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ORIGINAL ARTICLE

Cytogenetic and dosimetric effects of ^{131}I in patients with differentiated thyroid carcinoma: comparison between stimulation with rhTSH and thyroid hormone withdrawal treatments

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Abstract A study directed to the cytogenetic and dosimetric aspects of radionuclides of medical interest is very valuable, both for an accurate evaluation of the dose received by the patients, and consequently of the genetic damage, and for the optimization of therapeutic strategies. Cytogenetic and dosimetric effects of ^{131}I in lymphocytes of thyroidectomized differentiated thyroid cancer (DTC) patients were evaluated through chromosome aberration (CA) technique: Euthyroid patients submitted to recombinant human thyroid-stimulating hormone (rhTSH) therapy (group A) were compared with hypothyroid patients left without levothyroxine treatment (group B). CA analysis was carried out prior to and 24 h, 1 week, 1 month and 1 year after radioiodine administration (4995–7030 MBq) in both groups. An activity–response curve of ^{131}I (0.074–0.740 MBq/mL) was elaborated, comparing dicentric chromosomes in vivo and in vitro in order to estimate the absorbed dose through Monte Carlo simulations. In general, radioiodine therapy induced a higher total CA rate in hypothyroid patients as compared to euthyroid patients. The frequencies of dicentrics obtained in DTC patients 24 h after treatment were equivalent to those induced in vitro (0.2903 ± 0.1005 MBq/mL in group A and 0.2391 ± 0.1019 MBq/mL in group B), corresponding to absorbed doses of 0.65 ± 0.23 Gy and 0.53 ± 0.23 Gy, respectively. The effect on lymphocytes of internal radiation induced by ^{131}I therapy is minimal when based on the frequencies of CA 1 year after the treatment, maintaining a higher quality of life for DTC patients receiving rhTSH-aided therapy.

Keywords ^{131}I · Chromosome aberration · Recombinant human thyroid-stimulating hormone · Thyroid hormone withdrawal · Biological dosimetry · Differentiated thyroid carcinoma

Introduction

Differentiated thyroid carcinoma (DTC) represents the majority of thyroid malignancies (~95 %): Papillary thyroid cancer accounts for 85 % and follicular thyroid cancer for 10 % (Carvalho et al. 2012). In recent decades, there has been much speculation about the increase in the incidence of DTC. This may reflect a true increase in the occurrence of DTC due to potential risk factors such as environmental, dietary or genetic causes (Chen et al. 2009) and/or more efficient detection of cancer as a result of improved diagnostic accuracy (Kent et al. 2007; Pacini and Castagna 2008).

The initial treatment for most DTC patients comprises a total or near-total thyroidectomy followed by administration of ^{131}I for remnant ablation (Borget et al. 2008; Zanotti-Fregonara et al. 2008; Carvalho et al. 2012). For their survival, thyroidectomized patients need thyroid hormone

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replacement via levothyroxine (L-T4) administration. Radioiodine ablation is then performed via L-T4 withdrawal (THW) for 4–6 weeks, in order to allow a sufficient increase in endogenous TSH. This procedure permits an increase in serum TSH concentrations (>25–30 mIU/L) that is believed to optimize the trapping and retention of radioiodine for diagnostic procedures, thyroid remnant ablation and treatment of patients with DTC (Luster et al. 2005; Pacini and Castagna 2008; Zanotti-Fregonara et al. 2008). During this period, patients suffer from symptoms of clinical hypothyroidism, resulting in a decreased quality of life (Borget et al. 2008).

