Radiat Environ Biophys (2008) 47:453–461
DOI 10.1007/s00411-008-0189-5

ORIGINAL PAPER

Evaluation of the cytogenetic effects of ^{131}I preceded by recombinant human thyrotropin (rhTSH) in peripheral lymphocytes of Wistar rats

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Received: 23 January 2008 / Accepted: 24 July 2008 / Published online: 19 August 2008

Abstract The present study was carried out to investigate the cytogenetic effects of therapeutic exposure to radioiodine preceded by rhTSH in an animal model. Three groups of Wistar rats ($n = 6$) were used: one group was treated only with ^{131}I (11.1 MBq/animal); the other two groups received rhTSH (1.2 μg/rat of either Thyrogen or rhTSH-IPEN, respectively) 24 h before administration of radioiodine. The percentage of lymphocytes with chromosome aberrations and the average number of aberrations and of dicentrics per cell were determined on blood samples collected 24 h, 7 and 30 days after administration of ^{131}I. The data show that the treatment with radioiodine alone or associated with rhTSH resulted in a greater quantity of chromosome alterations in relation to basal values after 24 h, with a gradual decline after 7 and 30 days of treatment. An increase in chromosome alterations was also seen after rhTSH treatment alone. Neither of the treatments, i.e., with ^{131}I alone or associated with hormone, resulted in an aneugenic effect or influenced the kinetics of cellular proliferation in rat blood lymphocytes. There was no significant difference between the cytogenetic effects of Thyrogen and rhTSH-IPEN treatment. These data suggest that the treatment with radioiodine, associated or not with rhTSH, affects to a limited extent a relatively small number of cells although the occurrence of late stochastic effects could not be discarded.

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Introduction

During recent decades, increasing emphasis has been given to the cytogenetic effects of radiopharmaceuticals in concert with their increasing utilization for diagnostic as well as therapeutic purposes. Targeted therapy has several potential advantages over external beam therapy, including the possibility of delivering higher doses of radiation directly to the tumor [1].

Radionuclides of medical interest include alpha-emitters (^{211}At, ^{213}Bi, ^{225}Ac), Auger/internal conversion electrons-emitters (^{67}Ga, ^{201}TI) and mainly, beta emitters (^{186}Re, ^{153}Sm, ^{89}Sr, ^{166}Ho, ^{131}I). Among them, the beta emitters are more indicated for tumor therapy because of their low penetration power into tissues (1–12 mm with a maximum energy of 0.3 and 2.3 MeV), which enables them to reach the tumor specifically, without much damage to healthy tissues, thus reducing general toxicity [2, 3].

Among the therapeutic radionuclides, radioiodine (^{131}I) has been widely used for the last 50 years for the treatment of patients with thyroid diseases like hyperthyroidism to reduce the size of the thyroid gland or with differentiated thyroid cancer to eliminate remnant tumor cells after thyroidectomy [4, 5]. The widespread use of ^{131}I in nuclear medicine is due to its physical and radiochemical properties. It is a beta particle ($E_{max} = 0.61$ MeV and

E_{avg} = 0.20 MeV) and gamma ray (E = 0.36 MeV) emitter, with a physical half-life of approximately 8 days, which accumulates preferably in the thyroid tissue. About 90% of the secondary effects of its radiation are the result of the beta particles, whose track length is relatively short (~0.8 mm) in soft tissue [6].

In general, patients with differentiated thyroid carcinoma are submitted to thyroidectomy, to ablation of thyroid residues with radioiodine and to thyroid hormone suppression therapy to increase endogenous TSH levels (>25–30 μU/mL) and thus maximize the selective uptake of radioiodine by neoplastic cells [7, 8]. However, the withdrawal of levothyroxine (L-T_4) induces hypothyroidism symptoms for long periods (~10 weeks), causing physical and psychological problems. Besides these effects, long-term exposure to high TSH levels may increase the risk of neoplastic growth [6, 9, 10].

More recently, highly purified recombinant human thyrotropin (rhTSH), an exogenous source of hTSH, was proposed as an effective alternative for raising serum TSH concentrations in the follow-up of patients with well differentiated thyroid cancer, thus avoiding the undesirable symptoms of hypothyroidism associated with thyroid hormone withdrawal (THW). As a matter of fact hypothyroidism, in addition to impairing life quality, has negative effects on different organs and systems. It may exacerbate neuropsychiatric illness and cardiovascular diseases, induce dyslipidemia and increase atherosclerotic risk [11, 12]. Since rhTSH administration results in TSH elevation for several days rather than weeks it can decrease the risk of tumor growth stimulation that can rapidly occur in some patients [7, 13, 14].

