Radioiodine Therapy Induces Dose-Dependent In Vivo Oxidation Injury: Evidence by Increased Isoprostane 8-Epi-$PGF_{2\alpha}$

Roswitha M. Wolfram, MD[1]; Alexandra C. Budinsky, MS[2]; Barbara Palumbo, MD[3]; Renato Palumbo, MD[3]; and Helmut Sinzinger, MD[4,5]

[1]Department of Angiology, University of Vienna, Vienna, Austria; [2]Clinical Division of Oncology, Department of Internal Medicine I, University of Vienna, Vienna, Austria; [3]Institute of Nuclear Medicine, University of Perugia, Perugia, Italy; [4]Department of Nuclear Medicine, University of Vienna, Vienna, Austria; and [5]Wilhelm Auerswald Atherosclerosis Research Group (ASF) Vienna, Vienna, Austria

^{131}I is the treatment of choice for differentiated thyroid cancer and hyperthyroidism. A relationship between low-density lipoprotein oxidation and radioiodine therapy–related side effects, consequently inducing increased formation of 8-epi-prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) in situ, has recently been reported by several investigators. Isoprostanes, among them 8-epi-$PGF_{2\alpha}$, have been associated with increased oxidation injury due to various pathologic conditions in vivo. The aim of this study was to investigate the possible induction of oxidative stress as a consequence of ^{131}I therapy. **Methods:** 8-epi-$PGF_{2\alpha}$ was examined in plasma, serum, and urine in 42 patients undergoing radioiodine treatment of hyperthyroidism or thyroid cancer. The 8-epi-$PGF_{2\alpha}$ levels were analyzed daily for 1 wk and thereafter at different points up to 12 wk after treatment. **Results:** The isoprostane levels showed an increase after application of radioiodine in all investigated compartments. The effect was significantly higher and longer lasting after higher-activity therapy (2,960 or 7,400 MBq) than after lower-activity therapy (185 or 740 MBq). **Conclusion:** These findings document a significant, dose-dependent in vivo oxidation injury as a consequence of therapeutic radioiodine application to the salivary gland.

Key Words: radioiodine therapy; oxidation injury; isoprostanes; 8-epi-prostaglandin $F_{2\alpha}$

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For certain types of hyperthyroidism and for remnants after total thyroidectomy in patients with differentiated thyroid cancer, ^{131}I is the treatment of choice (*1*). Examination of lipoproteins after radioiodine therapy recently showed that low-density lipoprotein (LDL), temporarily, and high-density lipoprotein, later, are undergoing oxidative modification as a consequence of therapy (*2*). Oxidized LDLs in turn are known to be atherogenic (*3*), because they are rapidly taken up by monocytes or macrophages through pathways mediated by scavenger receptors (*3*). These pathways do not have a feedback control (*4*); thus, lipids may be taken up excessively. Consequently, the lipids are stored in macrophages, which are transformed into lipid-loaded foam cells (*5*). During LDL oxidation, the isoprostane 8-epi-prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), among other isoprostanes, is generated in situ after free radical–mediated peroxidation of arachidonic acid (*6*). These isoprostanes are stable prostaglandinlike compounds and have been shown to be reliable markers of in vivo oxidation injury (*7*). Immunochemical and immunohistochemical studies revealed that both oxidized LDL and 8-epi-$PGF_{2\alpha}$ are predominantly found along foam cells in the vascular wall (*8,9*). The aim of this study was to investigate whether therapeutic application of ^{131}I leads to an alteration of isoprostane levels in plasma, serum, and urine, reflecting possible oxidation injury from this therapy.

MATERIALS AND METHODS

Patients being treated with different activities of ^{131}I (185, 740, 2,960, or 7,400 MBq) were examined during and after radioiodine therapy. Samples of blood and urine were collected in the morning on the day before therapy and daily thereafter until the 8th day, as well as after 2, 3, 4, 6, 8, 10, and 12 wk. Smokers were excluded because of their severely elevated levels of 8-epi-$PGF_{2\alpha}$ (*10,11*). A total of 42 patients (18 men, 24 women) were investigated (Table 1).

Blood was drawn the morning after a fast of at least 12 h and was tested for levels of lipids, lipoproteins, thyroid-stimulating hormone, creatinine, and 8-epi-$PGF_{2\alpha}$ and for safety parameters (glutamyltranspeptidase, glutamate-oxalacetate-transaminase, creatine kinase, glutamate-pyruvate-transaminase, and LDH). A 24-h urinary sample was collected at the same time, and after total urinary volume had been assessed, an aliquot was stored at –70°C.