Recombinant human thyroid-stimulating hormone (rhTSH), in our case Thyrogen® from Genzyme Corporation (Framingham, MA, USA), is a highly purified heterodimeric glycoprotein, developed in 1993 to improve the diagnosis and treatment of patients with DTC when submitted to ^{131}I ablation (Cole et al. 1993; Luster et al. 2005; Mendonça et al. 2005; Pacini and Castagna 2008). The therapeutic use of rhTSH was approved by the European Medicine Agency and by the US Food and Drug Administration in 2005 and 2007, respectively (Pacini and Castagna 2008), and also in Brazil in 2006 (Rosário et al. 2008). Currently, stimulation by rhTSH has gained wide acceptance as an alternative to THW in the management of patients with DTC since it provides exogenous TSH stimulation without thyroid hormone withdrawal and the associated morbidity (Luster et al. 2005), allowing the patients to maintain the clinical euthyroid state on thyroid hormone therapy (Luster 2006; Borget et al. 2008). This avoids the clinical consequences of hypothyroidism, with a positive impact on the quality of life and work productivity (Zanotti-Fregonara et al. 2008). Other authors demonstrated that the use of rhTSH is associated with a significant decrease in whole-body irradiation, which may be relevant (Rosário et al. 2008), taking into account the collateral adverse effects of ^{131}I ablation therapy when healthy tissues are also irradiated. Many studies have also shown that the clearance of ^{131}I from the body is faster in the euthyroid status (Luster et al. 2005; Rosário et al. 2008), minimizing the risk of stochastic effects. Another important advantage of rhTSH-aided cancer treatment is that it avoids prolonged periods of high endogenous thyroid-stimulating hormone (TSH) stimulation, which may favor tumor growth. Several studies have found that the two regimens (euthyroid and hypothyroid status) are equally effective in preparing patients for ^{131}I remnant ablation, with a greater quality of life achieved when rhTSH is used (Borget et al. 2008; Pacini and Castagna 2008; Rosário et al. 2008).

There is no consensus as to the activity of ^{131}I to be administered in order to ablate the remaining glandular tissue; the values vary from one center to another, but usually 3.7 to 7.4 GBq (100–200 mCi) are prescribed for the first radioiodine therapy following partial or total thyroidectomy in newly diagnosed DTC patients (Sisson et al. 2003; Hänscheid et al. 2006).

Since ionizing radiation, including ^{131}I effects, can cause DNA damage in mammalian cells (Feinendegen and Pollycove 2001), various methodologies to detect DNA lesions have been proposed, including various types of cytogenetic assays. Thus, cytogenetic effects of ^{131}I have been evaluated in peripheral lymphocytes from patients with thyroid carcinoma or hyperthyroidism on the basis of chromosome aberrations (CA) (Baugnet-Mahieu et al. 1994; Gundy et al. 1996; M'Kacher et al. 1996; Gil et al. 2000), micronuclei (MN) (Gutiérrez et al. 1999; Watanabe et al. 2004), MN assay associated with fluorescence in situ hybridization (Ramirez et al. 1997), comet assay (Gutiérrez et al. 1998) and the sister chromatid exchange test (Erselcan et al. 2004).

In spite of the recognized therapeutic efficacy of rhTSH-aided cancer treatment associated with ^{131}I for patients with DTC, the data regarding the impact of this strategy are still scarce at the cellular level and, consequently, at the level of individuals under conventional treatment via THW. As far as we know, only two studies have been carried out on the cytogenetic effects of ^{131}I associated with rhTSH. The first was carried out in our laboratory (da Silva et al. 2008) utilizing an animal model that was studied via the chromosome aberration technique. The other study compared the frequency of chromosome translocations between DTC patients not receiving levothyroxine and those receiving rhTSH, measured before and 45 days after radioiodine treatment (Frigo et al. 2009). In this case, however, the authors analyzed only one type of CA (translocation) assessed only 45 days after exposure to ^{131}I. Thus, only a few dosimetric studies have specifically compared DTC patients treated with rhTSH with those under THW (Hänscheid et al. 2006; Frigo et al. 2009).

The present study was therefore carried out to evaluate chromosomal damage induced by internal irradiation due to ^{131}I treatment in peripheral blood lymphocytes of DTC patients, considering both the short- and long-term cytogenetic effects of radioiodine in patients that did or did not receive pretreatment with rhTSH. Another aspect of the study is the evaluation through biological dosimetry of the effects of ^{131}I in patients receiving radionuclide therapy in comparison with data obtained via in vitro activity–response curves based on Monte Carlo simulations. This approach derives from the importance of including biological parameters based on chromosomal modifications observed in patients undergoing treatment in the absorbed dose estimates, in addition to the mathematical models that are traditionally used and absorbed dose estimates based on physical methods.

