(Iso) Prostaglandins in saliva indicate oxidation injury after radioiodine therapy

R.M. WOLFRAEM1, B. PALUMBO6, E. CHENE1, R. PALUMBO1, A.C. BUDENSKY4, H. SINZINGER3

1Departments of Angiology, University of Vienna, Austria; 2Institute of Nuclear Medicine, University of Perugia, Italy; 3Department of Nuclear Medicine, University of Vienna, Austria; 4Clinical Division of Oncology, Department of Internal Medicine I, University of Vienna, Austria.

Summary.—As salivary glands concentrate radioiodine the radiation injury associated with 131I-therapy may result in salialadenitis and xerostoma leading to a lasting impaired quality of life. Recently we reported about prostaglandin concentration changes as biochemical markers for radiation injury. Isoprostanes, a new family of prostaglandin-like compounds, have been demonstrated to be reliable markers for oxidation injury in vivo. Patients and methods: In this study we examined the levels of 8-epi-PGF2α, the major member of the isoprostane family in 24 patients undergoing 131I treatment in different doses for hyperthyroidism and differentiated thyroid cancer. 6 healthy sex and age-matched volunteers were monitored in parallel. Saliva (iso)prostaglandins were determined before 131I treatment, as well as 1, 3, 7, 14, 21, and 28 days, and 2, 3, and 6 months after therapy. Results: 8-epi-PGF2α showed a significant 131I dose-dependent temporary increase. The alterations were comparable in all investigated patients and significantly higher in cigarette smokers. TXB2 and 6-oxo-PGF2α showed a dose-dependent increase too. TXB2 was higher in cigarette smokers and 6-oxo-PGF2α, lower as compared to non-smokers. Conclusion: These results clearly demonstrate a dose- and time-dependent decrease (TXB2, 6-oxo-PGF2α) and oxidation injury (8-epi-PGF2α) after 131I-therapy in the salivary glands.

KEY WORDS: Isoprostanes. 8-epi-PGF2α. Oxidation injury. Lode radioisotopes therapy.

INTRODUCTION

Isoprostanes (IP) are prostaglandin-like compounds formed by a non-enzymatic, cyclooxygenase-independent process during lipid peroxidation, catalyzed by free oxygen radicals in-vivo1,2. IP levels, detected among others in human serum, plasma and urine, do reflect and are therefore a valuable indicator of oxidation injury1 in a variety of clinical conditions. IP, among these especially 8-epi-PGF2α, display a potent biological activity in contracting smooth muscle cells4, enhancing platelet activity2 and as acting as a proliferative and mitogenic compound. 6-oxo-PGF2 α is the stable derivative of PGI2. PGI2 increases intracellular cAMP and inhibits platelet activity. TXA2 measured via its degradation product TXB2 exhibits exactly the opposite biological properties. During ra-
dioiodine-therapy about 2% of the administered dose of $^{131}$I is absorbed by the salivary gland and unfortunately side effects such as sialoadenitis, as an acute, and xerostomia, as a long-term consequence of this treatment are common. In an earlier study we were able to demonstrate that approximately 2-3 months after $^{131}$I-therapy salivary prostaglandins were significantly affected. Kinetics immediately after radioiodine therapy and the role of isoprostanes in human saliva as a measure of in-vivo oxidation injury has not been the subject of scientific investigations so far. We performed this study in order to monitor the potential effects of different doses of $^{131}$I on the salivary (iso)prostanes during a 6-months period, under consideration of sex, age, cigarette smoking and the administered dose, respectively.

**MATERIALS AND METHODS**

**Patients and control group**

Seventeen patients who received $^{131}$I for the treatment of hyperthyroidism and 7 patients for the treatment of differentiated thyroid carcinoma were included in the study (for patients characteristics see table 1). A healthy control group of 6 volunteers receiving no medication and being free of any oral or salivary gland disease was examined in parallel. Each patient was treated once orally with $^{131}$I activities ranging from 148 – 4440 MBq. None of the patients had undergone any type of preventive dentistry program and/or had been taken any medication influencing the PG-system for at least 2 weeks. The patients with thyroid carcinoma had undergone total thyroidectomy prior to $^{131}$I-therapy and all of them were receiving thyroid hormone replacement. No patient had previously undergone chemo- or radiotherapy. Seven out of 24 patients and 2 out of controls were smokers (all smoking more than 10 cigarettes a day). Saliva was collected before $^{131}$I-therapy, 1, 3, 7, 14, 21 days as well as 1, 2, 3 and 6 months after therapy. Written informed consent was obtained from all patients and healthy volunteers prior to their inclusion in the study.