Serum 8-Epi-$PGF_{2\alpha}$

Blood was drawn into glass vials, which were placed immediately into a water bath at 37°C for exactly 60 min. Serum was then

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For correspondence or reprints contact: Helmut Sinzinger, MD, Wilhelm Auerswald Atherosclerosis Research Group (ASF) Vienna, Nadlergasse 1, A-1090 Vienna, Austria.

E-mail: Helmut.Sinzinger@akh-wien.ac.at

TABLE 1
Patient Characteristics

Activity (MBq)	*n*	Sex (M/F)	Age (y)
185	17	8/9	34–68
740	11	4/7	44–71
2,960	6	3/3	36–70
7,400	8	3/5	38–65

removed after centrifugation (4°C, 1,000*g*, 10 min) and stored until determination (no longer than 2 wk at less than −70°C) (*12*). To evaluate oxidation injury in vivo, the most prostaglandinlike isoprostane compartment was chosen and 8-epi-$PGF_{2\alpha}$ was determined by a specific immunoassay. To evaluate interassay variability, the respective sample was determined several times in several assays. Intraassay variability was evaluated by assaying the same sample several times during the same assay procedure. Interassay variability within the normal range amounted to 3.8% ± 1.2%, whereas intraassay variability was 1.9% ± 0.7% (normal range, 150–250 pg/mL, $n = 17$).

Plasma 8-Epi-$PGF_{2\alpha}$

Blood samples were anticoagulated with 2% ethylenediaminetetraacetic acid and 1 mg/mL (final blood volume) acetylsalicylic acid. Immediate centrifugation at 4°C to obtain plasma was done at 1,000*g* for 10 min. Plasma was removed and stored at less than –70°C for no longer than 2 wk before determination. Interassay variability within the normal range was 5.5% ± 1.7%, whereas intraassay variability was 2.5% ± 0.7% (normal value, <20 pg/mL, $n = 11$).

Urinary 8-Epi-$PGF_{2\alpha}$

Urine was collected over a period of 24 h. Aliquots (10 mL) were taken for extraction and adjusted to pH 4.0 with formic acid. The eluate was subjected to silicic acid chromatography and further eluted. This final eluate was dried, recovered in buffer, and assayed after dilution. Cross-reactivity of the antibody with prostaglandins was less than 2%. Values are given in picograms of 8-epi-$PGF_{2\alpha}$/mg creatinine. Interassay variability within the normal range was 6.4% ± 2.3%, whereas intraassay variability was 2.7% ± 0.8% (normal range, 150–250 pg/mg creatinine, $n = 14$).

8-Epi-$PGF_{2\alpha}$-Assay

8-epi-$PGF_{2\alpha}$ was determined after extraction and purification by chromatography. In vitro artifactual formation of 8-epi-$PGF_{2\alpha}$ (which eventually could easily be generated by in vitro autooxidation of arachidonic or other fatty acids) was excluded by comparison with immediate measurements showing no difference in the respective eicosanoid. Cross-reactivity of the antibody was 8.6% for 8-epi-$PGF_{2\alpha}$.

Statistical Analysis

Values are presented as mean ± SE; significance was determined using the Student *t* test; $P < 0.05$ was considered statistically significant.

RESULTS

Plasma 8-Epi-$PGF_{2\alpha}$

In patients treated with 185 MBq, 8-epi-$PGF_{2\alpha}$ increased continuously until reaching a significant maximum by days 3 and 4 after treatment and then dropped continuously until reaching pretherapeutic levels after 3 wk (Fig. 1). In patients treated with 740 MBq, a significant increase of 8-epi-$PGF_{2\alpha}$ was found after day 2, reaching the highest levels on days 3 and 4. After 3 wk, the levels continuously decreased until reaching pretherapeutic levels. From day 2 until week 2, a significantly higher plasma 8-epi-$PGF_{2\alpha}$ was observed, with the highest levels occurring on days 3–5. The 8-epi-$PGF_{2\alpha}$ plasma levels on days 2, 3, 4, 5, 6, 7, and 8, as well as after 2 wk, were significantly higher in the 740-MBq treatment group than in the 185-MBq treatment group. In the 7,400-MBq treatment group, we found a significant increase of plasma 8-epi-$PGF_{2\alpha}$ after day 2 until week 8, and this increase also reached a maximum on days 3 and 4. The levels of 8-epi-$PGF_{2\alpha}$ on days 2, 3, 4, 5, 6, 7, and 8, as well as after weeks 2, 3, and 8, were significantly higher in the 7,400-MBq treatment group than in the 185-MBq treatment group.