Materials and methods

Patients

The study was performed with 21 patients who had undergone near-total thyroidectomy for DTC with no evidence of metastasis and did not suffer from nephrological or urological diseases. None of the patients had been previously treated with external radiotherapy or ^{131}I. The patients were randomly divided into two groups: group A (euthyroid patients) ($n = 11$; 10 females and 1 male; 10 papillary carcinomas and 1 follicular carcinoma; mean age of 45.4 years) was submitted to rhTSH (Thyrogen, Genzyme Corp., Cambridge, MA, USA) stimulation (0.9 mg intramuscular on 2 consecutive days prior to ablative therapy) while receiving L-T4 therapy, whereas group B (hypothyroid patients) ($n = 10$; 9 females and 1 male; 8 papillary carcinomas and 2 follicular carcinoma; mean age of 48.2 years) received radioiodine treatment following the standard protocol of L-T4 (levothyroxine) withdrawal (THW) for 4 weeks. A thyroid remnant ablation dose of 4995 to 7030 MBq ^{131}I was administered orally. The individual data are given in Table 1. Blood samples (peripheral lymphocytes) of each patient were collected for cytogenetic evaluation before administration of rhTSH and ^{131}I (basal), 24 h, 7 and 30 days and 1 year after radioiodine administration. All patients provided written informed consent for the study protocol. All blood samples were supplied by the University Hospital of São Paulo Medical School (FMUSP), and the study was approved by the Research Ethics Commission of the Institution on February 24, 2011 (no. 0741/10).

Healthy individuals

Blood samples from 5 non-smoking healthy donors of both sexes (2 males and 3 females, mean age of 37.4 years), with no irradiation history and no drug treatment at the time of blood sampling, were used for the elaboration of the in vitro activity–response curve.

In vitro irradiation

In order to produce the in vitro activity–response curve, blood samples obtained from healthy donors were exposed to different activity levels of Na^{131}I (0.074 to 0.740 MBq/mL) and maintained during 24 h at 37 °C. This period of time was chosen by taking into account the maximum 24-h

Table 1 Data from patients undergoing therapy with rhTSH before administration of radioiodine (group A, $n = 11$) and patients maintained in the hypothyroid state with radioiodine only (group B, $n = 10$)

Group	Donor	Age (year)	Sex	Weight (kg)	Total administered activity (MBq)	TSH level (mIU/L)
Group A	P1	27	F	136.0	5032	76.30
rhTSH + ^{131}I	P2	62	F	56.0	5797	183.10
	P3	31	F	56.0	6031	113.52
	P4	54	F	96.2	4995	144.46
	P5	48	F	98.0	5735	86.66
	P6	30	F	76.9	5735	81.91
	P7	64	F	61.5	6327	220.10
	P8	30	M	95.0	5735	154.06
	P9	50	F	110.3	5772	83.68
	P10	54	F	69.5	5994	–
	P11	50	F	76.0	6068	78.32
Mean ± SD		45.4 ± 13.56	10F/1 M	84.67 ± 25.01	5747.45 ± 407.61	122.21 ± 50.86
Group B	P12	59	F	–	6105	96.31
^{131}I	P13	37	F	–	5846	163.83
	P14	51	F	87.0	6660	91.27
	P15	58	M	–	5402	150.63
	P16	25	F	48.0	5957	254.66
	P17	56	F	70.0	7030	94.75
	P18	60	F	70.0	6031	150.28
	P19	32	F	64.0	6068	141.07
	P20	47	F	70.1	5846	87.15
	P21	57	F	63.0	6068	102.19
Mean ± SD		48.2 ± 12.58	9F/1 M	67.44 ± 11.63	6101.30 ± 449.94	133.21 ± 51.67

^{131}I thyroid uptake in humans (Zanzonico 1997). The activity range of ^{131}I was chosen by considering as reference a male of 70 kg body weight with 5 L of blood receiving an oral activity of 3.7 GBq ^{131}I. The solutions of Na^{131}I used in the assays were provided by the Radiopharmacy Center of the Nuclear and Energy Research Institute–National Nuclear Energy Commission (IPEN-CNEN) (São Paulo, Brazil).

