Thyroid problems in pediatric oncology: damage, prevention and consequences
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Citation for published version (APA):
van Santen, H. M. (2005). Thyroid problems in pediatric oncology: damage, prevention and consequences
Amsterdam

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Endocrine intervention during irradiation does not prevent damage to the thyroid gland

HM van Santen, JE van Dijk, H Rodermond, F Vansenne, E Endert, JJM de Vijlder, J Haveman, T Vulsma.
Abstract

PURPOSE Radiation to the head-neck region may damage the thyroid gland, leading to hypothyroidism or thyroid carcinoma. Outcomes of radiation protection by lowering plasma TSH have thus far been ambiguous. Our aim was to evaluate the radio-protective effect of inhibiting the thyroid gland's activity during X-radiation (XR).

METHODS Of 80 5-week old Wistar rats, 64 received 15 Gy XR (single dose). During XR, endocrine intervention was done, using thyroxine (T₄), T₄ & NaI or NaI alone, compared to placebo. During XR and follow-up, TSH and T₄ concentrations were measured periodically. Histological examination of thyroid, pituitary gland or the hypothalamus and any suspect lymph nodes, lungs and liver was performed after 6 and 54 weeks.

RESULTS Due to the endocrine intervention, plasma levels of TSH were lower in rats given T₄, and higher in rats given NaI, during XR. After 6 and 54 weeks, no significant reduction in hypothyroidism or thyroid carcinoma was found between the different groups of rats given any endocrine intervention or no intervention. A significantly higher number of adenoma was found in rats given NaI during XR after 54 weeks.

CONCLUSIONS The administration of T₄, NaI or the combination during X-irradiation does not protect against radiation-induced thyroid damage.
Introduction
Thyroid damage as late adverse event after head/neck irradiation has been extensively reported, which can result in thyroid dysshormonogenesis or dysmorphogenesis. The latency time between radiation exposure and the clinical, biochemical or morphological expression of thyroid damage can vary from several months to many years. Due to awareness of these late effects, the use of radiotherapy is limited whenever possible. Other preventive actions are reducing the total dose, (hyper) fractionation, reducing the size of the radiation-field and placing protective lead blocks. Because radiotherapy remains indispensable in the treatment of childhood cancer, prevention of thyroid damage must be investigated by alternative ways, for example by endocrine intervention. In animals as well as in humans, radiation effects on thyroid tissue are dose-dependent. The occurrence of hypothyroidism increases linearly with increasing radiation dose; in rats after doses of 12-20 Gy, a reduction of goitrogenic growth is seen, indicating loss of thyroid cells, and in children hypothyroidism is frequent after doses exceeding 25 Gy. For carcinoma, in children, the risk increases from 0.1 Gy, and levels off after 10 Gy (in children irradiated for benign diseases) or after 60 Gy (children with malignancies). In rats, the dose-response curve for radiation-induced thyroid tumors has its maximum after about 11 Gy of X-rays. Next to radiation dose, risk factors are a young age and a proliferative state of the thyrocytes.

A major role contributing to the pathogenesis of thyroid tumors has been ascribed to the level of plasma thyroid stimulating hormone (TSH). It has been demonstrated, in rats, that radiation-susceptibility of the thyroid is increased when TSH during radiation is increased. In contrast, after hypophysectomy no thyroid tumors developed at all, suggesting that the absence of TSH prevents against the development of thyroid tumors. TSH can also be suppressed by the administration of thyroxine (T4). Koritnik demonstrated, in rats, that administration of T4 during irradiation resulted in a lower incidence of thyroid tumors. However, data of 9 out of 20 animals in the T4 group were lacking and no results on thyroid function were given. In humans, the administration of T4 during exposure to X-radiation did not prevent hypothyroidism. However, in this study it was impossible to say anything about the occurrence of thyroid tumors, because the follow-up time was too short. Considering these partially
contradictive results and the fact that the studies on endocrine intervention to prevent X-radiation induced thyroid damage are limited, our aim was to evaluate different endocrine preventive strategies in a pre-clinical in vivo model.