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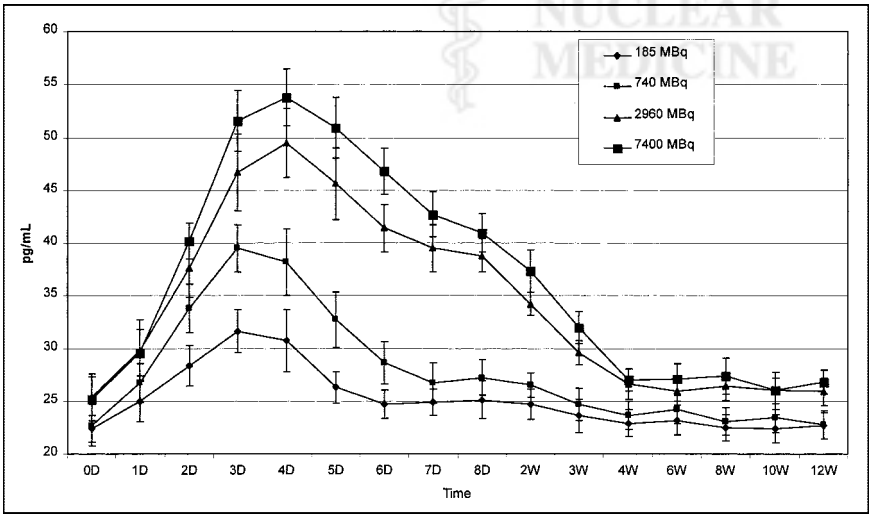

FIGURE 1. Kinetic of plasma 8-epi-$PGF_{2\alpha}$ after radioiodine therapy. Dose-dependent increase in 8-epi-$PGF_{2\alpha}$ is seen, with maximum at days 3 and 4 after therapy. Decline seems also to be dose dependent. D = day; W = week.

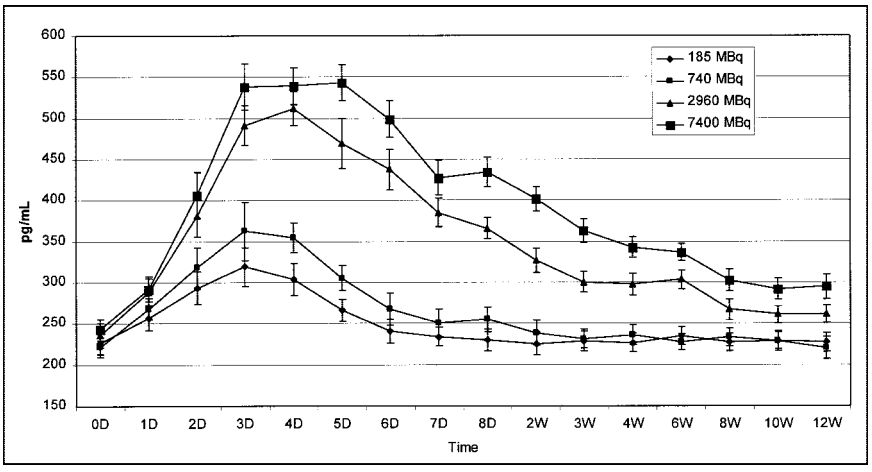

FIGURE 2. Kinetic of serum 8-epi-$PGF_{2\alpha}$ after radioiodine therapy. Dose-dependent increase in 8-epi-$PGF_{2\alpha}$ is seen, with maximum at days 3 and 4 after therapy. Decline seems also to be dose dependent. D = day; W = week.

