

The effects of thyroid scintigraphy studies on oxidative damage in patients

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Received: February 13, 2006

Accepted: May 2, 2006

The majority of radiation injury in cells depends on oxidative stress. Irradiation and absorbed doses, duration of the irradiation and the susceptibility of the tissue against radiation are the factors that cause variations on living cells. The aim of this study was to investigate gamma radiation-induced oxidative damage in erythrocytes after thyroid scintigraphy with Tc-99m pertechnetate. Fifteen patients (8 women and 7 men) who performed thyroid scintigraphy with Tc-99m pertechnetate were included in this study. The median age was 52±8 years (range 33–65). The blood samples were taken from patients just before, 1 hour after and three hours after injection of radiopharmaceutical. Malondialdehyde (MDA) and antioxidant enzymes such as glutathione peroxidase (GPX), superoxide dismutase (SOD), and catalase (CAT) levels were measured to evaluate the gamma radiation induced oxidative damage. No difference was detected in any final measurement activities of erythrocyte antioxidant enzyme such as SOD and GPX in the direct comparison between the before and after injection of the radiopharmaceutical groups, except erythrocyte CAT activities measured 1 hour after and 3 hours after injection of the radiopharmaceutical ($p<0.05$). MDA levels were decreased 1 hour after and 3 hours after injection of the radiopharmaceutical.

Keywords: ionizing radiation, free radicals, oxidative stress, thyroid scintigraphy

Hydroxyl radicals are generated by ionising radiation either directly by oxidation of water or indirectly by the formation of secondary partially reactive oxygen species (ROS) (4, 16).

Cells have developed a defense against ROS, the antioxidant system, which includes enzymatic and non-enzymatic components (20). The process of lipid

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peroxidation is one of oxidative conversion of polyunsaturated fatty acids to products to native aldehydes such as malondialdehyde (MDA) and 4-hydroxynonenal by a well-studied, biologically relevant free radical reaction. MDA itself, because of its high cytotoxicity and its inhibitory action on protective enzymes, has been suggested to act as a tumor promoter and a co-carcinogen (9). MDA, an end product of lipid peroxidation, has been used as index of oxidative damage (5).

The aim of this study was to investigate gamma radiation-induced oxidative damage in erythrocytes after thyroid scintigraphy with Tc-99m pertechnetate.

Materials and Methods

The study was approved by the ethics committee of our hospital. MDA and several antioxidant enzymes such as (glutathione peroxidase) GPX, superoxide dismutase (SOD), and catalase (CAT) were measured to evaluate gamma radiation induced oxidative damage.

Fifteen patients (8 women and 7 men) who had thyroid scintigraphy with Tc-99m pertechnetate were included to our study. The median age was 52 ± 8 years (range 33–65). The blood samples were taken from patients before, 1 hour after and three hours after injection of the 185 MBq Tc-99m pertechnetate.

Oxidative stress studies in erythrocytes

The MDA concentrations as an indicator of lipid peroxidation and antioxidant enzymes such as GPX, SOD and CAT were used to evaluate gamma radiation induced oxidative damage. Blood samples were obtained in EDTA. The tubes were centrifuged at 3000 g for 3 minutes. Erythrocyte pellets were obtained immediately from the EDTA blood by centrifuging at 3000 g for 3 minutes. Plasma and buffy coat were removed and the erythrocytes were washed three times in 5 ml of cold 0.9% NaCl solution. Erythrocytes were hemolysed by adding cold distilled water. Hemolysate samples were stored at -80 and all measurements were made within 2 months. The erythrocytes were thawed and the levels of MDA and the activities of SOD, GPX, and CAT were assessed.

MDA was determined by the double heating method of Draper and Hadley (2). The principle of the method was spectrophotometric measurement of the color produced during the reaction to thiobarbituric acid (TBA) with MDA. For this purpose 2.5 ml 100 g/l trichloroacetic acid solution was added to 0.5 ml erythrocytes in a centrifuge tube and placed in a boiling water bath for 15 min. After cooling in tap water the mixture was centrifuged at 1000 g for 10 min, and 2 ml supernatant was added to 1 ml 6.7 g/l TBA solution in a test-tube and placed in a boiling water bath for 15 min. The solution was then cooled in tap water and its absorbance was measured using a Shimadzu UV-1601 spectrophotometer (Japan) at 532 nm. The concentration of MDA was calculated by the absorbance coefficient of MDA-TBA complex $1.56 \times 10^5 \text{ cm}^{-1} \text{ M}^{-1}$ and expressed in nmol/g hemoglobin.

The measurement of SOD was based on the principle in which xanthine reacts with xanthine oxidase to generate superoxide radicals which react with 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride to form a red formazan dye. The SOD activity is then measured by the degree of inhibition of this reaction (21).

The determination of GPX activity was based on the method described by Paglia and Valentine (14). The principle of the method was as follows: GPX catalyzes the oxidation of glutathione by cumene hydroperoxide. In the presence of glutathione reductase and NADPH the oxidized glutathione is immediately converted to the reduced form with a concomitant oxidation of NADPH to NADP⁺. The decrease in absorbance of NADPH was measured at 340 nm.

