Oxidative stress in patients with differentiated thyroid cancer: Early effects of radioiodine therapy

Olgica B Vrndić1*, Snezana D Radivojević2, Marina D Jovanović3, Svetlana M Djukić1,2, Ljiljana C Mijatović Teodorović1,2 and Snezana T Zivancevic Simonović1

1Institute of Pathophysiology, Faculty of Medical Sciences, University of Kragujevac, Svetozara Markovica 69, 34000 Kragujevac, Serbia, 2Clinical Center Kragujevac, Kragujevac, Serbia, 3Institute for Medical Research, Military Medical Academy, Belgrade, Serbia

Received 06 November 2013; revised 18 April 2014

Ionizing radiation in differentiated thyroid cancer (DTC) patients treated with radioiodine (131-I) produces reactive oxygen species (ROS), which could induce oxidative stress with disturbance of redox balance. The aim of this study was to evaluate oxidative stress in DTC patients treated with 3.7 or 5.5 GBq of 131-I using values for serum malondialdehyde (MDA, a marker of oxidative stress), uric acid (to determine antioxidant status) and total antioxidative status (TAS). The study population included 20 DTC patients and 20 healthy controls. Significant differences in MDA concentrations were found between DTC patients before 131-I therapy and control subjects (p = 0.001), while TAS values were similar in both populations (p>0.05). There was a negative correlation between MDA concentrations and TAS in the DTC group before therapy (R² = 0.2973, p = 0.013). Three days after 131-I therapy, MDA concentrations were higher than the pretreatment values (3.36 ± 1.69 nmol/mL vs. 2.93 ± 1.31 nmol/mL; p = 0.006), while serum uric acid concentrations declined progressively from 341.0 ± 80.39 µmol/L to 304.25 ± 77.25 µmol/L (p = 0.026) in 3 days and 291.2 ± 88.86 µmol/L (p = 0.009) in 7 days after 131-I therapy. There was no dose-dependent effect on MDA, or uric acid concentrations and TAS. Thus, 131-I therapy in DTC patients induced oxidative stress, which was accompanied by a simultaneous and extended reduction in uric acid concentration, but without significant disturbances in TAS. This is the first study that evaluated TAS capacity in DTC patients before and 7 days after 131-I therapy. The relatively stable TAS values in these patients indicated a good protection from oxidative stress induced by high doses of ionizing radiation.

Keywords: Differentiated thyroid cancer, Radioiodine therapy, Oxidative stress, Malondialdehyde, Uric acid, Total antioxidant status

Reactive oxygen species (ROS) are produced during normal cellular metabolism, in the process of cellular respiration and during phagocytosis. A low level of ROS is necessary in many biochemical processes. In addition, ROS are formed as a consequence of various external factors, including ionizing radiation. Living cells have very strong antioxidant protection mechanisms that eliminate any excess of ROS. Oxidative stress is a state of imbalance between production and elimination of ROS where ROS generation dominates, which can lead to cell damage and death.

Lipid peroxidation is one of the most studied consequences of ROS action. It can be defined as oxidative deterioration of unsaturated lipids in cell membranes with the formation of many cytotoxic end-products, such as malondialdehyde (MDA). As a highly reactive organic compound, MDA is often used as a biological marker of oxidative stress. MDA production can be reduced by uric acid, a powerful antioxidant that contributes as much as two-third of all free radical scavenging capacity in plasma.

Thyroid cancer is the most common malignancy of the endocrine system, the incidence of which has been increasing over the past 20 years. Differentiated thyroid carcinomas (DTCs) account for more than 90% of all thyroid cancers and include papillary and follicular histological types. As DTCs originate from the follicular cells of the thyroid, with the ability to accumulate iodine, the treatment of DTC patients with radioactive iodine (131-I) is a standard procedure for ablation of remnant thyroid tissue and for treatment of...
DTC metastases\(^{10}\). Exposure to radiation causes different lesions in affected cells and body fluids because of direct ionization or free radical attack\(^{11}\). Therefore, the administration of 131-I (3.7 or 5.5 GBq) provides ideal conditions for assessing the \textit{in vivo} effects of prolonged irradiation by radionuclide incorporation and subsequent systemic \(\beta\)- and \(\gamma\)-ionizing radiation exposure.