Cytogenetic assay

For the chromosome damage evaluation, 1 mL of whole blood from each patient or healthy donor was cultivated with 3 mL of RPMI (Roswell Park Memorial Institute) medium (Cultilab, Campinas, Brazil) supplemented with 10 % fetal calf serum (Sigma, St. Louis, MO, USA), 100 µL of phytohemagglutinin (5 µg/mL, Gibco MRL., USA) and 60 µL of bromodeoxyuridine (BrdU) (5 µg/mL, Sigma, St. Louis, MO, USA) at 37 °C for 48 h.

Nearly 2 h before fixation, 40 µL of colcemid (0.7 µg/mL) (Sigma, St. Louis, USA) was added. At the end of incubation, the cells were harvested by centrifugation, submitted to hypotonic treatment with 0.075 M potassium chloride and 1 % sodium citrate (Merck, Darmstadt, Germany) and then fixed in a fresh solution of methanol and acetic acid (Merck) 3:1. This cell suspension was transferred to microscope slides in a preheated humid atmosphere at 65 °C and then air-dried overnight at room temperature. The slides were stained with Hoechst 33258 (Sigma, St. Louis, MO, USA), covered with 0.5 mL of McIlvaine buffer, pH 8.0, exposed to UV (ultraviolet) light (254 nm) for 20 min at 60 °C, rinsed with distilled water, air-dried and stained for 15 min with 5 % Giemsa (Cultilab, Campinas, Brazil) in Sorensen phosphate buffer, pH 6.8, and analyzed under an optical microscope (Nikon, Eclipse, 80i, Japan).

For the identification of different types of structural chromosome aberrations, the criteria adopted by the International Atomic Energy Agency (2011) were used. For each time point (before and after administration of ^{131}I), about 200 cells per patient (groups A and B) were analyzed. Similarly, for each radioactive concentration of ^{131}I, 50–380 cells per healthy donor (group C) were analyzed. Only the metaphases containing 44 or more chromosomes were scored. Three parameters were considered: incidence of affected cells (percentage of cells with aberrations), degree of intracellular damage (number of CA/cell) and the occurrence of dicentric chromosomes, a specific type of chromosome aberration. The number of cells with structural chromosome aberrations, the types of structural alterations, the number of cells in different cycles of mitotic division and the number of chromosomes for each metaphase were registered.

In vitro activity–response curve

In order to estimate the absorbed dose received by the DTC patients after 24 h of ^{131}I therapy, the frequencies of dicentrics observed in lymphocytes in vivo were compared with data for healthy donor lymphocytes obtained after 24 h of in vitro ^{131}I exposure, employed for the elaboration of the activity–response curve. The Monte Carlo N-Particle Transport Code (MCNP-4C), version 4.0 of the program (Briemeister 2000), was used to correlate the administered activity of ^{131}I (MBq) with the corresponding absorbed dose (Gy). This software utilizes a statistical method to estimate absorbed dose in which the physical process of the interaction of radiation with matter is simulated by considering the photon–electron coupled system. The code requires an input file, which allows the user to specify all the information about geometry modeling, source specifications, material compositions and the specific quantities to be estimated (tallies). Photoelectric effect, Rayleigh scattering, pair production and Compton scattering effects are all considered in the photon transport. Secondary electrons are also considered for the dose estimate. The energy cutoff for both photons and electrons was 1 keV, which is the inferior energy limit for photons and electrons transport simulation. The energy deposition in the culture was calculated using the pulse height tally, *F8, which is based on the particles collision, where the energy transferred to the media is subtracted from the particle energy. The photon cross section library utilized was the MCPLIB04. In the MCNP-4C code, the energy deposition is given per particle and per unit target mass, so that the final absorbed dose is obtained dividing the energy deposited in the target by its mass and multiplying by the total number of particles emitted from the source. In order to extrapolate in vitro, radioiodine activities of 370–3700 MBq received by the human body, different volumes of Na^{131}I solution were added to samples containing 1 mL of blood and 3 mL of culture medium to obtain ^{131}I activities of 0.074–0.740 MBq/mL, and the resulting samples then maintained 24 h at 37 °C. For the Monte Carlo simulation, the ^{131}I was assumed to be homogeneously distributed in the volume in direct contact with the blood for 24 h. Also, a correction factor of 0.9585 was applied, taking into account the 24-h decay correction. The photon energy emission spectrum was taken from the National Nuclear Data Center (NNDC).[1]