Human TSH is a heterodimeric glycoprotein consisting of two noncovalently linked subunits: an alpha subunit with 92 amino acid residues containing two glycosylation sites, common to other glycoprotein hormones (FSH, LH and CG), and a hormone-specific beta subunit with 118 amino acid residues, containing one glycosylation site. The amino acid sequence of rhTSH is identical to that of native pituitary hTSH and is produced by Chinese hamster ovary cells (CHO) transfected with plasmids containing the cDNA sequences coding for the alpha and beta subunits, whose genes are located in chromosomes 6 and 1, respectively [15–17]. The binding of rhTSH to its receptors, which are present in normal and neoplastic epithelial cells of the thyroid, stimulates iodine uptake and metabolization and the synthesis and secretion of thyroglobuline (Tg) and of the thyroid hormones triiodothyronine (T_3) and tyroxine (T_4). Studies point to a safe and promising use of rhTSH in association with ^{131}I in the treatment of nodular goiter [18, 19] and of differentiated thyroid cancer patients [7–9, 13].

In spite of the advantages of therapy, no study has been carried out on the effects of hTSH or of ^{131}I preceded by rhTSH at the cellular level in either humans or an animal model. ^{131}I is, like any radiation emitter, a mutagenic and carcinogenic agent. Since most of the information about the effects of radiation on chromosomes comes from in vitro studies where the cells are submitted to several levels of external irradiation, study of the effects of internal and continuous radiation of beta particles at the cellular level, particularly when of medical interest, is of great importance. Although ionizing radiation mainly induces structural alterations in chromosomes (clastogenic effect), it may also cause changes in the chromosome number in vivo and in vitro (aneugenic effect) [20].

Cytogenetic techniques to detect DNA or chromosome damage have been widely used as a sensitive indicator of exposure to genotoxic agents [21]. Cytogenetic alterations induced by ^{131}I have been detected by analysis of chromosome aberrations [22, 23], micronuclei [24–26], chromosome aberrations and micronuclei associated with fluorescence in situ hybridization (FISH) [27–29], sister-chromatid exchange [30] and comet assay [31] in peripheral lymphocytes from patients with thyroid carcinoma or hyperthyroidism. However, the data from several investigations are contradictory. Some studies reported a gradual decrease of the chromosome damage as a function of time in hyperthyroidism or thyroid carcinoma patients, although the cytogenetic damage persisted for 1 or 2 years after the therapeutic exposure to ^{131}I [25, 30, 32–34]. Other studies showed a significant and persistent increase of chromosome anomalies induced by ^{131}I treatment in patients with thyroid diseases [24, 25, 35]. On the other hand, Gutiérrez et al. [31], did not find any significant difference 1 week after the treatment concerning radio-induced DNA damage in relation to basal values, as evaluated by the comet assay in patients with thyroid carcinoma receiving therapeutic doses of radioiodine.

Several studies concerning to the cytogenetic effects of radioiodine in peripheral lymphocytes have been carried out when the thyroid gland has been totally or partially excised (thyroid cancer patients) or when the whole gland accumulates radioiodine (thyrotoxic patients). Gundy et al. [23] and Gutiérrez et al. [24, 25] found a difference in the response with respect to the frequency of cells with chromosome damage, after ^{131}I exposure, between patients with hyperthyroidism and with differentiated thyroid carcinoma. The patients with hyperthroidism, hyperactive and with an intact thyroid gland, showed a significantly higher frequency of cells with chromosome damages, even though the activity of radioiodine administrated was lower than for thyroidectomized patients with little or no functioning thyroid tissue. This was probably due to the difference in biological half-life of radioiodine. In this sense, we believe that animal models, even based on euthyroid mice and rats, can be useful for evaluating genotoxic exposure. The rat was chosen here as the animal model because karyotype analysis is easier than with mice and because rats have a

larger blood volume; in addition, rats have a diploid chromosome number ($2n = 42$) and a lymphocyte radiosensitivity close to that of humans ($2n = 46$) [36]. No study has been carried out, as far as we known, on the cytogenetic effects of hTSH or of ^{131}I preceded by rhTSH in either humans or an animal model.

