (5 mg/kg BW). Sacrifice was performed by the decapitation method.

**CT examination protocols**

All CT examinations were performed using the following examination protocols:

- **Groups $I_1$ and $I_2$:** tube voltage ($U$) 110 kV; tube current and rotation time product ($It$) 63 mAs; rotation time (trot) 1 s; slice thickness 10 mm.
- **Groups $II_1$ and $II_2$:** tube voltage ($U$) 130 kV; tube current and rotation time product ($It$) 63 mAs; rotation time (trot) 1 s; slice thickness 10 mm.
- **Groups $III_1$ and $III_2$:** tube voltage ($U$) 110 kV; tube current and rotation time product ($It$) 105 mAs; rotation time (trot) 1 s; slice thickness 10 mm.
- **Groups $IV_1$ and $IV_2$:** tube voltage ($U$) 130 kV; tube current and rotation time product ($It$) 105 mAs; rotation time (trot) 1 s; slice thickness 10 mm.

Rabbits from the groups $I_1$, $I_2$, $III_1$, and $III_2$ were sacrificed 2 hours after the CT procedure while rabbits from the groups $I_2$, $II_2$, $III_2$, and $IV_2$ were sacrificed af-
ter 7 days. Sacrifice was performed by the decapitation method. Immediately following sacrifice, blood samples were collected by exsanguination in order to determine the parameters of the oxidative stress in red blood cells.

Dose quantities

The dosimetry concept in CT is well-established and based on the practical dose quantities: weighted CT dose index (CTDIw), volume weighted CT dose index (CTDIvol) and dose-length product (DLP). The CT dose index is an indication of the average dose in the central part of a scanned region when slices are contiguous [23]. In addition, it enables comparisons between scanners and scan protocols and can be easily measured. All CT vendors are now required to display CTDI and DLP values on the user interface [24].

Prior to data collection, the CTDIw values were verified by measurements, using a well-established protocol [23]. Values obtained from the CT console differed by less than 10% of the measured values. Data were collected in terms of CTDIw. Data on the rabbits and scan protocols were also collected, including length and weight of animals, tube voltage (U), tube current and rotation time product (I), rotation time (trot), and technique used (acquisition mode, gantry angle, collimation, and pitch).

Determination of antioxidant enzyme activity

Whole blood samples were taken after decapitation in heparinized vacutainers. Erythrocytes and plasma were immediately separated by centrifugation (10 min, 5000 rpm, 4 °C). Aliquots of three-times washed erythrocytes with saline (0.9% w/v) were lysed in ice-cold distilled water. Antioxidant defence enzyme activities were measured in lysate. For determination of SOD activity, haemoglobin was removed by the method of Tsuchihashi [25] and values were estimated by the method of Drabkin and Austin [26].

Enzyme assays

Activity of SOD was determined using the adenine method [27] based on the increase in absorbance at 480 nm. This method is based on the capacity of SOD to inhibit the autoxidation of adrenaline to an adrenochrome. The reaction was carried out in a 50 mmolL\(^{-1}\) sodium carbonate buffer, pH 10.2, and was initiated by the addition of 0.3 mmolL\(^{-1}\) adrenaline. One unit of SOD was defined as the amount of protein required to halve the rate of substrate autoxidation.

Activity of CAT was measured spectrophotometrically, according to Beutler [28]. CAT activity was determined by the rate of H\(_2\)O\(_2\) disappearance measured at 230 nm. Specific activities were expressed as IU/mg\(^{-1}\) protein.

Activity of GSH-Px was measured following the spectrophotometric method [29] based on the NADPH consumption [i.e., NADPH oxidation by GR, at 340 nm]. The reaction mixture consisted of a 50 mM potassium phosphate buffer (pH 7.0), 1 mM EDTA, 1 mM GSH, 1 mM sodium azide, 1 IU/mL \(^{-1}\) GR from baker’s yeast, 0.2 mM NADPH and 3 mM \(t\)-butyl hydroperoxide (as a substrate and appropriate amounts of the sample). The blank did not contain a sample and the activity was calculated after subtraction of the blank value. The rate of NADPH oxidation was monitored at 37 °C on the basis of the decrease in absorbance at 340 nm. One unit of enzyme activity was defined as the amount of enzyme required to transform 1 nmol of substrate [\(\text{min}^{-1}\)] under the above described assay conditions. Specific activities are expressed as IU/mg\(^{-1}\) protein.

