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The role of thyroxin in thyroid radiation carcinogenesis in rats

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The aim of this study was to test the hypothesis on the protective role of thyroxin administration before and during irradiation on the occurrence of thyroid carcinoma in rats. Application of thyroxin before and during irradiation was expected to decrease production of thyrotropin by the hypophyseal feedback mechanism, caused by radiation damage of thyroid tissue. Stabilizing the thyroid cells in this way during irradiation would thus make them less radiosensitive.

In the experiment, we first divided 81 three to four week old Wistar strain rats of both sexes into two groups, i. e. thyroxin (T_4) and water (H_2O). The T4 rats were injected 1% thyroxin solution (0.01 mg / 100 g body weight) twice a day for 15 days, while the H_2O rats received saline in the same way. After ten days, the two main groups were divided each into two subgroups. The rats from both irradiated subgroups (T_4/X and (H_2O/X) received 10 Gy to the neck area. They were irradiated with a telecobalt machine for five consecutive days with one direct field. During a two years follow - up, all moribund animals were sacrificed and their thyroid glands taken. The rest of the thyroid glands were taken at the end of the experiment. All glands were pathohistologically analysed. Besides, all suspicious and enlarged extrathyroid organs and tissues were examined and the occurrence of tumors was noted. Pathohistological examination revealed the occurrence of 8 thyroid carcinomas and 7 adenomas in the H_2O/X group, and 3 adenomas in the T_4/X group. In the irradiated group of rats without thyroxin, significantly ($P = 0.01$) more thyroid carcinomas occurred than in the irradiated group without thyroxin.

The experiment confirmed the hypothesis about a protective role of thyroxin administration before and during the irradiation in postirradiation thyroid carcinogenesis in rats.

Key words: neoplasms, radiation induced; thyroid neoplasms; thyroxine; rats

Introduction

The carcinogenic effect of ionizing radiation in human thyroid gland has been well established.[1,2] Thyroid cancer was the first solid tumor that showed an increased incidence among the Japanese A-bomb survivors,[3] and a great increase of thyroid carcinoma has been recently reported among the children exposed to nuclear fallout in the areas around Chernobyl.[4] Long ago, the data implicating radiation as an etiologic factor in thyroid cancer rendered the practice of irradiat-

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ing benign childhood disorders obsolete,[5] but in radiotherapy of malignant tumors in the head and neck region, the exposure of the thyroid gland usually cannot be avoided. Subclinical or overt hypothyroidism, diffuse thyroid enlargement and benign nodules were frequently observed in patients after the irradiation of the thyroid region for Hodgkin's disease,[6] and most outsatnding was the occurrence of thyroid carcinoma. The relative risk of thyroid cancer after irradiation for Hodgkin's disease was 15.6%.[7] In the study of late effects after radiotherapy for childhood malignancies, the relative risk for secondary thyroid cancer was 53%.[8] Careful evaluation of thyroid status is required in all patients who were irradiated in the neck region and, in cases of elevated TSH level, substitutional therapy with thyroxin is recommended.[9]

Thyroid tumours can also be induced in experimental animals by the administration of radioactive iodine or external radiation. The induction of thyroid carcinoma by exposure to X-rays in rodents was first described by Frantz *et al.*[10] Lindsay and Sheline[11] studied the development of thyroid tumors in rats after the glands had been irradiated with 5, 10 and 20 Gy. Benign nodules and adenomas were frequently observed in irradiated rats and appeared to have originated as foci of nodular regeneration and hyperplasia. Thyroid carcinomas occurred in 22 - 45% of irradiated animals and apparently arised from pre-existing benign adenomas. Similar incidence of thyroid carcinomas in rats after external irradiation was reported from experiments on laboratory animals by Christov[12] and Lee *et al.* [13]

Clinical evidence that TSH has an important role in thyroid carcinogenesis was clearly confirmed by experimental studies. If the irradiation was followed by long-term goitrogen treatment, the yield of tumours was higher[14] and; on the other hand, if animals were treated with thyroxin after the irradiation, no thyroid tumours occurred.[15] In the experiments on tissue cultures, the frequency of cancer expression from initiated thyroid cell was greatly increased by certain chemicals, growth factors and hormones associated with elevated TSH level.[16-19] It is suggested that, in addition to radiation, elevated TSH promotes carcinogenesis by increased number of cell divisions and stimulation of growth.[20,21]

The aim of our study was to test if reduced TSH level *before and during* irradiation could protect the thyroid glands from the development of radiogenic cancer. Thyroxin application would decrease production of thyrotropin by impeding the hypophyseal feedback mechanism[22,23] before exposing the thyroid gland to radiation. If the thyroid cells were stabilized by thyroxin they would be expected to be less susceptible to growth stimulation caused by radiation damage. Reducing the number of mitoses would diminish the possibility of radiogenic mutations and thus make the thyroid tissue less sensitive to carcinogenic initiation.

Material and methods

In the experiment 81 three to four week old Wistar strain rats of both sexes were used. They were first divided into two groups according to thyroxin administration, *i. e.* thyroxin (T_4) and water (H_2O). Each of the main groups was further divided into two subgroups according to irradiation (X and sham: X) (Table 1).