For this study, we postulated three ways of endocrine prevention. The first is the administration of T₄ to suppress TSH levels. As clinical hyperthyroidism is not a favorable state in children with malignant diseases, however, our aim would be to lower, but not suppress TSH during irradiation. The second way is the administration of high doses of (stable) iodide, which inhibit the thyroid's activity (also known as the Wolff-Chaikoff effect) and might make the thyroid tissue less radiosensitive. The third way is the administration of high doses of (cold) iodide with simultaneous administration of T₄. The acute phase of the Wolff-Chaikoff effect might be radio-protective, but will result in a sudden fall of the plasma T₄ concentration due to inhibition of the iodide organification. Due to this decrease in plasma T₄, the level of TSH will increase, which will counteract the protective effect. This rise in TSH can be prevented by simultaneous administration of T₄.

As both hypothyroidism and thyroid carcinoma are important late effects of radiation treatment in clinical practice, a rat model was chosen in which both these late effects, with serial plasma hormone measurements and evaluation of thyroid tissue could be evaluated.

Materials and Methods

Animals and radiation model

For the experiments, 80 Wistar strain rats, all male, were used. All experiments were approved by the Animal Ethical Committee of the Academic Medical Center (AMC) and were performed according to the Dutch Law for animal experiments.

The animals arrived at four weeks of age. After a week of acclimatization, the experiments were started. Rats were divided into 5 groups of 16 rats each (figure 1), and were housed in groups with tap water and food ad libidum (Hope Farms, irradiated breeding and maintenance diet for rats, mice and hamsters, Woerden, The Netherlands). Mean room temperature was 22 °C, relative air humidity 55-60 %, animals were subjected to a 12-h light: 12-h dark cycle. Each rat was given an earmark.

When the rats were six weeks of age, the irradiation was given. During the irradiation experiments, animals were anesthetized by halothane inhalation. Short anesthesia was
performed by giving 4% fluothane and 96% air. Longer anesthesia was performed by giving 4% fluothane and 96% air, followed by a maintenance dose of 2% fluothane and 98% air.

Group I received sham irradiation, group II-V received 15 Gray (Gy), single dose. Irradiation was given from the ventral side with an X-ray generator (Siemens stabiliplan 2-machine) operated at 250 kV, filtered with 0.5 mm Cu. Focus to skin distance was 40 cm. Radiation field was marked by light, with the center point being the anatomical site of the thyroid gland, the upper boundary being the lower boundary of the skull/middle ear, just below the pituitary region \(^{25}\) (determined by marking the middle ear boundary by X-ray in a separate Wistar rat). Field size was 2 by 3 cm. Animals were situated on their back, paws were spread and fixed. Time-schedules for irradiation with 15 Gy was 10 minutes and 49 seconds. Dosing speed was 138.60 cGy per minute.

Six weeks after irradiation, half of each group was sacrificed. All other rats were sacrificed 54 weeks after irradiation.

Figure 1. Study design: All rats were given daily injections intra-peritoneal. If no endocrine intervention was given, an injections was given with placebo (saline/ NaCl 0.9%\(^{30}\)). After 1 week of injection, X-Radiation (XR) was given. \(^1\)T\(_4\) administration was given daily, starting one week before XR. \(^2\)Na\(_1\) administration was given daily, starting one day before XR. \(^3\)T\(_4\) administration was given daily, until two weeks after XR. \(^4\)Na\(_1\) administration was given daily, until one week after XR. Fup= follow-up time.
Endocrine interventions (figure 1)

All rats were given daily intraperitoneal injections, (0.004 ml per gram body-mass) starting one week before until two weeks after irradiation, alternating on the right and left side of the abdomen. Groups I and II received placebo (NaCl 0.9 %). Group III was given injections with 20 μg T₄/100 g per day during 3 weeks. Group IV received 20 μg T₄/100 g per day, also starting one week before irradiation until two weeks thereafter, together with NaI (10 mg ¹²¹I⁻ per day) during 1 week, starting the day before irradiation. Group V received NaI, for 7 days, starting the day before irradiation. On the days that rats of group V did not receive NaI, sham-injections were given with saline, so that all rats received injections during a period of three weeks.

Clinical examination

All rats were scored daily by examination of body-mass, activity, fur (shiny/ fade/ hairs upright) and skin (loss of hair/redness/wounds) condition at the irradiation site.