Statistical analyses

The statistical analyses were performed using the GraphPad Prism program (version 5.0), which was also utilized

[1] http://www.nndc.bnl.gov/mird/.

for the elaboration of graphs and tables. The activity–response curve obtained for the induction of dicentrics was fitted by the linear-quadratic model, according to the equation $Y = C + \alpha A + \beta A^2$, where Y is the frequency of the dicentric chromosomes, C is the background frequency of dicentrics, α and β are constants of the model and A is the level of activity in MBq/mL. The data were expressed as mean ± SE (standard error). Comparisons between the data were made using the Student's t test with a limit for statistical significance of $p < 0.05$.

Results

The patients in the two groups of the present study were comparable in terms of gender, weight, age, ^{131}I activity and TSH level ($p > 0.05$) before radioiodine treatment (Table 1), thus avoiding any possible differences in chromosome aberration yield associated with clinical parameters. The median TSH baseline value was 8.47 mIU (International Unit)/L (range 0.03–37.75) in group A, and an elevation of the TSH serum level to an average value of 122 mIU/L was obtained after the second rhTSH injection. A similar average value of 133 mIU/L was found in the hypothyroid group before ^{131}I administration.

The cytogenetic analysis showed many types of structural CA in the peripheral lymphocytes of patients treated with ^{131}I with or without rhTSH, such as dicentrics, acentric fragments, rings, double minutes, gaps and breaks (Table 2). The dicentric chromosome was the most frequent aberration and is considered to be the best indicator of radiation damage; thus, it was utilized for the absorbed dose estimation. Figure 1 shows the individual frequencies of chromosome aberration in vivo as a function of the time after ^{131}I administration, obtained from the peripheral lymphocytes of the whole study group (groups A + B). An increase in the frequency of cells with CA was seen 24 h after ^{131}I administration, analyzed in relation to the basal values of both groups of patients. There was a gradual decline in the three parameters, i.e., percentage of cells with CA (Fig. 1a), number of CA/cell (Fig. 1b) and number of dicentrics/cell (Fig. 1c), after 7 and 30 days and 1 year.

Figure 2 presents the mean values in both groups of patients of CA obtained for each parameter considered as a function of the time after ^{131}I administration. The basal values were similar between the groups, and statistical tests showed no significant difference ($p > 0.05$). A maximum value in the frequency of CA was seen 24 h after ^{131}I administration in relation to the basal values of both groups ($p < 0.05$), with no statistical significance between the groups ($p > 0.05$). A tendency for a gradual decline starting from 7 days after radioiodine administration was found, with lower values corresponding to group A. Statistical analysis showed a significance between the groups ($p < 0.05$) at 30 days and 1 year for the percentage of cells with CA (Fig. 2a) and at 30 days also for the number of CA/cell (Fig. 2b). Furthermore, the frequency of dicentrics was still higher in hypothyroid patients than in euthyroid patients (Fig. 2c) at 7 and 30 days after treatment, although the difference was not yet statistically significant ($p > 0.05$). It should be noted that the treatment with only the hormone did not induce any change in relation to basal values. It is also important to emphasize that the

Table 2 Mean frequencies ± SD (standard deviation) of structural chromosome aberrations (CA) observed in lymphocytes of patients from group A (rhTSH + ^{131}I, $n = 11$) and group B (^{131}I, $n = 10$), analyzed before and after administration of ^{131}I