Serum 8-Epi-$PGF_{2\alpha}$

In the 185-MBq treatment group, the levels increased until reaching a peak on days 3 and 4; the increase was not, however, statistically significant at any time, and the levels dropped to pretherapeutic values after 6 d (Fig. 2). After patients received a treatment activity of 740 MBq, their serum 8-epi-$PGF_{2\alpha}$ increased significantly on days 1, 2, 3, 4, and 5; reached the highest levels on days 3 and 4; and dropped to pretherapeutic levels after 2 wk. No statistically significant difference in levels of serum 8-epi-$PGF_{2\alpha}$ was found between the 740-MBq treatment group and the 185-MBq treatment group. After patients received an activity of 2,960 MBq, a significant increase of serum 8-epi-$PGF_{2\alpha}$ was observed from day 2 until week 6, with maximum levels occurring on days 3 and 4. The levels of serum 8-epi-$PGF_{2\alpha}$ on days 2, 3, 4, 5, 6, 7, and 8, and also after weeks 2, 3, 4, and 6, were significantly higher in the 2,960-MBq treatment group than in the 185-MBq treatment group. After patients received 7,400 MBq, we found significantly increased levels of plasma 8-epi-$PGF_{2\alpha}$ from day 2 through the rest of the observation period, with the highest levels occurring on days 4 and 5. The serum levels of 8-epi-$PGF_{2\alpha}$ after day 2 and until week 12 were significantly higher in the 7,400-MBq treatment group than in the 185-MBq treatment group.

Urinary 8-Epi-$PGF_{2\alpha}$

In the 185-MBq treatment group, the urinary 8-epi-$PGF_{2\alpha}$ levels increased until reaching a maximum on days 3 and 4 (Fig. 3). Afterward, the urinary 8-epi-$PGF_{2\alpha}$ decreased continuously and reached pretherapeutic levels after day 6, but none of the measured levels was statistically significant. After patients received an activity of 740 MBq, a significant increase of urinary 8-epi-$PGF_{2\alpha}$ levels was observed on days 3, 4, 5, 6, 7, and 8, with a maximum occurring on days 3 and 4. The urinary 8-epi-$PGF_{2\alpha}$ levels decreased after day 4 and reached pretreatment levels after 3 wk. No statistically significant difference in levels was found between the 740-MBq treatment group and the 185-MBq treatment group. In the 2,960-MBq treatment group, a significant increase was observed after days 1, 2, 3, 4, 5, 6, 7, and 8, as well as after weeks 2, 3, and 4, with the highest levels of urinary 8-epi-$PGF_{2\alpha}$ occurring on days 3 and 4. The urinary 8-epi-$PGF_{2\alpha}$ levels were significantly higher in the 2,960-MBq treatment group than in the 185-MBq treatment group from day 2

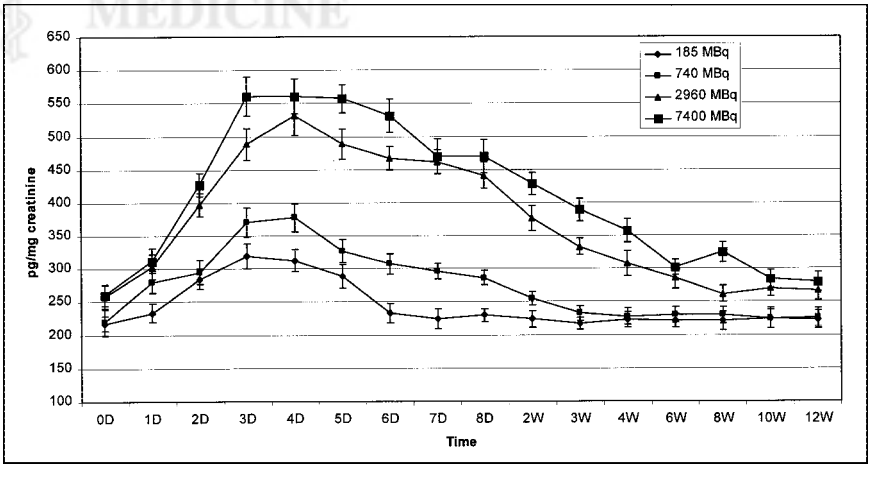

FIGURE 3. Kinetic of urinary 8-epi-$PGF_{2\alpha}$ after radioiodine therapy. Dose-dependent increase in 8-epi-$PGF_{2\alpha}$ is seen, with maximum at days 3 and 4 after therapy. Decline seems also to be dose dependent. D = day; W = week.

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through week 4. In the 7,400-MBq treatment group, we found a significant increase of urinary 8-epi-$PGF_{2\alpha}$ levels from day 1 through week 12, with the highest levels occurring on days 3 and 4. We found significantly higher urinary 8-epi-$PGF_{2\alpha}$ levels in the 7,400-MBq treatment group than in the 185-MBq treatment group on days 2, 3, 4, 5, 6, 7, and 8, as well as after weeks 2, 3, 4, and 6.