Thyroid function

In the first 6 weeks, weekly blood samples were collected for measurement of plasma T₄ and TSH concentrations. From six weeks after irradiation, these determinants were measured monthly. At sacrifice after 54 weeks, plasma TSH, T₄ and T₃ were measured. T₄ and T₃ were determined by an in-house radio immuno-assay, and TSH by a chemiluminescent immuno-assay adapted for rat TSH (Immumite, Diagnostic Products Corp., Los Angeles, CA). Intra-assay variation T₄ 2 - 4 %, T₃ 3 - 4 %, and TSH 2 - 4 %. Inter-assay variation T₄ 3 - 6 %, T₃ 7 - 8 %, and TSH 3 - 4 %. Detection limits: T₄ 5 nmol/L, T₃ 0.3 nmol/L, and TSH 0.01 mU/L.

Histological examination

At sacrifice, thyroid gland, pituitary gland, hypothalamus, lungs, liver and thymus were removed and stored in a buffered 4 % formaldehyde solution (40 mmol/L, pH 6.8-7.2) for histological evaluation of radiation effects, tumor growth and presence of metastases. Any suspicious lymph nodes were taken out and histologically examined. Both thyroid lobes were removed en bloc together with the adjacent trachea to prevent surgical damage of the tissue. Routinely paraffin-embedded, HE and PAS (for
glycoproteins) stained sections were examined for irradiation effects. All examinations were done by a blinded veterinary pathologist.

The histological classification score for activity of thyroid glands was based on the predominant aspects of four criteria (follicular size, colloid density, cell height and the number of desquamated cells in the follicular lumen) in two histological sections, all scores ranging from 1 to 5 (the scores are described in more detail together with assessment of inter-observer reliability in chapter 5). From the follicular size, colloid density, and cell height an activity-index was calculated, being the sum of these scores and reflecting hyperplasia and hypertrophy of thyroid cells. A high index indicates active cell metabolism and a high protein turnover. A low index represents resting thyroid tissue glands with a lot of thyroglobulin piled up in the follicles.

Also, the presence of follicular irregularity, hyperplasia, adenoma, cysts, carcinoma and thyroiditis was evaluated.

Histological classification of the pituitary glands was based on four criteria (high power field, objective 40 x): presence of ‘colloid droplets’, number of basophilic cells and the presence of pycnotic nuclei in the anterior pituitary gland, and the aspect of status spongiosus in the posterior pituitary gland (described in more detail in chapter 5).

Statistical analysis
Data were analyzed using MS Excel ‘00 and SPSS 11.5.1 Statistics UK software. Differences between group I against all other groups were tested, and the differences between group II and the other groups which received different kinds of thyroid protection (group III-V), and the differences between the three groups was tested. Statistical analysis for numeric data was performed using the Student's t-test (parametric) and Mann Whitney U, 2-independent test (non-parametric data). For nominal data, statistics were performed using Chi-square tests. Follow-up data on multiple thyroid function determinants were analyzed using LSD, repeated measures. The level of statistical significance was set at p<0.05.
Results
Thyroid function before X-irradiation (XR)
Mean plasma concentration of TSH of all animals one week before XR was 1.1 mU/L (range 0.7-2.7) and of $T_4$ it was 62 nmol/L (range 48-83).

Concentrations of plasma TSH and $T_4$ during endocrine interventions
Mean concentrations of plasma TSH and $T_4$ in the 5 different groups during the endocrine interventions and exposure to irradiation are shown in table 1. Due to injections with $T_4$, a significant decrease in mean TSH concentration is found in group III compared to group I ($p = 0.001$) and compared to group II ($p=0.05$). In both groups that were given $T_4$, significant increases in mean $T_4$ concentrations were found (both $p < 0.000$). In group V, a significant increase of TSH was observed ($p < 0.000$), with a significant decrease of $T_4$ ($p < 0.000$) compared to groups I and II.

Table 1. Mean concentrations of TSH and $T_4$ during the endocrine interventions, at the moment of (sham)-irradiation with 15 Gy.