Lipid peroxidation products and antioxidant molecules in DTC patients before and after radioiodine (131-I) therapy have already been studied\(^{12-14}\). However, the findings are not consistent, primarily because different parameters have been used to assess the intensity of oxidative stress and antioxidant status, e.g. MDA + 4-hydroxyalkenals\(^{15}\), erythrocyte MDA and erythrocyte reduced glutathione\(^{16}\), MDA, selenium, total superoxide dismutase and glutathione peroxidase\(^{17}\), ROS levels and intracellular glutathione\(^{18}\). In addition, the time elapsed between the application of radioactive 131-I and measurement of the intensity of oxidative stress and antioxidant status varies from several days\(^{16,19}\) to several months\(^{20}\).

Until now, the early effects of radioactive iodine therapy are very little investigated. As the most intense oxidative stress can be expected within the first few days after administration of 131-I, this study has been aimed to evaluate the level of oxidative stress products (as MDA) and antioxidant status (as serum uric acid concentrations) and total antioxidative status (TAS) in DTC patients 3 and 7 days after therapy with 3.7 or 5.5 GBq of 131-I. Protein concentrations have also been determined before, as well as 3 and 7 days after 131-I therapy of DTC patients and compared with values for healthy controls.

Material and Methods

Study population

The study was approved by the Ethical Committee of the Clinical Center Kragujevac (number 01-5868). All patients and control subjects gave written (informed) consent, according to the Helsinki Declaration.

The study population included 20 DTC patients (14 females and 6 males) of mean age 53.30 ± 15.78 yrs. Among them, 15 (75\%) had papillary carcinoma, 4 (20\%) had the follicular variant of papillary carcinoma and 1 patient (5\%) had follicular carcinoma. Nineteen of 20 DTC patients included in this study underwent the total thyroidectomy alone and only one patient underwent the total thyroidectomy with right neck dissection. Four to six weeks after surgical thyroidectomy and 10 days after a low-iodine diet, the patients were treated at the Nuclear Medicine Department of the Clinical Center, Kragujevac, according to EANM guidelines\(^{21}\), with fixed nominal activities of 3.7 GBq (100 mCi) (11 patients) or 5.5 GBq (150 mCi) (9 patients) of sodium [131-I] iodide administered orally. At the time of 131-I administration, all patients were hypothyroid after thyroid hormone withdrawal (concentration of thyroid-stimulating hormone, TSH > 30 mIU/L). None of the patients had been exposed to potentially confounding factors, such as other ionizing radiations (radiographic examination or scintigraphy) within 3 months before therapy. They were not administered salicylates or other agents that could influence the oxidative state for 3 weeks before the study and had normal liver and renal function. Patients were released from hospital 3 days after 131-I therapy or later, when measured residual activity had reached a value below 2 mR/h.

Control group comprised of 20 healthy subjects, 15 (75\%) females and 5 (25\%) males of mean age 46.76 ± 12.89 yrs. The control subjects had not been exposed to radioactive sources for a minimum of 3 months before the study. All control subjects were evaluated for thyroid function and thyroid antibodies. The mean concentration of TSH was 1.46 ± 0.72 mIU/L (range 0.4-3.5 mIU/L) and thyroid antibodies were negative. The subjects who were diabetic, hypertensive or those with liver disease, renal failure, malignant diseases and dysproteinemia/dyslipoproteinemia were not included in the study. All control subjects provided their written consent according to the Declaration of Helsinki. Blood samples from control subjects were taken only once, while blood samples from DTC patients were obtained three-times: before, as well as 3 and 7 days after 131-I therapy of DTC patients and compared with values for healthy controls.