**Collection of saliva**

After rinsing the mouth with tap water, unstimulated whole saliva was collected into sterile tubes with the patient’s head tipped forward and the nose pointing to the floor using a procedure reported before. Saliva specimens were stored and frozen at $< -70 \, ^\circ \text{C}$ until analyzed. Tests were performed within 2 weeks of saliva collection.

**8-epi-PGF$_{2\alpha}$ measurement**

Samples were mixed with 2 % EDTA and 1 mg/ml (final volume) acetylsalicylic acid (ASA). Centrifugation at 4 °C was done at 1000xg for 10 minutes. The supernatant was removed and stored at $-70 \, ^\circ \text{C}$ for not longer than 2 weeks until determination. 8-epi-PGF$_{2\alpha}$ was determined using an enzyme immunoassay. The interassay variability was 5.5 ± 1.7 %, the intraassay variability 2.5 ± 0.7 %. Values are given in pg/ml. Normal value: $< 25 \, \text{pg/ml} \ (n = 11)$.

Artificial in-vitro formation of 8-epi-PGF$_{2\alpha}$ which easily could occur due to autooxidation of arachidonic- or other fatty acids was excluded by immediate determination of some samples (showing no difference to the respective controls).

**TXB$_2$ determination**

Samples were mixed with 2 % sodium EDTA under the addition of 100 mg acetylsalicylic acid (ASA) for 1 ml final volume for cyclooxygenase inhibition. Samples were centrifuged at 4 °C and 1500xg for 15 minutes. The cell free supernatant was stored for

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean dose</th>
<th>n</th>
<th>f</th>
<th>m</th>
<th>S</th>
<th>NS</th>
<th>Age range x ± SD</th>
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<td>A</td>
<td>148-185</td>
<td>11</td>
<td>6</td>
<td>5</td>
<td>3</td>
<td>8</td>
<td>36-65</td>
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<tr>
<td></td>
<td>157 ± 7.3</td>
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<td></td>
<td>53.4 ± 6.9</td>
</tr>
<tr>
<td>B</td>
<td>555-740</td>
<td>6</td>
<td>4</td>
<td>2</td>
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<td>4</td>
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<td>625 ± 28.2</td>
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<tr>
<td>C</td>
<td>2960-4440</td>
<td>7</td>
<td>4</td>
<td>3</td>
<td>2</td>
<td>5</td>
<td>35.6 ± 6.0</td>
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<td></td>
<td></td>
<td></td>
<td>53.9 ± 6.4</td>
</tr>
</tbody>
</table>

Dose in MBq; n: number of patients as control; f: female; m: males; S: smokers; NS: non-smokers; x ± SD: mean ± standard deviation; A + B: patients with hyperthyroidism; C: patients with thyroid carcinoma; D: healthy volunteers.
no longer than 2 weeks at −70 °C. Specific RIA was performed in unextracted samples using the double-antibody technique for separation of free and antibody bound ligand. The interassay variation was 5.6 ± 1.4 %, the intraassay variation 3.9 ± 1.1 %. Values are presented in pg/ml.

Radioimmunoassay for 6-oxo-PGF$_{1\alpha}$

100 µl aliquots were used for RIA. RIA was performed using a specific antibody without extraction. A double antibody was used for separation of free and antibody bound ligand. The values are given in pg/ml. The intra- and interassay variation was 4.6 ± 1.0 % (at 20 pg) and 6.8 ± 1.8 %. Values are given in pg/ml.

Statistical analysis

Values are presented as mean ± SD; statistical analysis was performed by means of ANOVA; p < 0.01 was considered significant.

RESULTS

6-oxo-PGF$_{1\alpha}$

Immediately after $^{131}$I-therapy there was a sudden increase in 6-oxo-PGF$_{1\alpha}$, reaching its maximum after 1 day already (figs. 1A–1D). After that the values continuously decreased until finally reaching the pre-value levels within less than 2 weeks, and further declin-
ing the following 2 months. In the control group no change throughout the entire observation period was found. The values in the non-smoking group were at almost all time points significantly higher as compared to smokers. The increase in 6-oxo-PGF_{1α} showed to be dose dependent, additionally as in the long-lasting depression in 6-oxo-PGF_{1α} after 1 month, subanalysis showed no sex or age dependency.