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean TSH mU/L (range)</th>
<th>$T_4$ nmol/L (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>1.3 (0.9-2.1)</td>
<td>67 (55-89)</td>
</tr>
<tr>
<td>II</td>
<td>1.2 (0.6-2.0)</td>
<td>65 (50-91)</td>
</tr>
<tr>
<td>III</td>
<td>0.9 (0.6-1.4) *</td>
<td>142 (81-201) **</td>
</tr>
<tr>
<td>IV</td>
<td>1.1 (0.7-1.9)</td>
<td>149 (99-210) **</td>
</tr>
<tr>
<td>V</td>
<td>4.0 (2.0-7.1) **</td>
<td>41 (30-52) **</td>
</tr>
</tbody>
</table>

During the weeks of endocrine interventions following the irradiation, the mean concentration of TSH stayed significantly lower in group III compared to group II, but was not suppressed (figure 2). Also in the rats of group IV (compared to group II) in the weeks following radiation, during the endocrine intervention significant higher levels of $T_4$ are found, with significant lower levels of TSH.

Clinical and macroscopic morphological findings during follow-up
The mean weight of all rats at 5 weeks of age was 132 grams (range 109-199). Rats in group I had the lowest mean body-mass (mean 123 g) and group V the highest (mean 140 g). These differences were not significant. After 6 weeks and 1 year, significant differences in body-mass were found between group I and groups II to V (mean weight gain after 6 weeks 202 and 144 g, and after 1 year 405 and 327 g resp.), but not
between groups II, III, IV or V.

During follow-up of one year, all sham-irradiated rats of group I remained healthy. In the other groups, the following clinical signs or macroscopic abnormalities were observed:

Group II: one was panting at sacrifice, one had developed a cornifying epithelioma on the back of the neck and died 1 month before the end of the study-period due to a large and aggressive fibrosarcoma growing around the esophagus and trachea and with lung metastases; in two animals the thyroid gland was palpably enlarged, in 1 rat multiple suspect (slightly enlarged) lymph nodes were found at sacrifice (microscopically normal).

Group III: one rat (of the short term group) was lost during an anesthesia procedure; another rat died 4 days before the end of the study-period and showed, at obdution, a large tumor in the neck (most probably fibrosarcoma) and lung bleeding. No further thyroid histology could be obtained due to autolysis.

Group IV: in two rats large tumors were palpable in the neck, one of which had grown into the skin. Yet the animals did not show signs of illness. A 3rd rat had a tumor on the right shoulder. In 2, the tumors appeared to be mamma carcinoma and one was a large malignant fibrous histiocytoma.

Group V: one rat had severe body-mass loss and appeared ill at sacrifice (no microscopic explanation found, in according to protocol selected samples).
Thyroid function

Thyroid function 6 weeks after radiation exposure

In figure 2, the mean plasma concentrations of TSH are shown during the first 8 weeks of the experiment. The mean concentration of TSH of group I varies from 1.3 (1 week before sham-XR) to 1.5 mU/L (6 weeks after sham-XR). In group II, a significant increase in plasma TSH was seen, from the 3rd week onwards, to 4.8 mU/L at six weeks (p<0.000). The mean concentration of T4 in group II is significantly lower in the 1st week and from the 3rd week onwards when compared to the sham-irradiated animals (p<0.000) (data not shown).

After stopping the endocrine interventions, TSH increased in all irradiated groups (maximum value 13.3 mU/L) with a fall of plasma T4 (minimum value 42 mmol/L). In the groups given T4, the rise in TSH appeared to be slower than in the other groups, although this was not statistically significant. Six weeks after radiation exposure (4 weeks after stopping the endocrine intervention), no significant differences were found between the mean concentration of TSH and of T4 of groups II, III, IV or V.

![Figure 3. Mean concentration of plasma TSH, T4 and T3, 1 year after irradiation in various groups](image)

* = p ≤ 0.05, ** = p ≤ 0.001
Group I: no XR, group II: XR + saline, group III: XR + T4, group IV: XR + T4 + Nal, group V: XR + Nal

Thyroid function after 1 year

No significant differences were found in total mean concentration of TSH during the year of follow-up between groups II and III, IV or V.

At 54 weeks after exposure to radiation, the mean concentration of TSH of group I, II, III, IV, V were 1.2, 9.1, 16.2, 14.4, 17.0 mU/L respectively; mean T3 values were 0.56, 0.34, 0.26, 0.20 and 0.31 mmol/L respectively, and mean concentrations of T4 were 39,
27, 22, 16 and 23 mmol/L respectively (figure 3). A significant increase in TSH and a significant decrease in T₃ and T₄ were found between group I and all other groups (all p-values < 0.004). No significant differences were found in plasma TSH concentration between group II and the groups given T₄, NaI or the combination. Significant lower T₄ and T₃ concentrations were found in group III & IV compared to group II.