Thyroxin administration

The T4 rats were injected 1 % thyroxin solution (0.01 mg / 100 g body weight) twice a day for 15 days, while the H_2O rats received saline in the same way. The effectiveness of thyroxin mediated TSH suppression was tested in a preliminary study. Four rats were used, 1 % thyroxin solution or water was

Table 1. Number of rats in subgroups

Irradiation	Application	
	Water	Thyroxin
Irradiated	H_2O/X	T_4/X
	(n=37)	(n=20)
Sham irradited	$H_2O/\not{X}$	$T_4/\not{X}$
	(n=12)	(n=12)

H_2O/X - water injection + irradiation, T_4/X - T_4 injection + irradiation, H_2O/X - water injection + sham irradiation, T_4/X - T_4 injection + sham irradiation.

administered twice a day for 10 days; after that, they were sacrificed and the serum TSH level was measured. The mean value was 0.015 mU/L in thyroxin group and 0.31 mU/L in control group ($P < 0.01$), which was accepted as sufficient suppression.

Irradiation

After 10 days, the rats from both irradiated subgroups (T_4/X and (H_2O/X) received 10 Gy to the neck area. They were irradiated with a telecobalt machine for five consecutive days with one direct field. Daily dose was 2 Gy defined at a depth of 1.5 cm, FSD of 80 cm, and field dimension of 5 cm x 5 cm.

Follow up

The animals were kept in cages, 4 - 5 of the same subgroup together, with food and water ad libitum. They were monthly weighed and regularly examined. Special attention was paid to the animals' coats and general appearance indicative of altered thyroid function. The animals which looked unhealthy or had an obvious neoplasm were sacrificed for immediate post mortem examination. Two years later, the survivors were also sacrificed by chloroform.

Histopathology

At autopsy, the trachea with the whole thyroid gland was removed, fixed in Bouin's fixative, embedded in paraffin and sectioned serially. All sections were stained with hematoxylin and eosin and pathohistologically examined. In search for metastases, specimens from the lungs and lymph nodules of the neck region were obtained, as well as from all other organs displaying pathological changes. The diagnoses were made according to Murthy's classification.[24] All lesions were classified as follicular cysts and hyperplasia, follicular adenomas or follicular carcinomas. Carcinomas were diagnosed on the basis of nuclear pleomorphism, anaplasia and dedifferentiation, but the main criteria for malignancy were capsular and/or vascular invasion and tumor cell emboli in the vasculature.[25]

Results

During the initial 18-month latent period, 12 rats had to be sacrificed and further material of 11 animals was not diagnostically due for postmortem autolysis. Therefore, 58 animals were assigned for final analysis.

The incidence of thyroid lesions in different groups are shown in Table 2. Pathological changes were the most numerous in the H_2O/X subgroup, where also carcinomas occurred; a smaller number of lesions, but no carcinomas, were noticed in the T_4/X subgroup, while pathological changes in the $H_2O/\not{X}$ and $T_4/\not{X}$ subgroups were rare.

Table 3 shows the incidence of thyroid carcinomasin rats sacrificed in the last four months of follow-up. The statistical analysis was made by modified t - test for small samples by Bonferroni[26]; the difference between both irradiated subgroups was significant ($P < 0.01$).

The cumulative incidence of tumors in irradiated subgroups is shown in Figures 1 and 2. The first carcinoma occurred in the H_2O/X subgroup 20 months after irradiation.

Table 2. Number (and relative portion) of thyroid lesions in 58 rats by subgroups

Group	Carcinoma	Adenoma	Other lesions	Normal glands	Sum
H_2O/X	8 (0.33)	7 (0.29)	5 (0.20)	7 (0.29)	24
f	3 (0.25)	4 (0.33)	2 (0.16)	4 (0.30)	12
m	5 (0.41)	3 (0.25)	3 (0.25)	3 (0.25)	12
T_4/X	0	3 (0.27)	2 (0.18)	6 (0.54)	11
f	0	2 (0.40)	0	3 (0.60)	5
m	0	1 (0.16)	2 (0.33)	3 (0.50)	6
H_2O/✱	0	1 (0.08)	1 (0.08)	10 (0.83)	12
f	0	1 (0.16)	1 (0.16)	4 (0.66)	6
m	0	0	0	6 (1.00)	6
T_4/✱	0	0	0	11 (1.00)	11
f	0	0	0	6 (1.00)	6
m	0	0	0	5 (1.00)	5
	8	11	8	34	58*

H_2O - water, T4 - thyroxin, X - irradiation, ✱ - sham irradiation, f - female, m - male, Other Lesions - diffuse hyperplasia, nodular hyperplasia, follicular cyst; *as more than one sort of lesion may have occurred in the thyroid gland of one rat, the sum of all lesions is different from the number of rats.

Table 3. Number (and relative portion) of thyroid carcinomas in rats by subgroups - sacrificed in the last four months of observation

Group	H_2O (n=27)	T_4 (n=19)	p<
X (n=24)	8/16 (0.50)	0/8 (0.00)	0.01
✱ (n=22)	0/11 (0.00)	0/11 (0.00)	N.S.