The objetive of the present study was thus to analyse the cytogenetic effects of a therapeutic exposure to radioiodine (^{131}I) in peripheral rat lymphocytes in order to: (1) evaluate the presence of structural and numerical chromosome aberrations and the number of cells in the different mitotic cycles and (2) determine whether or not theses effects were influenced by a prior stimulus with of rhTSH (employing two different rhTSH preparations).

Materials and methods

Preparations of recombinant human TSH

Two preparations of rhTSH were used: the commercial preparation Thyrogen (Genzyme Transgenics Corp, Framingham, MA, USA) and a preparation that was synthesized, purified and characterized in our laboratory, rhTSH–IPEN [17, 37–39].

Animals

Female Wistar rats SPF (Specific Pathogen Free), 2 months old, were utilized. Their weight was ~200 g; food containing 1.5 μg iodine/g and water were given ad libitum. The animals were housed in metabolic cages, under controlled conditions of temperature (20–25°C) and photoperiodism (12 h light/12 h dark), and handled in accordance with ethical guidelines [40].

All animals were non-thyroidectomized and divided into three groups of six rats each: animals treated with ^{131}I (11.1 MBq administered by gastric gavage) only (G1) and animals submitted to rhTSH treatment (1.2 μg/animal or 6.4 μg/kg injected i.m.), with either Thyrogen (G2) or rhTSH–IPEN (G3), 24 h before ^{131}I administration. Hormone dosage and ^{131}I activity administered to the animals were calculated according to the protocol designed by the Center of Nuclear Medicine (São Paulo, Brazil) for the treatment of thyroid disease: 0.45 mg of Thyrogen (6.4 μg/kg) and about 3.700 MBq of ^{131}I (53 MBq/kg) in a reference man of 70 kg body weight. The solutions of Na ^{131}I used in the assays were provided by the Center of Radiopharmacy of IPEN–CNEN (São Paulo, Brazil).

In order to determine the 24-h thyroid uptake, 1.11 or 11.1 MBq of ^{131}I were administered to mice (female, Balb/c, ~20 g body weight) or rats (female Wistar, ~200 g body weight), respectively, with or without administration of rhTSH (6.4 μg/kg) prior to the dose of ^{131}I. A higher dose of Thyrogen was also used (3 μg/mouse and 30 μg/rat, i.e., 100 μg/kg). At 24 h after ^{131}I administration, the thyroid gland was excised and the retention of ^{131}I in the gland was measured in a radioisotope calibrator (Capintec, Inc., CRC–35R, USA). The 24-h thyroid uptake of ^{131}I was calculated as the percentage of the total administered radioactivity present in the thyroid at that time.

Cytogenetic assay

Blood samples were collected for cytogenetic evaluation from the orbital sinus, before administration of rhTSH (basal), 24 h after rhTSH, and 24 h, 7 and 30 days after ^{131}I administration. Blood cells were cultivated in RPMI 1640 (Cultilab, Campinas, Brazil), supplemented with 20% bovine fetal serum (Gibco, Invitrogen, UK), 5 μg/ml BrdU (Sigma, St Louis, USA) and stimulated with 100 μg/ml concanavalin-A (Amersham, Uppsala, Sweden), as described previously (specifically for rats) [41] and maintained for 72 h at 37°C. The cells were then treated with 0.7 μg/ml colcemid (Sigma, St Louis, USA) for 2 h, hypotonized with 0.075 M KCl and 1% sodium citrate (Merck, Darmstadt, Germany), fixed and spread on histological slides pre-heated at 65°C.

For the evaluation of cell cycle kinetics the fluorescence plus Giemsa (FPG) staining technique that allows unequivocal identification of cells from first, second or later mitoses was utilized [21, 42]. The slides were stained with 5 μg/ml Hoechst 33258 (Sigma, St Louis, USA), covered with 0.5 ml McIlvaine buffer and exposed to UV light (254 nm) for 20 min at 60°C on a heated plate. They were then washed with distilled water and stained with 5% Giemsa (Sigma, St Louis, USA) in Sorensen buffer, pH 6.8, and analyzed under an optical microscope (Carl Zeiss, Germany). All the metaphases containing a diploid number of up to $2n - 2$ chromosomes were considered. For the purpose of investigating the possible influence of the radiation emitted by ^{131}I on the number of chromosomes, the frequency of metaphases with modal ($2n = 42$) and hypomodal ($2n = 40$ and $2n = 41$) chromosome numbers was taken into account. The structural chromosome aberrations were classified according to the criteria established by the International Atomic Energy Agency [21] and were evaluated with regard to three main parameters: incidence of affected cells (percentage of cells with aberrations), degree of intracellular damage (number of aberrations/cell) and the occurrence of dicentric chromosomes, a specific type of chromosome aberration.