8-epi-PGF_{2α}

The isoprostanes exhibited a slower increase reaching a maximum at about 7 days after therapy (table 2). Thereafter, the levels slowly decreased and even after half a year values were (sometimes still significantly) above the pre-therapeutic ones. 8-epi-PGF_{2α} was higher in smokers and the increase was more pronounced with higher doses of ¹³¹I administered. Again no influence of age and sex in the sub- or total group was found.

TXB₂

TXB₂ showed the highest values 3 days after ¹³¹I-therapy after that it decreased significantly within 2 weeks (see table 3). During the 6 months follow-up no more significant change could be observed. Comparable to 6-oxo-PGF_{1α} and 8-epi-PGF_{2α}, an influence of administered dose and cigarette smoking but not of age and sex was observed.
Table 2
KINETICS OF 8-EPI-PGF2α AFTER RADIOIODINE THERAPY

<table>
<thead>
<tr>
<th>Group</th>
<th>P</th>
<th>1 D</th>
<th>3 D</th>
<th>7 D</th>
<th>14 D</th>
<th>21 D</th>
<th>28 D</th>
<th>2 M</th>
<th>3 M</th>
<th>6 M</th>
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<tbody>
<tr>
<td>A</td>
<td>57.6±5.3</td>
<td>60.8±6.7</td>
<td>89.7±10.3</td>
<td>147.8±12.3</td>
<td>126.3±10.7</td>
<td>106.8±12.3</td>
<td>110.2±15.8</td>
<td>93.8±14.6</td>
<td>84.7±12.2</td>
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<td>90.8±12.6</td>
<td>97.6±6.7</td>
<td>142.4±8.6</td>
<td>204.2±14.0</td>
<td>184.9±13.1</td>
<td>155.9±12.6</td>
<td>106.4±10.7</td>
<td>122.7±11.6</td>
<td>138.3±19.4</td>
<td>102.7±7.6</td>
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<tr>
<td>B</td>
<td>53.5±6.7</td>
<td>63.8±5.7</td>
<td>94.8±9.6</td>
<td>158.0±12.4</td>
<td>131.8±10.6</td>
<td>107.9±14.2</td>
<td>108.6±11.3</td>
<td>94.2±9.7</td>
<td>83.9±11.0</td>
<td>64.2±8.8</td>
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<td></td>
<td>94.2±7.7</td>
<td>147.8±6.7</td>
<td>208.8±13.2</td>
<td>191.6±16.7</td>
<td>165.5±18.4</td>
<td>160.3±22.1</td>
<td>125.9±10.3</td>
<td>124.8±16.4</td>
<td>117.3±12.2</td>
<td>NS</td>
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<tr>
<td>C</td>
<td>50.0±5.9</td>
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<td>97.9±5.9</td>
<td>164.9±12.3</td>
<td>140.2±8.6</td>
<td>167.9±10.3</td>
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<td>87.6±9.3</td>
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<td>208.5±14.7</td>
<td>171.8±15.3</td>
<td>162.4±21.5</td>
<td>151.8±10.7</td>
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<td>127.8±14.2</td>
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<tr>
<td>D</td>
<td>54.3±6.2</td>
<td>61.7±5.9</td>
<td>95.8±8.6</td>
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<td>87.8±5.3</td>
<td>90.0±11.0</td>
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</tbody>
</table>

Values are expressed as mean ± standard deviation.

*p < 0.01 (vs. prev.; P: pre(therapeutic) value; in subgroups of less than 3 no SD is given; D: day; M: months; for further abbreviations see table 1.

Table 3
KINETICS OF TXB2 AFTER RADIOIODINE THERAPY

<table>
<thead>
<tr>
<th>Group</th>
<th>P</th>
<th>1 D</th>
<th>3 D</th>
<th>7 D</th>
<th>14 D</th>
<th>21 D</th>
<th>28 D</th>
<th>2 M</th>
<th>3 M</th>
<th>6 M</th>
</tr>
</thead>
<tbody>
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<td>64.4±4.8</td>
<td>53.2±9.2</td>
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<td>37.8±4.7</td>
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<td>117.2±10.8</td>
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<td>64.3±9.5</td>
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<td>63.7±4.2</td>
<td>62.7±5.3</td>
<td>61.9±5.5</td>
<td>60.1±4.8</td>
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<tr>
<td>B</td>
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<td>77.3±5.6</td>
<td>63.9±5.1</td>
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<td>74.8±7.0</td>
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<td>89.4±4.2</td>
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<tr>
<td>D</td>
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<td>186.5±2.6</td>
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Values are expressed as mean ± standard deviation; abbreviations see tables 1 and 2.