H_2O - water, T_4 - thyroxin, X - irradiation, ✱ - sham irradiation.

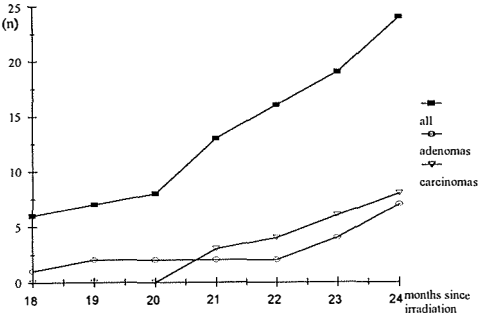

Figure 1. Cumulative incidence of thyroid tumors in the H_2O/X subgroup (water + irradiation).

Discussion

In thyroid carcinogenesis, the moment of induction is followed by a latent period before the first thyroid tumors occur.[13] It is assumed that the latent period after irradiation is one to one and a half years long.[11,12] In our material, the first carcinoma was observed in the rat sacrificed 21 months after irradiation, while the overall time of observation was 24 months. Statistical analysis of carcinoma incidence for the period of the last three months revealed significant differences between the two irradiated groups. In the irradiated subgroup without T_4, the number of carcinomas was significantly higher than in the subgroup treated with T_4, where no carcinomas were found. Based on the results

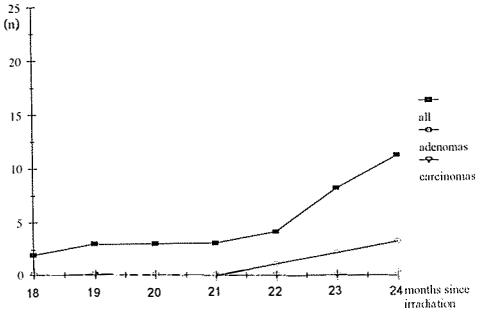

Figure 2. Cumulative incidence of thyroid tumors in the T_4/X subgroup (thyroxin + irradiation).

of our experiment, the hypothesis of radioprotective role of thyroxin in thyroid carcinogenesis was confirmed.

Similar findings are reported from the experiment on animals by Doniach.[14] The author studied histological changes in the thyroid glands of irradiated rats after administration of T_4 and water, respectively. In the animals which received single doses of 1 Gy, 2.5 Gy or 5 Gy and were after that given every day 20 μg T_4 until they were sacrificed 20 months later only one adenoma occurred. On the contraty, in the irradiated group drinking water 5 adenomas and two carcinomas occurred. The small number of tumors in the whole group was probably due to a relatively low dose of irradiation.

In our experiment, the parenteral administration of T4 was started before the irradiation to prevent an enhanced growth and mitotic activity during the action of mutagen. Thyrotropin suppression was measured in the preliminary test. Since no carcinomas occurred after irradiation, we can conclude that the stabilization of thyroid cells was achieved and cocarcinogenic stimulation of thyrotropin in the phase of initiation was abolished.

Bause *et al.*[27] reported a significant reduction of tumor incidence by an immunostimulation with xenogenic, lyophilized fetal cells administered twice after whole body irradiation of rats. The incidence of carcinomas was 25% in the immunized group versus 55% in the controls. In spite of the promising results, this method was never described in a clinical trial.

The use of T_4 as radioprotective agent was tested in humans by Bantle *et al.*[28] They have administered exogenous T_4 to lymphoma patients receiving radiation therapy, in an attempt to suppress serum thyrotropin and prevent radiation induced thyroid damage. Twenty patients in experimental group were treated with 200 μg T_4 1 to 13 days (average 5 days) before the beginning of mantle radiation. The level of thyrotropin suppression was documented by measuring T_4 index in the serum and by performing TRH (thyrotropin realising hormone) test. In all 20 patients of the experimental group, T_4 index rise was achieved and, in 19 of 20 patients, TSH (thyrotropin) response to TRH was demonstrated before radiotherapy was initiated; only in one case, the test was not performed. T_4 application was discontinued after the completion of the radiation and the 20 patients without T_4 served as controls. After a mean follow-up of 19 months, 35% of patients in the experimental group had a higher level of serum thyrotropin and, in the control group, only 25% of patients developed hyperthyrotropinaemia. From these results, it may be concluded that the suppression of serum thyrotropin during neck irradiation should not prevent subsequent thyroid dysfunction. This finding is contrary to the results of our study, but the end-point observation in this clinical trial, *viz.* thyroid dysfunction, was different from ours, *viz.* the detection of thyroid tumors.

In addition to carcinomas, other lesions were observed in the thyroid glands of our experiment. The histopathologic examination revealed the occurrence of adenomas, diffuse or nodular hyperplasia and follicular cysts. The largest proportion of these lesions was found in the irradiated rats not receiving T_4.

A smaller number of such changes occurred also in the irradiated subgroup of rats which were given T_4. In the non-irradiated subgroups, such lesions were rare. This additionally confirms the protective role of T_4 in thyroid radiation carcinogenesis.

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