Measurement of lipid peroxidation

The level of lipid peroxidation was estimated from generation of MDA. A standard curve was prepared
by acid hydrolysis of 1,1,3,3-tetramethoxypropane. One volume of sample was mixed thoroughly with two volumes of a stock solution of 15% w/v trichloroacetic acid, 0.375% w/v thiobarbituric acid and 0.25 M HCl. The combination of sample and stock solution was heated for 5 min in a bath of boiling water. After cooling, the precipitate was removed by centrifugation at 1000 × g for 5 min. The absorbance of clear sample was determined spectrophotometrically at 535 nm using Ultrospec 2000 spectrophotometer (Pharmacia Biotech, England). MDA values were expressed as nmol/mL using a molar extinction coefficient of 1.56 × 10^5 M⁻¹ cm⁻¹.22

Measurement of serum uric acid and protein concentrations

Serum concentrations of uric acid and proteins were determined using commercially available enzymatic reagents (OSR6198 and OSR6232 Beckman Coulter, respectively) adapted to an auto-analyzer (Beckman Coulter Olympus AU 400), according to the instruction of the manufacturer. The method for determination of proteins is based on the enzymatic reaction in which cupric ions in an alkaline solution react with proteins and polypeptides containing at least two peptide bonds to produce a violet colored complex. The absorbance of complex is directly proportional to the concentration of protein in the serum sample. Reference ranges are as follows: uric acid: 154-428 µmol/L and proteins: 64-89 g/L.

Measurement of total antioxidant status (TAS)

TAS was determined colorimetrically using the TAS kit (Fortress Diagnostics, UK). This assay relies on the ability of antioxidants in the sample to inhibit the formation of ABTS⁺ by oxidation of ABTS [2,2′-azino-di-(3-ethylbenz-thiazoline sulfonate)] with metmyoglobin (a peroxidase). ABTS⁺ has a stable blue green color that can be measured at 600 nm. Antioxidants in the sample suppress the formation of the color in proportion to their concentration. Values of TAS were expressed as mmol Trolox equivalent/L.

Statistical analysis

All values were expressed as mean ± standard deviation (SD). The commercial SPSS version 20.0 for Windows was used for statistical analysis. Differences of the determined parameters between control subjects and DTC patients before therapy were evaluated by the independent samples t-test or U-test (depending on distribution), while those between DTC patients before and after radioiodine therapy were evaluated by ANOVA or Friedman test, in the case of non-parametric distribution. Probability values less than 0.05 were considered to be statistically significant and those less than 0.01 highly significant.

Results

The MDA concentrations and TAS values found in DTC patients and control subjects are shown in Fig. 1. The difference in MDA concentrations between DTC patients before 131-I therapy and control subjects (2.93 ± 1.31 nmol/ml vs. 1.82 ± 0.33 nmol/mL; p = 0.001) was highly significant. In contrast, TAS values were similar in both populations (1.88 ± 0.26 mmol/L vs. 1.87 ± 0.31 mmol/L; z = -0.071; p>0.05). There was a negative correlation between MDA concentrations and TAS in the DTC group before therapy (R² = 0.2973, p = 0.013).

MDA concentrations in DTC patients were increased 3 days after 131-I therapy, when compared to the values before treatment (3.36 ± 1.69 nmol/mL vs. 2.93 ± 1.31 nmol/mL; z = -2.745, p = 0.006) with a subsequent fall to 2.71 ± 1.13 nmol/mL (z = -2.396, p = 0.017) at 7 days after therapy. Friedman’s test showed a statistically significant difference concerning MDA in DTC patients during 131-I therapy (p = 0.016). One week after administration of 131-I, MDA concentrations were somewhat lower than before therapy, but without statistical

![Fig. 1—Concentrations of MDA and TAS in DTC patients (before, 3 and 7 days after 131-I therapy) and control subjects [Highly significant differences in MDA concentrations between DTC patients before 131-I therapy and control subjects (p = 0.001) and between MDA concentrations in DTC patients 3 days after and before therapy (p = 0.006) were seen: *highly significant difference in MDA concentration between DTC patients before 131-I therapy and control subjects; **significant difference of MDA concentration in DTC patients 3 days after and before 131-I therapy]
significance (2.71 ± 1.13 nmol/mL vs. 2.93 ± 1.31
nmol/mL; z = -0.926, p>0.05). TAS values in the
DTC group did not change significantly after I-131
therapy (p>0.05).