Group	Sample	Number of analyzed cells	Structural CA						Cells with aberration (%)	Number of aberration/cell	Frequency of dicentric/cell
			Dic	Ring	Ace	Dm	Gap	Break			
Group A	Basal	2134	9	0	6	6	10	8	1.8 ± 1.0	0.018 ± 0.011	0.0042 ± 0.0038
	After rhTSH	1840	10	0	3	7	9	5	1.8 ± 1.1	0.018 ± 0.011	0.0054 ± 0.0042
	24 h	1801	46	1	16	20	24	11	6.3 ± 2.6	0.065 ± 0.027	0.0255 ± 0.0140
	7 days	2200	32	1	13	18	14	8	3.7 ± 1.6	0.039 ± 0.018	0.0145 ± 0.0099
	30 days	1904	13	2	11	9	12	3	2.4 ± 1.4	0.024 ± 0.015	0.0068 ± 0.0064
	1 year	1917	12	0	6	3	4	8	1.7 ± 1.0	0.017 ± 0.011	0.0063 ± 0.0058
Group B	Basal	2000	10	0	7	5	10	5	1.9 ± 1.4	0.019 ± 0.014	0.0050 ± 0.0053
	24 h	2000	42	1	20	20	18	9	5.5 ± 2.3	0.055 ± 0.023	0.0210 ± 0.0141
	7 days	1785	29	1	13	15	8	10	4.1 ± 2.2	0.043 ± 0.024	0.0162 ± 0.0113
	30 days	2100	24	0	10	10	13	7	3.0 ± 1.6	0.031 ± 0.017	0.0114 ± 0.0117
	1 year	1781	10	0	4	6	10	3	1.8 ± 1.3	0.019 ± 0.013	0.0056 ± 0.0047

Dic = dicentric, Ring = centric and acentric ring, Ace = acentric fragment, Dm = double minute, Gap = chromatic and chromosomic gaps, Break = chromatic and chromosomic breaks