Statistical analysis

A Student *t* test was used for the comparison of the data obtained between the different groups of animals (G1, G2

and G3) and between the different times periods following radioiodine administration (24 h, 7 and 30 days). P values less than 0.05 were considered to be statistically significant.

Results

In view of the data of Colzani et al. [43] data concerning the effects of rhTSH on thyroid function of rats and mice, we determined the 24-h ^{131}I thyroid uptake for these two animals, with or without rhTSH administration, in order to confirm the validity of our rat model. As shown in Table 1, in rats the 24-h uptake of ^{131}I was unaffected by rhTSH (6.4 μg/kg). In mice, a slight decrease was observed after administration of the smaller dose of rhTSH (1.2 μg/mouse, i.e., 6.4 μg/kg), inline with the results reported by Colzani et al. [43] for the 2-h thyroid uptake of ^{125}I in mice. These authors, however, used a considerably higher dose of rhTSH (∼100 μg/kg). In our hands, a dose equivalent to theirs greatly increased the 24-h thyroid uptake, i.e., ∼1.9-fold ($P < 0.01$) and ∼2.8-fold ($P < 0.05$), respectively, for rat and mouse. We therefore decided to maintain the rat model for the reasons already mentioned in the introduction, there being no particular advantage in utilizing the mouse model, once that we used dosages of ^{131}I and rhTSH comparable to those utilized in humans (see "Materials and methods").

Table 1 Twenty-four hour thyroid uptake in rats or mice after administration of 11.1 MBq (rat) or 1.11 MBq (mice) of ^{131}I preceded or not by rhTSH

Animal model	^{131}I only (% uptake)	rhTSH (6.4 μg/kg) + ^{131}I (% uptake)	rhTSH (100 μg/kg) + ^{131}I (% uptake)
Rat	7.3 ± 1.5 ($n = 6$)	7.7 ± 1.6 ($n = 4$)	14.3 ± 4.6 ($n = 3$)
Mouse	13.2 ± 6.5 ($n = 7$)	12.6 ± 5.0 ($n = 4$)	36.1 ± 8.4 ($n = 3$)

The cytogenetic analysis of lymphocytes of rats treated with ^{131}I showed many types of structural chromosome aberrations, among them dicentrics, double minute, acentric fragments, gaps and chromosome breaks. Representative examples are shown in Fig. 1. Tables 2 and 3 show the data for the animals of the three groups studied with respect to structural chromosome aberration frequency and chromosome number. The basal percentage of cells with chromosome aberrations was around 1.7% in the three groups. Results of the statistical analysis and graphical presentation of the cytogenetic data obtained are shown in Table 4 and Fig. 2, respectively.

We observed an increase in the percentage of cells with chromosome aberrations (Fig. 2a) and in the number of chromosome aberrations per cell (Fig. 2b) 24 h after ^{131}I administration, when compared to the basal values in animal group G1 (only ^{131}I treatment), but the difference was statistically significant ($P < 0.05$) only for the number of chromosome aberrations per cell. Between 7 and 30 days after radioiodine administration, a gradual decline in the two parameters was found. Although the values were still higher than basal values, they were not significant ($P > 0.05$) for the percentage of cells with chromosome aberrations.

An increase in the frequency of cells with chromosome aberrations and in the frequency of aberrations per cell was also seen 24 h after rhTSH treatment in animals of groups G2 and G3 (before treatment with radioiodine), although the difference was statistically significant only with respect to the frequency of cell with aberrations in group G2.