Histological evaluation of thyroid tissue

Thyroid histology after 6 weeks

In the thyroid glands, 6 weeks after radiation exposure, no hyperplasia, adenoma, carcinoma or thyroiditis were observed. Between group I and the other groups, significant differences were found in colloid density for all groups and in the activity index for groups II, IV and V. No significant difference was found in the activity index for group I and group III.

No significant differences were found between the irradiated groups in any of the histologic classification points.

Thyroid histology after 1 year

Regarding the cell activity indices of the thyroid, only significant differences were found between the sham irradiated rats and each of the other groups. No differences were found between the irradiated groups given saline or any kind of endocrine intervention. Also, no significant differences were found between numbers of desquamated cells.

In group I no follicular size irregularity, hyperplasia, adenoma, carcinoma, or any other malignancies were found, which was significantly different from all other groups regarding regularity in follicular size and presence of hyperplasia (table 2). This was also significantly different regarding adenoma compared to group II and V, but not to group III and IV. The number of adenomas in group V was also significantly higher than in group IV (p=0.039).

In the irradiated rats, 3 thyroid carcinomas were found, each in a group with a different endocrine intervention. None of these 3 rats had metastases to the lungs, cervical lymph nodes or liver. Furthermore, in 18 irradiated thyroid glands at least one lesion with hyperplasia was found, in 11 at least one 1 adenoma, in 1 a thyroid cyst, and in 1
Figure 4. Morphology of thyroid tissue one year after irradiation with 15 Gy
A: Normal thyroid gland, activity index low (3), obj. 20 x, B: Thyroid gland after irradiation: high activity index; PAS staining pale due to low density colloid, obj. 20 x, C: Thyroid hyperplasia, obj. 10 x, D: Thyroid adenoma with thin capsule and some compression, obj. 10 x, E: Thyroid carcinoma, solid trabecular growth, obj. 10 x, F: Thyroid carcinoma, not encapsulated, partly follicular, partly solid, obj.10 x

lymphocytic infiltration (sign of thyroiditis). Only in 2 irradiated animals (5 %) no thyroid abnormalities were found.

No correlation was found between plasma TSH at radiation and the concentration of TSH, T₄ and/or T₃ levels at 6 weeks and 54 weeks after irradiation. No correlation was found between TSH concentration during radiation and the activity index of thyrocytes or the development of structural thyroid abnormalities after 54 weeks.
Table 2. Induction of structural thyroid abnormalities one year after radiation with 15 Gy and different kinds of endocrine intervention.

<table>
<thead>
<tr>
<th>Group</th>
<th>Irregular follicular size (n)</th>
<th>Hyperplasia (n)</th>
<th>Adenoma (n)</th>
<th>Cyst (n)</th>
<th>Carcinoma (n)</th>
<th>Thyroiditis (n)</th>
<th>No thyroid abnormalities ( ^{1} ) (n)</th>
<th>Other Malignancy (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>0 ((*; all))</td>
<td>0 ((*; all))</td>
<td>0 ((*; all))</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>8 ( ^{*} )</td>
<td>0</td>
</tr>
<tr>
<td>II</td>
<td>7</td>
<td>4</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1 ( ^{1/2} )</td>
</tr>
<tr>
<td>III</td>
<td>5</td>
<td>5</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0 ( ^{2/2} )</td>
</tr>
<tr>
<td>IV</td>
<td>7</td>
<td>5</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>3 ( ^{2/2} )</td>
</tr>
<tr>
<td>V</td>
<td>7</td>
<td>5</td>
<td>11</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Sum: 

\( N=38 \) 26 19 11 3 1 10 5 \( ^{2/2} \)

XR: radiation, \( T_{4} \): thyroxine, NaI: sodium-iodide, +: with, -: without. \(^{1}\) No thyroid abnormalities, indicating no irregular follicle size, adenoma, cysts, carcinoma of thyroiditis. \(^{2}\): fibrosarcoma, \(^{2/2}\): in one rat of whose thyroid microscopy is missing a large tumor around the aorta was found, probably fibrosarcoma. \(^{2/2}\): two mamma carcinomas and one malignant fibrous histiocytoma (MFH). \( ^{*} p < 0.05 \) (Chi-square)

Pituitary glands and hypothalamus

Histological analysis of pituitary glands 6 weeks after irradiation showed no significant differences in presence of colloid droplets or number of pyknotic nuclei (p=0.112 and p=0.778 respectively). A significantly higher amount of basophilic cells was seen in the irradiated animals (1.5 and 2.2, p=0.038). Neither abnormalities in the pars intermedia nor status spongiosus in the posterior pituitary lobe were found.