DISCUSSION

8-epi-$PGF_{2\alpha}$ belongs to a series of bioactive prostaglandin F_2–like compounds that are generated in vivo by free radical and LDL particle–mediated, nonenzymatic peroxidation of arachidonic acid (*13*). 8-epi-$PGF_{2\alpha}$ is mitogenic and a vasoconstrictor and displays a potential proaggregation effect on human platelets measured in plasma, serum, and urine. 8-epi-$PGF_{2\alpha}$ has been shown to be a reliable marker for oxidation injury and to increase in association with cigarette smoking (*14*), hypercholesterolemia (*12,15*), diabetes (*16,17*), and advanced age. The potential role of 8-epi-$PGF_{2\alpha}$ in radiation therapy and radioiodine therapy as a new, noninvasive method of assessing radiation injury has not been investigated so far. The increased levels of 8-epi-$PGF_{2\alpha}$ in plasma, serum, and urine in patients undergoing radioiodine therapy reflect the oxidative and possible radiation injury in these patients. In our study, we could demonstrate a dose-dependent increase of 8-epi-$PGF_{2\alpha}$ after radioiodine therapy. The isoprostane values showed a comparable behavior in all investigated compartments. Although in the 185-MBq group no statistically significant increase of 8-epi-$PGF_{2\alpha}$ could be found, an administered activity of 740 MBq led to significantly elevated values, further increasing with administration of 2,960 MBq and 7,400 MBq. In the last 2 groups, significance was found not only in comparisons with pretherapeutic levels but also in comparisons with levels after administration of 185-MBq activities. Furthermore, serum and urinary 8-epi-$PGF_{2\alpha}$ values showed a significant increase after treatment with an activity of 7,400 MBq, consequently dropping after a peak at days 4 and 5 and days 3 and 4, respectively, but not reaching pretherapeutic values after 12 wk of follow-up. The peak concentrations reached in plasma, serum, and urine are comparable with results obtained from recent studies investigating the increase of 8-epi-$PGF_{2\alpha}$ in cigarette smokers (*18*) and hyperlipoproteinemia patients (*12*). The somewhat elevated values of 8-epi-$PGF_{2\alpha}$ seen before therapy in patients receiving a high ^{131}I dose may be due to the athyrotic stage and the associated hyperlipidemia in these patients, who had mean cholesterol values at the time of therapy of 308 ± 36 and 322 ± 39 mg/dL, respectively. Our results indicate that ^{131}I therapy of the thyroid gland can induce oxidative stress in vivo and is not restricted to the area of administration but is detectable, in a dose-dependent manner, even in plasma, serum, and urine. These results further suggest that the increase of isoprostanes after radioiodine therapy is partially reversible at low activities. However, in patients receiving an ^{131}I activity of as high as 7,400 MBq, the values did not decrease to pretherapeutic values throughout the 12-wk follow-up period (as is analogous to observations in former heavy smokers, whose isoprostane levels dropped significantly after smoking cessation but never reached the values of healthy nonsmokers (*14*)). Long-term observations are therefore necessary. Alterations in circulating isoprostanes have been found in a variety of diseases. Isoprostanes, with their proaggregation (*19*), proliferative vasoconstriction (*12,20*), and mitogenic action, may favor impairing actions on various tissues.

Isoprostanes are established as reliable biomarkers for evaluating the extent of oxidative stress in atherosclerosis-associated diseases, including concomitant risk factors and patterns such as chronic lung disease (*21*). Isoprostanes have been detected in various tissues and body fluids such as blood (*22*) and lymph vessels (*20,23*). Measurement of 8-epi-$PGF_{2\alpha}$ levels in patients undergoing radioiodine therapy might reflect the extent of oxidative or, in this special case, radiation injury resulting from different forms of treatment, but further investigation is required.

CONCLUSION

The assessment of isoprostanes, especially 8-epi-$PGF_{2\alpha}$ levels, in various tissues and body fluids represents a new minimally invasive technique to evaluate the extent of oxidation injury in vivo. This method might allow monitoring of various treatment effects and evaluation of the possible benefits of using antioxidant therapy as an adjuvant to conventional treatment for minimizing therapy-related side effects.

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