Uric acid and protein concentrations measured in
serum of DTC patients and control subjects are
shown in Fig. 2. Before therapy DTC patients
had a significantly higher concentration of uric acid
than the healthy subjects (341.0 ± 80.39 μmol/L vs.
256.28 ± 86.56 μmol/L ; z = -2.516, p = 0.012)
(Fig. 2a). There was also a significant difference
in protein concentrations (85.85 ± 5.66 g/L vs.
81.68 ± 4.71 g/L; p = 0.012) between DTC patients
before therapy and control subjects (Fig. 2b).
Uric acid values showed a progressive decline after
therapy (p = 0.011). Post-hoc comparison revealed a
10.77% reduction to 304.25 ± 77.25 μmol/L
(p = 0.026) at 3 days and a 14.60% reduction to
291.2 ± 88.86 μmol/L (p = 0.009) at 7 days after 131-I
therapy. There was also a change in the protein
concentrations in DTC patients after treatment
(p<0.001), but in contrast to uric acid, the decrease in
protein concentration was observed only at 3 days
after 131-I therapy, with a trend to return to
pretreatment values at 7 days after therapy. Thus, the
average decline in protein concentration was 9.08%
3 days after 131-I therapy (78.05 ± 6.73g/L; p<0.001),
but only 4.54% (81.95 ± 4.67 g/L; p = 0.011) 7 days
after 131-I therapy, when compared to the value
before therapy.

The stratification of the study subjects based on
the thyroid cancer subtype and their corresponding
MDA, TAS and uric acid concentrations are shown in
Table 1. There was an evident increase in MDA and
decrease in uric acid concentrations, with slightly
modified TAS values in all subgroups of DTC
patients. Due to large differences in the number of
subjects, comparison between subgroups of DTC
patients was not done.

DTC patients treated with 3.7 GBq or 5.5 GBq of
131-I had significant decrease in uric acid
concentration shortly after therapy (p = 0.005, partial

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Time of examination</th>
<th>Histology of DTC (P/F*)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>P (n = 15)</td>
<td>P/F (n = 4)</td>
</tr>
<tr>
<td>MDA (nmol/mL)</td>
<td>Before therapy</td>
<td>2.98 ± 1.34</td>
</tr>
<tr>
<td></td>
<td>3rd Day</td>
<td>3.46 ± 1.82</td>
</tr>
<tr>
<td></td>
<td>7th Day</td>
<td>2.77 ± 1.75</td>
</tr>
<tr>
<td>TAS (mmol/L)</td>
<td>Before therapy</td>
<td>1.88 ± 0.30</td>
</tr>
<tr>
<td></td>
<td>3rd Day</td>
<td>1.88 ± 0.33</td>
</tr>
<tr>
<td></td>
<td>7th Day</td>
<td>1.90 ± 0.36</td>
</tr>
<tr>
<td>Uric acid (μmol/L)</td>
<td>Before therapy</td>
<td>345.5 ± 86.64</td>
</tr>
<tr>
<td></td>
<td>3rd Day</td>
<td>314.6 ± 81.04</td>
</tr>
<tr>
<td></td>
<td>7th Day</td>
<td>309.8 ± 87.39</td>
</tr>
</tbody>
</table>

*P- Papillary carcinoma
P/F- Follicular variant of papillary carcinoma
F- Follicular carcinoma