Activity of GR was assayed by the method described previously [30] and based on NADPH oxidation concomitant with GSSG reduction. The reaction mixture consisted of 0.5 M sodium phosphate buffer (pH 7.5), 0.1 mM EDTA, 0.1 mM NADPH, 0.1 mM GSGG and appropriate amounts of samples. The rate of NADPH oxidation was monitored at 37 °C after the decrease in absorbance at 340 nm (\(e_{340 \text{ nm}} = \text{mmol} \text{L}^{-1}\text{cm}^{-1}\)). The blank did not contain GSSG and the activity was calculated after subtraction of the blank value. Specific activities are expressed as IU/mg\(^{-1}\) protein.

Statistical analysis

All values are expressed as mean ± SEM. Statistical evaluation was calculated by two ways ANOVA (factors: anaesthesia (A) and time (T) as well as dose (D) and time (T)). For all comparisons, \(p < 0.05\) was considered as significant.

RESULTS

Radiation dose

The radiation dose in terms of CTDIw for used scan protocols is presented in tab. 1. Gantry angulation was zero for all examination protocols and all image acquisitions were performed using the axial scanning mode.

The effect of anaesthesia on the antioxidant enzymes activity in rabbit erythrocytes

The results indicate that anaesthesia had no influence on the activity of antioxidant enzymes (fig. 1, \(e\) is molar extinction coefficient...
ANOVA, effects of anaesthesia and days, non-significant. Furthermore, the activity of studied antioxidant enzymes in non-treated (NT) animals was similar to values registered in anaesthetized animals after 2 hours ($A_1$) as well on the 7th day ($A_2$). It can be concluded that the applied anaesthesia had no influence on the activity of antioxidant enzymes in erythrocytes.

The effect of different CTDI values on the antioxidant enzymes activity in rabbit erythrocytes following computed tomography

The results showed that the applied treatment changed the activity of antioxidant enzymes and the effects depend on the dose applied and time of the particular enzyme activity.

There is no significant general trend in the level of SOD activity in erythrocytes after the CT scan (fig. 2, ANOVA no significant effect of neither doses nor time), but a very low level of significance between general groups (interaction $D \times T$, $p < 0.05$) was found. However, post hoc Tukey’s HSD test showed no significant differences between individual groups.

Statistical analysis of the measurements of CAT activity showed no general trend of response after CT scan exposure (fig. 3, ANOVA, no significant effects of treatment and time). However, there are significant differences between individual groups (ANOVA, interaction, $p < 0.001$). There is a significant increase in CAT activity ($p < 0.001$) after 2 hours of exposure of 29.8 mGy compared to the 17.9 and 25.2 mGy groups. Moreover, CAT activity in that group decreased after 7 days when compared to the other groups.

Table 1. Scan parameters and assessed CTDIw values for different examination protocols

<table>
<thead>
<tr>
<th>Groups</th>
<th>$U$ [kV]</th>
<th>It [mAs]</th>
<th>trot [s]</th>
<th>CTDIw [mGy]</th>
</tr>
</thead>
<tbody>
<tr>
<td>$I_1$ and $I_2$</td>
<td>110</td>
<td>63</td>
<td>1</td>
<td>17.9</td>
</tr>
<tr>
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<td>63</td>
<td>1</td>
<td>25.2</td>
</tr>
<tr>
<td>$III_1$ and $III_2$</td>
<td>110</td>
<td>105</td>
<td>1</td>
<td>29.8</td>
</tr>
<tr>
<td>$IV_1$ and $IV_2$</td>
<td>130</td>
<td>105</td>
<td>1</td>
<td>42.1</td>
</tr>
</tbody>
</table>

Figure 1. The effect of anaesthesia on antioxidant enzyme activity in rabbit erythrocytes measured 2 hours and 7 days after the application

Figure 2. The effect of different CTDIw values on SOD activity in rabbit erythrocytes measured 2 hours and 7 days following computed tomography

Figure 3. The effect of different CTDIw values on CAT activity in rabbit erythrocytes measured 2 hours and 7 days following computed tomography (a vs. b – statistically significant differences; ab vs. a or b – statistically non-significant differences)

Figure 4. The effect of different CTDIw values on GSH-Px activity in rabbit erythrocytes measured 2 hours and 7 days following computed tomography (a vs. b – statistically significant differences; ab vs. a or b – statistically non-significant differences)
Figure 5. The effect of different CTDIw values on GR activity in rabbit erythrocytes measured 2 hours and 7 days following computed tomography (a vs. b – statistically significant differences; ab vs. a or b – statistically non-significant differences)

Statistical analyses of GSH-Px activity showed that, in general, the activity increased after the day 7th (fig. 4, ANOVA, significant day effect, p < 0.001, no significant changes of dose effect). However, 2 hours after exposure GSH-Px activity in the group of 29.8 mGy was significantly lower than the control. There is no particular statistically significant individual difference when comparing 2 hours vs. the 7th day, but each GSH-Px activity on the 7th day was higher to some extent compared to its 2 hours counterpart except controls.