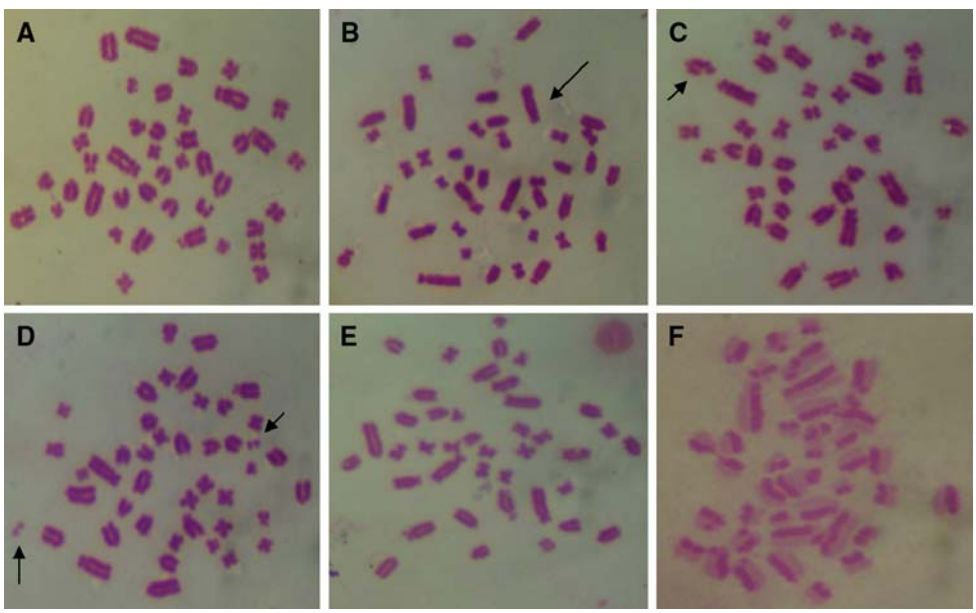

Fig. 1 Photomicrographs showing structural chromosome aberrations observed in rat peripheral lymphocytes treated with ^{131}I: **a** normal metaphase, $n = 42$; **b** dicentric, $n = 42$; **c** chromatidic break, $n = 41$; **d** acentric fragments, $n = 42$; **e** metaphase, $n = 40$; **f** metaphase in the second division, $n = 42$, n number of chromosomes (×1,000)

Table 2 Frequencies of structural chromosome aberrations observed in Wistar rat lymphocytes from groups G1 (with ^{131}I), G2 (with Thyrogen and ^{131}I) and G3 (with rh-TSH-IPEN and ^{131}I)

Group	Sample	Number of analyzed cells	Dic	Ring	Dm	Break	Gap	Ace	Tri	Number of aberration/cell	Cells with aberration (%)	Frequency of dicentric/cell
G1	Basal	1,200	1	0	3	4	7	5	0	0.017 ± 0.008	1.7 ± 0.8	0.0008 ± 0.0020
	24 h after^{131}I	1,132	6	1	5	10	6	6	2	0.032 ± 0.012	2.8 ± 1.1	0.0050 ± 0.0077
	7 days after ^{131}I	1,295	6	0	6	11	5	7	0	0.026 ± 0.006	2.6 ± 0.7	0.0047 ± 0.0008
	30 days after ^{131}I	1,200	6	0	4	10	5	3	0	0.023 ± 0.008	2.3 ± 0.7	0.0050 ± 0.0045
G2	Basal	1,200	3	0	5	5	4	3	0	0.017 ± 0.007	1.7 ± 0.7	0.0025 ± 0.0027
	24 h after rhTSH	1,194	7	0	6	7	6	6	0	0.027 ± 0.006	2.7 ± 0.6	0.0058 ± 0.0038
	24 h after^{131}I	1,061	12	0	6	9	9	6	0	0.039 ± 0.010	3.9 ± 1.0	0.0118 ± 0.0081
	7 days after ^{131}I	1,158	6	0	6	5	7	7	0	0.027 ± 0.004	2.7 ± 0.4	0.0055 ± 0.0052
	30 days after ^{131}I	1,191	5	0	4	13	3	5	0	0.025 ± 0.010	2.5 ± 1.0	0.0042 ± 0.0038
G3	Basal	1,134	2	0	5	6	4	3	0	0.018 ± 0.005	1.7 ± 0.5	0.0017 ± 0.0026
	24 h after rhTSH	1,200	6	0	8	10	5	6	0	0.029 ± 0.013	2.9 ± 1.3	0.0050 ± 0.0032
	24 h after ^{131}I	1,001	8	0	6	11	5	8	0	0.038 ± 0.005	3.8 ± 0.5	0.0078 ± 0.0070
	7 days after ^{131}I	1,073	2	0	4	11	6	11	0	0.033 ± 0.007	3.1 ± 0.5	0.0020 ± 0.0032
	30 days after ^{131}I	1,135	8	0	6	6	3	4	0	0.024 ± 0.007	2.4 ± 0.7	0.0070 ± 0.0024

Dic Dicentric with or without fragments, *Ring* centric and acentric ring, *Dm* double minute, *Break* chromatidic and chromosomic breaks, *Gap* chromatidic and chromosomic gaps, *Ace* excess of acentric fragment, *Tri* triradial