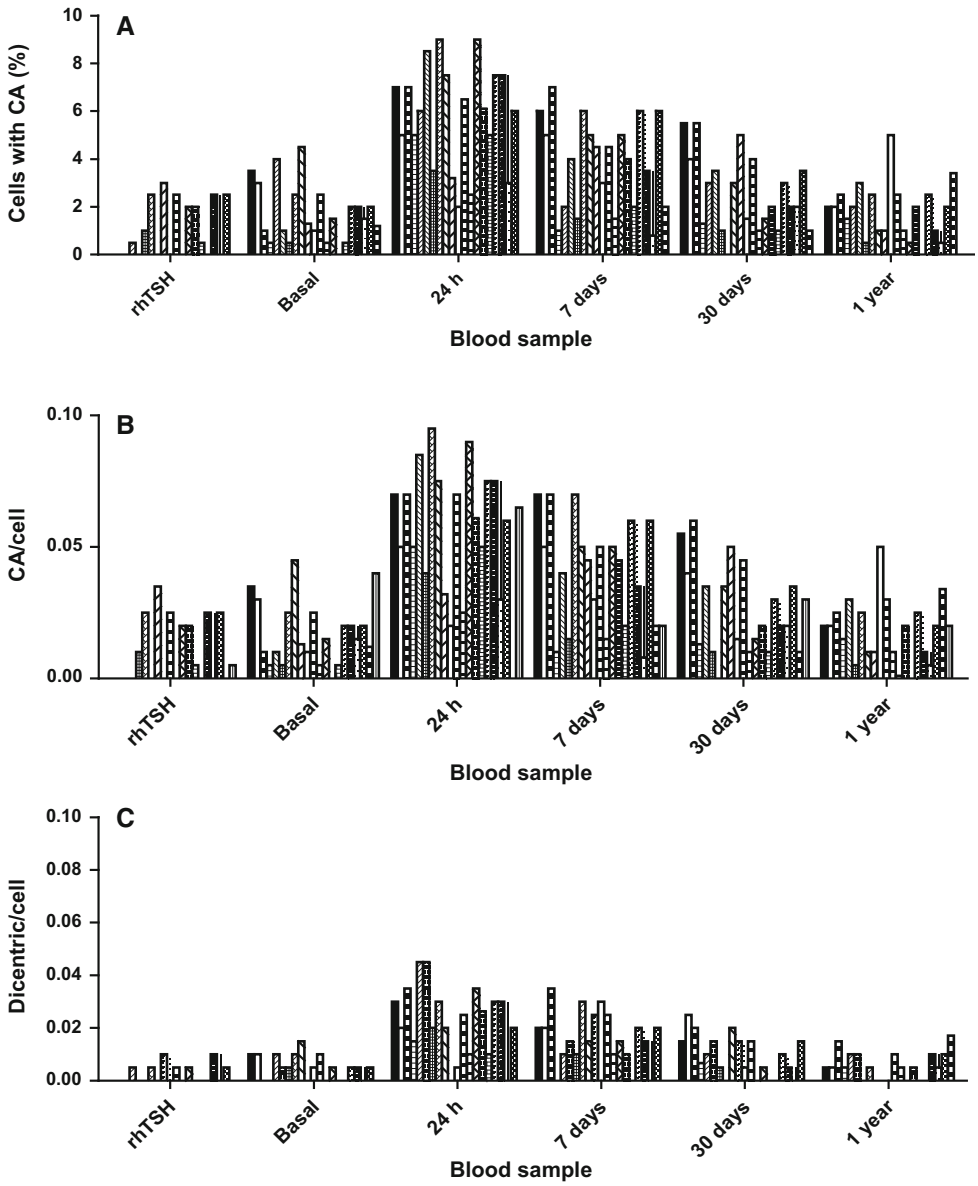

Fig. 1 Frequencies of chromosome aberrations (CA) observed in peripheral blood lymphocytes for each patient of the whole study group (Group A + B) ($n = 21$) before (rhTSH and basal) and after ^{131}I treatment (24 h, 7 days, 30 days and 1 year). **a** Percentage of cells with CA, **b** number of CA/cell, **c** number of dicentrics/cell

frequencies of CA declined to near-baseline levels 1 year after the ^{131}I administration in both groups.

The modal chromosome number ($2N = 46$) varied from 90.0 to 94.0 % (Table 3), and no positive correlation was observed with either ^{131}I or rhTSH administration. Table 3 also shows that the percentage of cells in the first mitotic division was higher than 93.0 % in both the basal and treated samples, suggesting that neither ^{131}I nor exogenous hTSH interferes in the cell cycle kinetics of the lymphocytes of DTC patients.

Cytogenetic data obtained from lymphocytes of healthy donors submitted in vitro to the different levels of activity of ^{131}I are given in Table 4. An increase in the frequency of CA was seen as a function of the activity of radioiodine 24 h after exposure. Figure 3 shows the activity–response curve for the induction of dicentrics in vitro as a function of the level of activity of Na^{131}I (0.074–0.740 MBq/mL), which corresponds to an absorbed dose range of 0.17 to 1.67 Gy, as estimated by the Monte Carlo program (Table 5). The data in Fig. 3 were fitted by a linear-quadratic model ($Y = C + \alpha A + \beta A^2$): $Y = 0.0066 \pm (0.0076) + 0.0374 \quad (\pm 0.0562)A + 0.0954 \quad (\pm 0.0743)A^2$, $R^2 = 0.9467$.

When the number of dicentrics/cell found in the peripheral lymphocytes of the two groups of patients 24 h after administration of radioiodine was introduced in the equation above, radioactive concentrations of 0.2903 ± 0.1005 MBq/mL for group A and of 0.2391 ± 0.1019 MBq/mL for group B were found. It can thus be considered that the cytogenetic damage caused in the two