Statistical analyses of GR activity showed that GR activity increased after 2 hours upon exposure to 29.8 mGy compared to other groups, but after 7 days, decreased to a level not significantly different to controls and 17.9 and 25.2 mGy groups (fig. 5, ANOVA interaction, p < 0.001). On the other hand, there was an increase of GR activity in the group exposed to 42.1 mGy after 7 days compared to its 2 hours counterparts and other groups on the 7th day (ANOVA significant dose, p < 0.05, and interaction effect, p < 0.001).

DISCUSSION

Numerous studies show that the radiation dose received during CT examination may have adverse effects on living subjects and the International Agency for Research on Cancer (IARC) has classified X-rays as carcinogenic to humans on the basis of sufficient evidence for carcinogenicity [31]. The International Commission on Radiological Protection (ICRP) in a publication from 1990 also suggested that a low level of radiation exposure could result in cancer [32, 33]. Contrary to this, some authors suggested that below 10 mSv, which is a dose range relevant to radiography, no direct epidemiological data support increased cancer risk. However, this does not mean that this risk is not present, as even large epidemiological studies would not have the statistical power to detect increased risk, if present, at a low radiation dose [34]. Despite some controversy over the excess cancer risk of low-dose radiation, the linear no-threshold theory is widely used because an alternative method for assessing the potential risks of low-dose radiation is lacking. In addition, the epidemiological data directly suggest increased cancer risk in the 10 mSv to 100 mSv range, which is relevant to many CT studies [35]. X-rays and γ-rays have also been tested for carcinogenicity at various doses and under various conditions in a range of animal species. In adult animals, the incidences of leukaemia and of mammary, lung, and thyroid tumours were increased in a dose-dependent manner with both types of radiation. Prenatal exposure also gave rise to increased incidences of various types of tumours [31]. Low radiation doses lead to cellular reactions [9] triggering oxidative stress, and changes in the antioxidant enzymes activity were demonstrable when the applied dose ranged from 0.1 mGy [13, 14] to 50 mGy [12], which corresponds to the doses received during the CT examination [32].

The presented results originate from the first ever performed study on radiation effects during CT examinations of rabbits. CT scan exposure of rabbits led to a different response of the activity of antioxidant enzymes regarding both dose and time. The results indicate that there is a dose threshold that is about 25 mGy despite the fact that changes in the antioxidant enzymes activity were demonstrable when the applied dose ranged from 0.1 mGy [13, 14] to 1 mGy [13].

Doses below 25 mGy do not produce any significant changes in the level of antioxidant enzymes activity compared to controls. Higher doses had an impact on antioxidant enzymes, but different components are involved.

A dose of 29.8 mGy led to changes of CAT and GR activities after 2 hours that decreased to values not significantly different compared to the controls and lower dose exposure groups after 7 days. The same group (exposed to 29.8 mGy) had lower GSH-Px activity after 2 hours of exposure, but not different compared to the other groups after 7 days. Both CAT and GSH-Px decompose hydrogen peroxide (H₂O₂), but with different enzyme kinetics i.e. CAT operates at a higher H₂O₂ concentration than GSH-Px. This indicates that in 29.8 mGy, erythrocytes were exposed to a high impact of H₂O₂ after exposure to the CT scan. However, after 7 days the levels of antioxidant enzymes in that group were similar to controls, except CAT that was lower compared to other groups. It seems that GSH-Px activity after 7 days was efficient to in establishing a normal oxidative status and lower amount of H₂O₂ in this, as well as in other groups. Namely, ANOVA showed that GSH-Px was elevated to a certain extent in the all the examined groups (except the control), suggesting this enzyme as generally protective after 7 days of CT scan exposure.
On the other hand, exposure to 42 mGy led to the increase of GR activity after 7 days. This means that the reduction of oxidized glutathione was accelerated, suggesting the glutathione mediated way of antioxidant protection as dominant in the group that received the highest dose of irradiation.