Table 3 Frequencies of cells with modal and hypomodal number of chromosomes and different cell cycles observed in Wistar rat lymphocytes from groups G1 (with ^{131}I), G2 (with Thyrogen and ^{131}I) and G3 (with rhTSH-IPEN and ^{131}I)

Group	Sample	Number of analyzed cells	Number of chromosomes (%)			Mitotic cycles (%)		
			42	41	40	First division	Second division	Third division
G1	Basal	1,200	82.5	12.6	4.9	96.5	3.4	0.1
	24 h after ^{131}I	1,132	85.2	9.6	5.2	96.0	4.0	0.0
	7 days after ^{131}I	1,295	84.5	11.2	4.3	94.0	5.9	0.1
	30 days after ^{131}I	1,200	94.2	5.8	0.0	98.4	1.6	0.0
G2	Basal	1,200	90.7	8.3	1.0	94.5	5.3	0.2
	24 h after rhTSH	1,194	89.9	8.6	1.5	93.2	6.7	0.1
	24 h after ^{131}I	1,061	88.3	9.7	2.0	95.6	4.2	0.2
	7 days after ^{131}I	1,158	90.9	8.0	1.1	94.6	5.3	0.1
	30 days after ^{131}I	1,191	88.5	9.2	2.3	94.4	5.3	0.3
G3	Basal	1,134	79.5	12.9	7.6	95.2	4.6	0.2
	24 h after rhTSH	1,200	94.6	5.4	0.0	97.8	2.2	0.0
	24 h after ^{131}I	1,001	92.1	6.9	1.0	91.2	8.4	0.4
	7 days after ^{131}I	1,073	93.9	5.5	0.6	93.5	6.5	0.0
	30 days after ^{131}I	1,135	89.2	8.4	2.4	94.5	5.5	0.0

In animals stimulated with exogenous rhTSH followed by ^{131}I administration (G2 and G3), the percentage of cells with chromosome aberrations and the number of aberrations per cell increased when compared to the treatment with rhTSH only, although differences were not always statistically significant (Fig. 2; Table 4).

With regard to the frequency of dicentric chromosomes (Fig. 2c), a maximum value was observed again 24 h after treatment with radioiodine in all three groups, without or with prior treatment with rhTSH. The G2 and G3 groups presented a slight tendency to decline after 7 and 30 days, whereas G1 exhibited a relatively stable value. The treatment with hormone alone (24 h after TSH) did not induce a significant change relative to the basal values or to ^{131}I preceded by rhTSH (24 h after ^{131}I) in G2 and G3.

The modal chromosome number ($2n = 42$) varied from 82.5 to 93.9% (Table 3) and no positive correlation was