Of the long term animals, none of the pituitary glands could be evaluated as, by mistake, the brains without pituitary glands were sampled at sacrifice. For this reason, the hypothalamus and its vasculature were evaluated. No signs of radiation damage were observed.

Discussion

Exposure to X-radiation in rats, 6 weeks of age, with 15 Gy single dose, leads to hypothyroidism, follicular irregularity, thyrocyte hyperplasia and adenoma of the thyroid gland. We could not confirm earlier results that lowering TSH during exposure to radiation prevents hypothyroidism or the induction of thyroid neoplasms. In contrast, 54 weeks after radiation exposure, 3 thyroid carcinomas were observed in all three groups that were given an endocrine intervention.

The first question is whether the dose of XR that we used was correct. Too high a dose may damage all cells, blunting the occurrence of malignancies. In contrast, too
low a dose may blunt the occurrence of hypothyroidism. An attempt was made to develop a model which can be linked to the radiation doses that are used in the clinical pediatric setting after which both hypothyroidism and neoplasms are observed. One year after exposure to 15 Gy, it is seen that histologically and biochemically the rat thyroid glands were not ablated indicating that this radiation dose was appropriate for studies on hypothyroidism. No carcinoma developed in group II. This is however, not probably caused by too high a dose as no severe damage of the thyroid cells was found at 6 weeks. Considering the fact that neoplasms did develop in the other groups, also the follow-up time seems adequate.

A second question is, whether the suppression of plasma TSH was sufficient. As we did not aim to induce clinical hyperthyroidism, because this is not a favorable state in children with malignant diseases, we did not suppress TSH levels completely. With a T<sub>4</sub> dose of 20 µg/100 g per day, we achieved more than doubling of the plasma concentration of T<sub>4</sub>, with a significantly lower mean TSH concentration, but maintaining clinically euthyroid rats (subclinical hyperthyroidism). Apparently, inducing subclinical hyperthyroidism does not prevent against radiation-damage.

A third question is whether stray irradiation to the pituitary gland may have resulted in such an impairment in TSH secretion, that all rats experienced comparable (TSH-related) thyroid radiation effects. However, considering our results in which only small and mainly secondary changes were seen in the basophilic cells (hypertrophy and hyperplasia related to hypothyroidism) and the lack of changes in the hypothalamic region, there is no evidence for pituitary radiation damage.

It has been suggested that it is possible to prevent the occurrence of thyroid malignancies after irradiation by life-long administration of thyroxine. In humans, a decrease in palpable thyroid nodules was demonstrated. No evidence has been provided regarding the prevention of thyroid malignancies after irradiation, though. In our opinion, the development of a thyroid malignancy is the most severe late effect of radiation on the thyroid gland for the patient and also most important for medical care. In case of a very high risk to develop a thyroid carcinoma, a preventive strategy that one could consider is to remove the thyroid gland prophylactically (surgical or with radio-iodide). This would also imply life-long thyroxine supplementation, but
then with the absolute certainty not to develop a thyroid malignancy. Supplementation of thyroxine to children without a thyroid is very well feasible: experience has been gained from children born without a thyroid gland (agenesis) and in children with the syndrome MEN-2A, whose thyroid is prophylactically removed for prevention of medullary thyroid carcinoma. For these reasons, the preventive strategy to administer life-long T\textsubscript{4} and still a risk to develop a thyroid carcinoma is, in our opinion, not an option and we did not include such a study-group.

Of special attention is the fact that the levels of T\textsubscript{3} are decreased with about 50 % after 1 year (figure 3). Even in moderate hypothyroidism, normally levels of T\textsubscript{3} are maintained within the normal values for a long time. Considering the fact that the animals were, clinically, not deeply hypothyroid, other reasons for the lower T\textsubscript{3} must be considered. An explanation could be the fact that these irradiated animals (group II-V) were in a worse clinical condition than group I. A bad clinical condition can result in low T\textsubscript{3} levels due to an inhibition of the peripheral conversion of T\textsubscript{4} (in combination with a low plasma TSH), which is called the syndrome of non-thyroidal illness (NTI). Our results might fit with the diagnosis thyroidal hypothyroidism in combination with non-thyroidal illness (NTI), explaining both the increase in TSH with the combination of a low T\textsubscript{3} and T\textsubscript{4}.