DISCUSSION

PGs exhibit an active role in salivary gland metabolism. They actually play a key role in salivary gland function by stimulating salivary flow and secretion and by regulating electrolyte concentrations. Salivary glands are capable to concentrate radioiodine such as 131I within the epithelial cells of the intrabuccal ducts, the uptake of the latter significantly influencing PG levels in saliva. The quantitative and qualitative changes resulting from an increased absorbency of 131I include, among others, alterations in salivary fluid viscosity and pH, thus leading to an increased rate of cations and oral mucositis patients undergoing radioiodine therapy. Finally, due to reduced levels of PGE2, this may result in ulcerations of the oral mucosa. Sialoadenitis is present in about 11.5% of patients after 131I-therapy, generally manifesting within a week after therapy and its symptoms lasting between 3 weeks and 2.5 years. 2 patients in the high-dose group showed mild salivary symptoms. Their isoprostane values, however, were well among the ones of patients showing no symptoms. The ultimate consequence of radiation injury may be xerostomia, reflecting a non-reparable damage of the acinar cells.

Various data have indicated that cigarette smoking heavily influences the PG-system, therefore, we separately analyzed our data, dividing them into a smoking and a non smoking group. Respective analysis in saliva, have according to our knowledge, not been reported so far. In recent trials isoprostanes have shown to be involved in the pathogenesis of various diseases actually reflecting the extent of oxidative injury. A significant dose-dependent increase of 8-epi-PGF2α in plasma, serum and urine as a consequence of radioiodine therapy already has been reported in another group of patients. We were able to confirm these results by our own findings, as our data showed a severe influence of 131I-therapy upon salivary (iso)prostaglandins. After 131I-therapy 6-oxo-PGF2α, the stable
metabolite of PGs, suddenly increased followed by an instant continuous drop, these results confirming oth-
er previously published data reflecting oxidative injury in salivary glands as well as decreased 6-oxo-PGF
levels in vessels affected by atherosclerosis, compared to unaffected arteries.14 There was a 131I dose-
dependent decrease in 6-oxo-PGF1α levels and levels were found to be almost double in non-
smokers compared to smokers. Interestingly, values decreased within the follow-up period significantly
below the pre-therapeutic values, thus indicating an ir-
reversible damage of the salivary gland. Additionally
we were able to prove that radioiodine therapy lead to
an increase in 8-epi-PGF2α levels which was signifi-
cant as compared to pre-therapeutic values and com-
pared to healthy controls, however, no significant cor-
relation between the administered radioiodine dose
and 8-epi-PGF2α levels could be demonstrated within
all the investigated groups. The values obtained for
TXB2 showed a dose-dependent increase with a peak
after 3 days, followed by a continuous decrease with
in 2 weeks and remaining stable throughout the whole
follow-up period, its values again lasting significantly
above the pre-therapeutic ones being in correlation to
the administered dose. The values found for TXB2 and
6-oxo-PGF1α in saliva after 2 months are comparable to
the ones reported earlier.

Serum alpha amylase and tissue polypeptide anti-
gen were also assessed as markers for radiation injury
in the salivary gland. The highest values having been
found 1 day after therapy returning to original levels
after 3 days already. Again their increase was more
pronounced and longer lasting the higher the admin-
istered dose and the retention4. We do not have data on
these 2 markers from our patients. To our knowledge, there is only one report on 8-epi-PGF2α in saliva in
normotensive vs. preeclam-
ptic patients. These authors found normal control val-
ues of 150 ± 27 pg/ml.

Our data show that salivary (iso)prostaglandins are severely influenced by 131I-therapy, thus suggesting
them to be reliable biomarkers for the assessment of
the extent of radiation injury. Whether the extent in
saliva PG’s and oxidation injury is related to the later
clinical symptoms in patients, such as sialoadenitis
and xerostomia, still requires further investigations.

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