Fig. 2—Serum uric acid and protein concentrations in DTC
patients (before, 3 and 7 days after 131-I therapy) and control
subjects (a) *significant differences in uric acid concentrations
between DTC patients before 131-I therapy and control subjects
(p = 0.012); ** significant difference of uric acid concentration
in DTC patients 3 days after and before 131-I therapy (p = 0.026);
and ***significant difference of uric acid concentration in DTC
patients 7 days after and before 131-I therapy (p = 0.009); and
(b) *Significant difference in protein concentrations between DTC
patients before 131-I therapy and control subjects (p = 0.012); and
**highly significant difference of protein concentration in DTC
patients 3 days after and before 131-I therapy (p<0.001)]
eta squared = 0.465). The dose of 5.5 GBq had a slightly more intense influence, but the difference between the two doses was not statistically significant (p>0.05, partial eta squared = 0.016). There was no effect of the doses (3.7 or 5.5 GBq) on MDA concentrations or TAS values 3 and 7 days after 131-I therapy (p>0.05).

Discussion

In this study, we evaluated oxidative stress and antioxidant status in DTC patients treated with 3.7 GBq or 5.5 GBq of 131-I. The oxidative stress was estimated from the concentration of MDA and antioxidant status on the basis of serum uric acid concentration and TAS values. All baseline parameters were compared to those for a control group of healthy subjects. In radioiodine-treated DTC patients, we unequivocally demonstrated an increase of MDA 3 days after 131-I therapy accompanied by declining serum uric acid concentrations lasting for 7 days, but without significant changes of TAS. Our results indicated that the whole body ionizing radiation in DTC patients induced oxidative stress with the disturbances of some elements of the antioxidative protection, whereby the TAS remained unchanged.

Following surgery, treatment of DTC patients with 131-I is a standard procedure for the ablation of remnant thyroid tissue and for the treatment of iodine-avid metastases. This treatment is based on the accumulation of 131-I in thyroid tissue and radiation-induced cell damage. There are literature data indicating that overall risk of secondary malignancies in thyroid cancer survivors is slightly increased, which some authors postulated could be due to 131-I therapy. After 131-I therapy, ROS and their products can react with polyunsaturated fatty acids in cell membranes, causing lipid peroxidation. The level of oxidative stress indicators (for example, MDA) and antioxidative defense changes (such as uric acid and TAS) might, at least, to some extent indicated the possibility of side effects in radioiodine-treated DTC patients.

It has been shown that parameters of oxidative stress might increase even before therapy in patients with different types of cancers, including DTCs. However, published data about oxidative stress in DTC patients treated with 131-I are not consistent. Some studies have demonstrated an increase oxidative stress markers in DTC patients after 131-I therapy, while others do not. Thus, in order to get a more comprehensive insight into the redox state of such patients, we measured MDA, uric acid and TAS concentrations.

MDA is a stable product of lipid peroxidation of cell membranes, which can react with the free amino groups of proteins and nucleic acids, causing further damage to cells. Therefore, the degree of damage to biological structures induced by lipid peroxidation in vivo and in vitro can be estimated by the measuring of MDA concentration. MDA was used as a marker of oxidative stress in this study, as well as in many previous studies. In one study, a transitory increase of MDA concentration is reported 5 days after 131-I therapy of DTC patients, while in another study a small initial increase has been observed in MDA one month after administration of 131-I, followed by a significant decrease at 6 months after the therapy. In our study, we evaluated the early effects of 131-I therapy during the first week after administration of 131-I. These results were in agreement with those of Konukoglu et al., who reported significantly elevated level of erythrocyte MDA 2 days after radioiodine therapy. Although the maximum intensity of oxidative stress can be expected in the first hours and days after administration of 131-I, we did not examine the earliest changes for technical reasons. In our study, patients were isolated for at least 3 days after application of 131-I, or until the residual activity had reached a value below 2 mR/h.