Next to changes in histology, also chromosome aberrations and RET/PTC rearrangements have been described to occur after radiation exposure\textsuperscript{27,28}. We did not include molecular genetic studies for two reasons. Firstly, we wanted to examine to entire gland for microscopy, because a local histological aberration could be present in just one thyroid lobe. Secondly, although an increase of mainly RET/PTC-1 rearrangements has been shown after external radiation\textsuperscript{29,30} and of RET/PTC-3 in the Chernobyl tumors\textsuperscript{27,28}, this could not be confirmed by others\textsuperscript{31,32}. So, although scientifically very challenging, these genetic rearrangements are not directly clinically useful for studies of prevention.

A comment may be made on the fact that carcinoma developed in the groups with (slightly) lower TSH during XR. This may suggest that our endocrine interventions have “lowered” the threshold for radiation-exposure resulting in an increase of neoplasms and a decrease of hypothyroidism (figure 3). If this is true, it would mean
that endocrine intervention during irradiation (in rats but probably also in children) is more disadvantageous than protective: inducing carcinoma instead of hypothyroidism. The results of our study indicate that alternative ways than the administration of T₄ or Na-I should be searched for to prevent the thyroid against X-radiation. If the assumption is made that the presence of TSH is required for the development of thyroid tumors, an alternative way to block the action of TSH is the administration of TSH-antagonists. These compounds are mainly designated for the treatment of Graves’ disease and TSH-secreting pituitary adenoma, but its possible radio-protective abilities should also be examined in animal models. However, it must also be considered that TSH is not the only factor responsible for the thyroid tumor development. In a study performed on the relation between iodine-(in)sufficiency and tumor development after external radiation in rats also no relation was found between the level of TSH and adenoma formation. Insulin and Insulin-like growth factor (IGF) may be very important for tumor growth. As also growth hormone is withdrawn by hypophysectomy, this might explain why hypophysectomy is a more effective protection for the development of thyroid tumors than the administration of T₄.

Another, non-endocrine, pharmacological radio-protective intervention that may be considered is the administration of free radical scavengers. An example is amifostine that has been shown to protect the salivary glands during X-radiation. No data on its protective action on the thyroid have been reported yet. Also, other anti-oxidants or free radical scavengers of which previous results indicate that they might be radio-protective for the thyroid gland, such as selenium, vitamin E, C or S-phenethyl formamidino 4 (N-ethyl isothiamide) morpholine dihydrochloride, a sulfur-containing compound, should be further evaluated in animal studies. However, an important aspect that must be guarded, when protecting the thyroid from radiation with these non-thyroid specific compounds, is that the malignancy itself is not protected against the irradiation.

We could confirm the fact that an increased level of TSH during exposure to irradiation leads to an increase in thyroid radiation damage in the group of rats that received NaI only. The rise in TSH during ¹²³I administration can be explained by the fall in T₄ reflecting the acute Wolff-Chaikoff effect. In general, however, due to the escape
mechanism, the production of $T_4$ will quickly be restored and TSH and $T_4$ levels will not be disturbed during exposure to excess iodine $^{43}$. This short elevation of TSH during the administration of high doses of iodide can be explained by a failing escape mechanism (more likely to be present in very young children or in the elderly). This phenomenon of a failing escape-mechanism, together with the subsequently increased occurrence in adenoma, should be a point of attention for those who administer high doses of iodide for thyroid protection against radio-iodide to young children $^{44}$.

In conclusion, endocrine inhibition of the thyroid's metabolic activity with the administration of $T_4$, NaI or both during X-radiation does not prevent radiation induced function loss or structural abnormalities of the thyroid gland in young Wistar rats. This implies that the current strategies undertaken to minimize radiation-exposure to the thyroid gland must be continued (hyperfractionation, reduction of total radiation dose and field, and applying lead shields) until novel preventive strategies evolve.

**Acknowledgments**

This work was partly funded by Pfizer BV. and the Stichting Kindergeneeskundig Kankeronderzoek (SKK).
References