The presented results suggest that CT exposure led in general to the activation of a protection against hydrogen peroxide production, which depends on irradiation doses as a determinant of hydrogen peroxide production. Moreover, the level just above that threshold had significant impact on the antioxidant defence, but changes were time dependent (2 hours vs. 7 days after exposure), suggesting that the higher dose requires a longer adaptive period.

Although erythrocytes are not very radiosensitive [36], low radiation doses can trigger the oxidative stress in these cells, resulting in elevated activity of the main antioxidant enzymes [10, 11]. According to the literature data, changes in the antioxidant enzymes activity were demonstrable when the applied dose was 50 mGy [12] or less, which is confirmed by our results. Despite the fact that the results related to the effect of low-dose radiation on the activity of oxidative enzymes in erythrocytes are presented in the available literature, they mostly relate to chronic irradiation.

Several studies were conducted with the aim of clarifying the oxidative stress status in radiology workers but their results are quite contradictory. Recently, some authors investigated changes of antioxidant enzymes activity in erythrocytes of hospital staff occupationally exposed to ionizing radiation with radiation doses ranging between 0.10 and 3.8 mGy per month [37]. Their results showed that activities of erythrocyte CuZn-SOD and Se-GPx, observed for the exposed group, were significantly higher than in the control group. It was also indicated that the activities of antioxidant enzymes were significantly increased in radiation-exposed subjects compared with control individuals. The activities of erythrocyte CAT levels in the exposed group were found to be significantly lower than in the control group. The authors assume that these findings may be explained in terms of low-dose radiation-induced hormesis. According to other authors [17], erythrocyte CAT activities are slightly increased in radiographers when compared to the controls.

Long-term exposure to low radiation doses in workers operating X-ray equipment reduced antioxidant enzyme activities (SOD, CAT, and GPx) in erythrocytes as compared to controls [38]. These results suggest that long-term exposure to a low radiation dose in workers operating X-ray equipment diminishes their antioxidant defence.

Blood samples obtained from the rabbits, before and after X-rays irradiation, that was continued for a week in daily sessions of 100 rad/min until a total dose of 550 rad was reached, were analyzed in order to determine the differences in the content of reduced glutathione (GSH), and glutathione peroxidase activity (GP). In blood samples that were taken following irradiation, the activity of GP was increased, whereas the levels of GSH were decreased [39].

As previously stated, there are no available literature data regarding activity of antioxidant enzymes in rabbit erythrocytes following acute low dose X-ray irradiation during CT examination.

**CONCLUSION**

The presented study investigated the influence of the radiation dose during CT examination to antioxidant enzyme activity in rabbit erythrocytes. The obtained results show that the antioxidant enzyme response follows dose mediated ROS production. According to this, the highest dose had a lag period for adaptive response compared to the lower one, and the dose threshold is about 25 mGy. The level just above that threshold, had significant impact on the antioxidant defence, but in a relatively short time period (2 hours after exposure), compared to the highest dose that requires a longer adaptive period (7 days). Furthermore, antioxidant protection is established by different antioxidant enzymes and depends on the level of irradiation.

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**REFERENCES**


[38] Klucinski, P., et al., Erythrocyte Antioxidant Parameters in Workers Occupationally Exposed to Low Levels of Ionizing Radiation, Experientia, 30 (1974), 6, pp. 9-12

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УТИЦАЈ ДОЗЕ ЗРАНЕ ПРИЛИКОМ КОМПЈУТЕРИЗОВАЊЕ ТОМОГРАФИЈЕ НА АКТИВНОСТ АНТИОКСИДАТИВНИХ ЕНЗИМА У ЕРИТРОЦИТАМА КУНИЋА

Циљ ове студије био је да се изврши процена доза зрачења током компјутеризоване томографије куних обављене при различитим протоколима прегледа, као и да се добијене вредности упореде са активношћу антиоксидативних ензима у еритроцитима ових животиња. Добијени резултати указују на то да након једнократног, рутинског СТ прегледа долази до промена активности антиоксидативних ензима у еритроцитима куних чији степен зависи од дозе зрачења и мења се у функцији времена. На основу добијених резултата такође се може закључити да постоји граница дозе која износи 25 mGy испод које нису уочене значајне промене у нивоу активности антиоксидативних ензима. С друге стране, вредност непосредно изнад тог прага, значајно је утицала на антиоксидативну одбрану, али само у релативно кратком временском периоду (2 часа након излагања зрачењу), у поређењу са већом дозом која је захтевала дужи адаптивни период (7 дана).

Кључне речи: компјутеризована томографија, СТ дозни индекс, еритроцит, антиоксидативни ензим, куних