Uric acid is a strong antioxidant that can inhibit lipid peroxidation, but in contrast to other antioxidant scavengers, this process does not involve uric acid oxidation. In our DTC patients, uric acid concentrations before 131-I therapy were higher than those in control subjects, which might point to the body's response to the disease. After administration of 131-I, serum uric acid concentrations decreased progressively, as measured at 3 and 7 days post-treatment. This was most probably a consequence of ionization-induced ROS production, and an inability of protective antioxidant mechanisms to control the oxidative stress. Indeed, our DTC patients had significantly higher concentrations of MDA before therapy, compared to controls. Our results were in agreement with the results of other studies.

We also estimated the protein concentrations in serum of DTC patients before and after 131-I therapy. The concentration of proteins in DTC patients before 131-I therapy was significantly higher than in controls. In fact, protein concentration in all control
subjects was in the reference range, while in a few DTC patients before 131-I therapy exceeded the upper limit of the reference range (89 g/L). The DTC patients included in this study had no known disease that could associate to the increased concentration of proteins in the serum. In these patients, we had not examined the concentrations of protein fractions.

Literature data indicate that mean values of albumin and alpha region glycoproteins are significantly lower in patients with oral precancerous conditions, while the gamma region glycoproteins are significantly elevated. In contrast, others have demonstrated significantly lower plasma protein levels in the patients with bladder cancer, as compared to the healthy control group. We clearly demonstrated the administration of 131-I in DTC patients led to the significant decrease of protein concentration 3 days after the therapy. After a decrease at 3 days after 131-I therapy, the values recovered near to those measured before therapy. The decrease of protein concentrations 3 days after 131-I therapy was possibly caused by oxidative stress. Earlier, it is shown that protein oxidation and oxidative damage might be responsible for the decrease in protein concentrations of cancer patients.

At the time of 131-I administration, all our patients were severely hypothyroid due to thyroid hormone withdrawal (TSH >30 mIU/L). Although Pereira et al. have shown that hypothyroidism tends to reduce the level of lipid peroxidation in rats, our results were consistent with previously published data showing intensive production of MDA in hypothyroid patients. An insufficient antioxidant defense and altered lipid metabolism could be responsible for such high levels of lipid peroxidation in these patients. In our DTC patients treated with 131-I, we unequivocally demonstrated an increase of MDA 3 days after therapy, with simultaneous and progressive decline in uric aci, which remained significantly depleted 7 days after the therapy.

According to our best knowledge, this is the first study which examined TAS level in DTC patients within a first week after 131-therapy. In a previous study, Konukoglu et al. have analyzed the individual components of antioxidative protection early after 131-I therapy and reported an elevated level of GSH-related antioxidant enzymes (GSH-Peroxidase and GSH-Reductase) two days after the administration of 131-I. In our study, although the serum uric acid concentrations decreased progressively 3 and 7 days after 131-I therapy, TAS values were reduced to a much smaller extent without statistical significance. This could be explained by the fact that the TAS capacity is not a simple sum of the activities of the various antioxidative substances. It is a dynamic equilibrium that is influenced by the interactions between each serum antioxidative constituent. It is thought that the cooperation of antioxidants in human serum provides greater protection against free-radical attacks than any antioxidant substance alone. Therefore, the determination of TAS capacity is useful in assessing the medical importance of free oxygen radicals effect and antioxidative defense.

In our DTC patients, there was no significant influence of dose on concentrations of uric acid, MDA or TAS. These results indicated that the difference between the doses was not sufficient to cause a difference in oxidative stress intensity. This confirmed the findings of Makarewicz et al., who showed that the dose of 131-I did not affect redox balance significantly.

In conclusion, 131-I therapy in DTC patients induced oxidative stress, which was accompanied by a simultaneous and extended reduction in serum uric acid concentrations, but without significant disturbances in TAS. The highest intensity of oxidative stress was found 3 days after 131-I therapy, irrespective of the dose of 131-I used. This is the first study evaluated TAS capacity in DTC patients before and 7 days after 131-I therapy. The relatively stable TAS values in these patients indicated a good protection from oxidative stress induced by high doses of ionizing radiation.

Acknowledgements
The study was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (Grant nos. III 41010 and ON 175069).

References