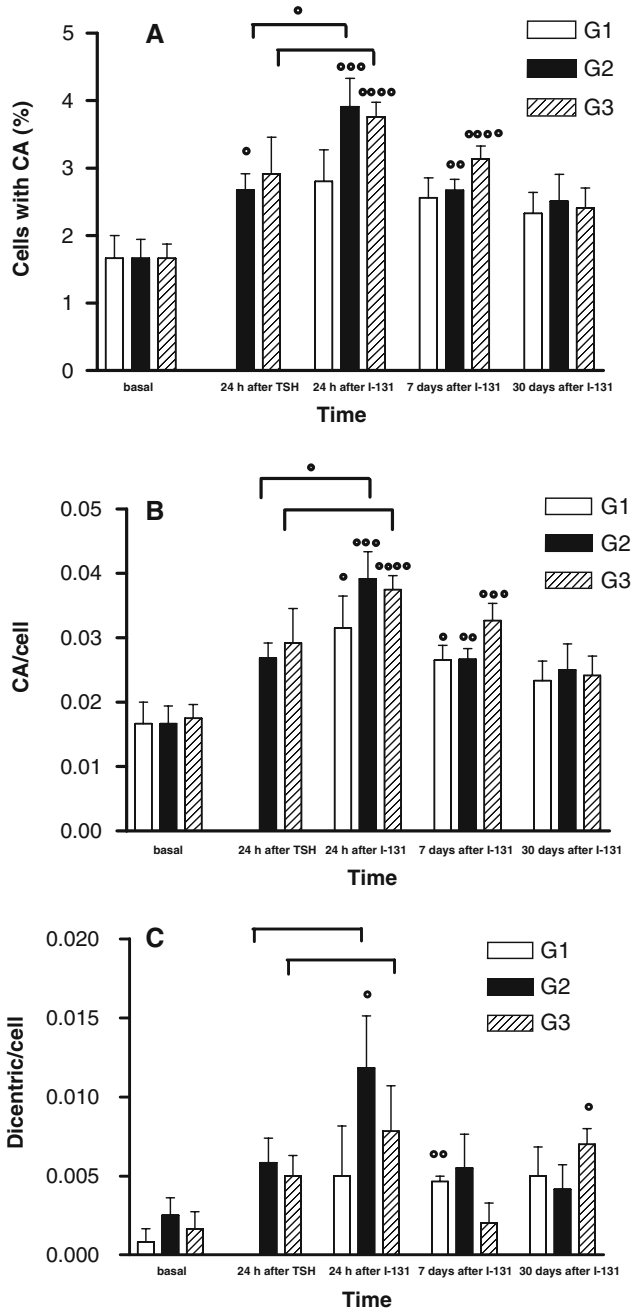

Fig. 2 Frequencies of chromosome aberrations (CA) observed in rat peripheral lymphocytes before and after ^{131}I treatment in three groups of animals (G1, G2 and G3). **a** Percentage of cells with chromosome aberrations, **b** number of chromosome aberrations/cell, and **c** number of dicentrics/cell. The Student's *t* test was used to compare the values at the three post-treatment times with the pre-treatment (basal) values: •$P < 0.05$; ••$P < 0.01$; •••$P < 0.005$; ••••$P < 0.001$; ⊓ not significant

observed with ^{131}I or rhTSH administrations. Table 3 also shows that the percentage of cells in the first mitotic division was higher than 90% in both basal and treated samples, suggesting that neither ^{131}I nor exogenous hTSH interfere in the cell cycle kinetics of rat lymphocytes.

Discussion

The effects of ionizing radiation on chromosomes have attracted a lot of attention in the last years as a consequence of the increasing utilization of various types of therapeutic radiopharmaceuticals and have stimulated discussion of the stochastic effects of radiation [24, 30]. However, there is no clear epidemiological evidence for an increase in the incidence of late effects with any of the radiopharmaceuticals used in nuclear medicine. In this context, it is of special interest to analyze at the cellular and tissue level the effects of radionuclides of medical interest, particularly radioiodine (^{131}I) which is widely applied in the treatment of patients with thyroid diseases. Here, an investigation was carried out to determine to what extent treatment with radioiodine preceded by rhTSH administration induces cytogenetic damages in non-thyroidectomized rat peripheral blood lymphocytes. Two different preparations of rhTSH (Thyrogen and rhTSH–IPEN) were tested.

Considering that extremely low doses of rhTSH (0.01 mg, i.e., ~0.14 μg/kg) can significantly increase the 24-h ^{131}I thyroid uptake in humans with nontoxic, nodular goiter [18], we believe that our data on ^{131}I thyroid uptake of rats confirm the low biological potency of rhTSH reported by Colzani et al. [43] for these rodents. We decided, however, to maintain doses of ^{131}I (53 MBq/kg) and rhTSH (6.4 μg/kg) equivalent to those used in humans, since the goal was to determine the cytogenetic effects in the animal model under conditions comparable to the treatment of humans. In the three groups of treated animals, there was an increase in the three parameter analyzed 24 h after ^{131}I administration, with a tendency for a gradual decline after 7 and 30 days, although the levels were still higher than the basal values determined before the treatment. There is no precise information on the destiny of damaged lymphocytes in vivo submitted to internal irradiation. The gradual decline with time of cells presenting genetic damage may be the result of various biological mechanisms: apoptotic process [28], repair mechanisms [44] and the normal turnover of lymphocytes, considering that the average half-life of rat lymphocytes is only 4 weeks [45].

The data obtained in the present study also showed that the dicentrics induced by treatment persist in all groups for 30 days after administration of radioiodine, although with statistical significance only for G3. This contrasts to some extent with the results obtained by Tucker et al. [46] on rat peripheral blood lymphocytes irradiated in vitro with ^{137}Cs gamma rays. These authors observed that the frequencies of dicentrics declined to near-baseline levels by the 4th day and suggested that the kinetics of loss is the same for dicentrics and for translocations and acentric fragments after external irradiation in vitro. The relative persistence of

Table 4 Statistical analysis of cytogenetic data obtained before (basal) and after administration of ^{131}I and rhTSH in the three groups of Wistar rats ($P < 0.05$ was considered to be significant)