CAT activity was measured according to the method of Aebi (1). The principle of the assay is based on the determination of the rate constant (k , per second) of hydrogen peroxide decomposition by CAT enzyme. The rate constant was calculated from the following formula: $k = (2.3/\Delta t)(a/b) \log(A_1/A_2)$. In this formula, A_1 and A_2 are the absorbance values of hydrogen peroxide at t_1 (0th second) and t_2 (15th second), a is the dilution factor, and b is the hemoglobin content of the erythrocytes. An autoanalyzer, Abbott Aeroset, was used to determine the activities of SOD and GPX, and the spectrophotometer, Shimadzu UV-1601 (Japan), was used to estimate MDA and CAT.

Statistical analysis

Data were analysed using the statistical package SPSS for Windows (Ref. 9.05, SPSS Inc., Chicago, IL.). Results were expressed as mean $\pm$ SD. Statistical significance was set at the 0.05 levels. Differences within the same group were tested by repeated measures of ANOVA since that are all time dependent data.

Results

Results are tabulated in Table I. The enzyme activities of SOD were increased 1 hour after ($p=0.091$) but decreased 3 hours after ($p=0.668$) injection of the radiopharmaceutical. The enzyme activities of GPX were decreased 1 hour and 3 hours after (respectively $p=0.315$, $p=0.039$) injection of the radiopharmaceutical. The enzyme activities of CAT were decreased 1 and 3 hours after (respectively $p=0.000$ and $p=0.002$) injection of the radiopharmaceutical. MDA levels were decreased 1 hour after and 3 hours after (respectively $p=0.008$ and $p=0.551$) injection of the radiopharmaceutical.

Table I
MDA and antioxidant levels ($Ort \pm SD$)

	MDA (nmol/mg Hb)	SOD (U/g Hb)	GPx (U/g Hb)	CAT (k/g Hb)
Before radiopharmaceutic	92.06 $\pm$ 13.38	1880.33 $\pm$ 43.64	72.059 $\pm$ 13.158	63.50 $\pm$ 16.77
1 hour after radiopharmaceutic	79.74 $\pm$ 9.43 ^a	2169.16 $\pm$ 15.46	67.660 $\pm$ 12.529	42.02 $\pm$ 10.34 ^a
3 hours after radiopharmaceutic	89.40 $\pm$ 5.76 ^b	1826.20 $\pm$ 381.59 ^b	61.648 $\pm$ 12.151	43.71 $\pm$ 13.04 ^a

^a p<0.05 compared to before radiopharmaceutical group.

^b p<0.05 compared to 1 hour after radiopharmaceutical group.

Discussion

Ionizing radiation causes harmful effects through the generation of free radicals (18). Radiation is a known producer of ROS. When water, the most abundant intra- and extracellular material, is exposed to ionising radiation, decomposition occurs through which a variety of ROS, such as the superoxide radical ($O_2^{\cdot-}$), the hydrogen peroxide (H_2O_2) and the hydroxyl radical ($OH^{\cdot}$), are generated. These ROS formed in cells contribute to radiation injury in cells. Although all respiring cells are equipped with protective enzymes such as SOD and CAT or GPX, increased oxidative stress in cells that stem from ionising radiation may overwhelm the protective systems, leading to cell injury (4, 19).

SOD converts superoxide anion radical ($O_2^{\cdot-}$) to H_2O_2 , thus decreasing the amount of $O_2^{\cdot-}$ and the formation of peroxynitrite anion ($ONOO^-$), a highly destructive product of the interaction between O_2 and nitric oxide ($NO^{\cdot}$) (9).

Sabitha and Shyamaladevi (17) demonstrated that activities of erythrocyte SOD, CAT and GPX enzymes were significantly lower after radiotherapy than before radiotherapy in their study. This suggests that ionising radiation causes enzyme deficiencies, arising as a result of enormous production of free radicals in the system. Lee et al. (11) reported that SOD was an important anti-oxidant protein in the protection of yeast cells against ionising radiation.

Greenstock (6) reported that ionising radiation increased the level of MDA. In agreement with these results, we found that gamma radiation increases erythrocyte MDA levels.

Nikishkin et al. (12) reported that levels of enzymatic and non-enzymatic antioxidants decrease after irradiation. SOD and GPX each play a role in the antioxidant defense system, but their response to radiation is unclear. Gren et al. (7) found that

radiation did not significantly affect GPX activities in the long term, while Kaya et al. (8) reported that GPX activities were not decreased significantly after irradiation compared with sham controls.

Our results demonstrate that the activities of GPX are not different in the before and after radiopharmaceutical groups. These results confirm those of Kaya et al. (8) and Reiter (15).

The enzyme activities of CAT were decreased 1 and 3 hours after injection of the radiopharmaceutical ($p < 0.05$). Exposure to hydrogen peroxide, a reactive oxidant, induces the activation of a set of genes whose transcripts are translated into the antioxidant enzyme CAT. Radiation induces changes in antioxidant activities expressed as a decrease in blood CAT activities (3). The enzyme activities of SOD were found increased in 1st hour samples ($p > 0.05$) decreased in 3rd hour samples ($p > 0.05$). MDA levels were decreased 1 hour after and increased 3 hours after injection of the radiopharmaceutical ($p > 0.05$).

In conclusion, it is known that ionized radiation affects the cells by increasing the levels of free radicals. On the other hand, the effects of nuclear medicine studies on free radicals are not clear. In this study, we found that radiation due to thyroid scintigraphy with Tc-99m pertechnetate applications decreased the erythrocyte antioxidant levels and almost no changes in MDA levels.

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