Parameter	Group	Sample				
		24 h after rhTSH versus basal	24 h after ^{131}I versus basal	7 days after ^{131}I versus basal	30 days after ^{131}I versus basal	24 h after rhTSH versus 24 h after ^{131}I
Cells with chromosome aberrations (%)	G1		$P > 0.05$	$P > 0.05$	$P > 0.05$	
	G2	$P < 0.02$ (•)	$P < 0.005$ (•••)	$P < 0.01$ (••)	$P > 0.05$	$P < 0.05$ (•)
	G3	$P > 0.05$	$P < 0.001$ (••••)	$P < 0.001$ (••••)	$P > 0.05$	$P > 0.05$
Number of chromosome aberrations/cell	G1		$P < 0.05$ (•)	$P < 0.05$ (•)	$P > 0.05$	
	G2	$P > 0.05$	$P < 0.005$ (•••)	$P < 0.01$ (••)	$P > 0.05$	$P < 0.05$ (•)
	G3	$P > 0.05$	$P < 0.001$ (••••)	$P < 0.005$ (•••)	$P > 0.05$	$P > 0.05$
Number of dicentrics/cell	G1		$P > 0.05$	$P < 0.01$ (••)	$P > 0.05$	
	G2	$P > 0.05$	$P < 0.05$ (•)	$P > 0.05$	$P > 0.05$	$P > 0.05$
	G3	$P > 0.05$	$P > 0.05$	$P > 0.05$	$P < 0.05$ (•)	$P > 0.05$

chromosome aberrations observed in the present study in rat peripheral blood lymphocytes can be, in part, a consequence of the combination of ^{131}I accumulation in the thyroid gland, which would act as an internal source of irradiation [24], with the concomitant selective loss of damaged lymphocytes in the peripheral bloodstream.

A possible explanation for this fact is the short penetration power of the beta particles, which would affect a restricted number of cells, but would deposit a significant fraction of the energy in a heterogeneous way in the nuclei, thus generating multiple damaged sites in the DNA molecule [47]. It is believed that these sites consist of various types of lesions, such as DNA double-strand breaks of various degrees of complexity, which are responsible for the formation of chromosome aberrations.

The data obtained in the present study with rhTSH alone (G2 and G3) have no ready explanation since, as far as we know, no data have been reported so far concerning chromosome aberrations produced by this hormone.

No aneugenic effect mediated by radioiodine was found in rat peripheral lymphocytes. Since there was no significant difference between treated and control samples, we suggest that the relatively high frequency of cells with a hypomodal chromosome number observed in this study is probably a reflection of the cytogenetic technique employed and due to spreading of the chromosomes on the slides. Hypotonic treatment (which may burst the cell membrane), brief fixation (which leaves the cytoplasm fragile) and metaphase spreading are all factors that contribute to chromosome loss from metaphase cells [48]. With respect to the frequency of cells in different mitotic cycles, the treatment with ^{131}I alone, or associated with hormone, did not appear to influence the kinetics of cellular proliferation in rat blood lymphocytes.

Most of the absorbed ^{131}I dose is delivered by beta particles and about 90% of their energy is absorbed within 0.8 mm of the source [6]. This relatively short range suggests that the extent of irradiation of other tissues should be small and, consequently, its collateral effects should also be small at the cellular level. Taking into account the spontaneous frequency of lymphocytes with aberrations (<3%) reported by various authors in rat peripheral lymphocytes [41, 49], the observed increase of this parameter can be considered in our case to be relatively small, even using an euthyroid animal model and therapeutic doses that could potentially cause severe damages. The animals in G2 and G3 showed, in fact, significantly higher percentages of cells with cytogenetic damage compared to the spontaneous rate (3.8–3.9% vs. 1.7%) 24 h after radioiodine administration, with a remarkable decline 30 days after the treatment. This suggests that radioiodine exposure, with or without association with rhTSH, affects a relatively small number of cells, without causing any major damage during the treatment, although in principle the occurrence of late adverse side effects cannot be discarded. The results also demonstrated that the analysis of chromosome aberrations is a very sensitive technique, capable to detecting subtle DNA damage associated with therapeutic exposure to radioiodine in peripheral lymphocytes.

Acknowledgments The authors are grateful to Maria Neide F. Mascarenhas for her careful and excellent assistance with the animals during experiments. We thank the "Centro de Radiofarmácia" of the IPEN-CNEN/SP, Brazil, for providing Na^{131}I for the experiments. The work was supported by FAPESP (Project 02/09872–6).

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