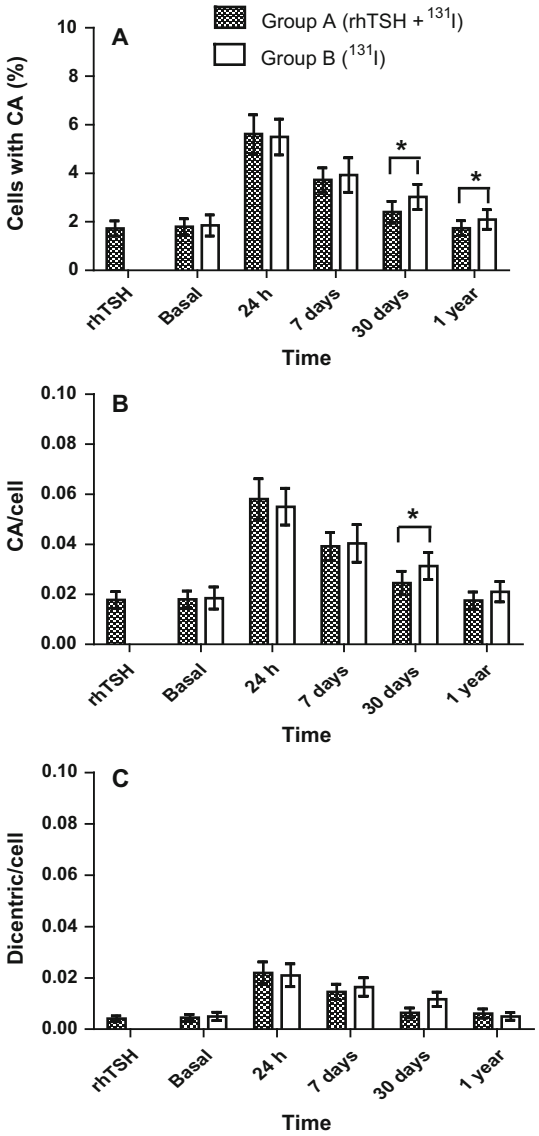

Fig. 2 Mean frequencies of chromosome aberrations (CA) obtained for the two groups of patients after ^{131}I treatment, as a function of time **a** percentage of cells with CA, **b** number of CA/cell, **c** number of dicentrics/cell. *Each error bar* indicates standard deviation of 11 (group A) and 10 (group B) independent individual determinations. $^{*}p < 0.05$

groups of DTC patients is equivalent to that induced in vitro by mean internal radiation doses of 0.65 ± 0.23 Gy and 0.53 ± 0.23 Gy, respectively, without statistical significance ($p > 0.05$), as calculated from Monte Carlo simulation.

Discussion

Ionizing radiation is a physical agent possessing a dual function: On the one hand, it acts as an inductor of cancer, but on the other it is a well-known therapeutic agent used in cancer treatment. Considering that our focus was the injury received by blood cells (lymphocytes), even recognizing the importance of the EANM guidelines (2008) for the internal physical dosimetry, we adopted a biological approach as an alternative strategy to assess the absorbed dose through blood lymphocytes, taking into account also that little information is available about the biological dosimetry in DTC patients subjected to internal irradiation, using Monte Carlo simulations.

The choice of the blood-collecting time points was based on literature data, taking into account the acute and late effects of radioiodine. According to the International Commission on Radiobiological Protection (ICRP 2012), the detectable tissue reactions in humans, resulting in substantial amount of damaged cells after relatively high doses of radiations, may occur early (days) or late (later than 90 days to years) after irradiation, depending on the specified level of injury on the particular tissue and on the sensibility of the method used to detect it. In the case of internal incorporation of radionuclides, IAEA (2011) recommends blood sampling 24 h after the radionuclides administration in order to reach equilibrium and thus allow the analysis of a homogenous and representative population of lymphocytes. Based on these observations, blood samples were collected to evaluate acute (24 h, taking into consideration the maximum 24-h ^{131}I thyroid uptake), early (1 week and 1 month) and late (1 year) cytogenetic damage in peripheral lymphocytes of DTC patients, as a function of time after ^{131}I administration.

In the present study, the incidence of CA induced in peripheral lymphocytes by radioiodine remnant ablation in DCT patients undergoing euthyroid (group A with rhTSH) or hypothyroid treatment (group B without rhTSH) was investigated and compared. Statistical analysis showed no difference ($p > 0.05$) between the groups in relation to the clinical parameters prior to radioiodine treatment. The high variability of the cytogenetic response observed between the patients (Fig. 1) can be due to the difference in radiosensitivity of each individual (Monsieurs et al. 1999).

Radioiodine therapy induced genotoxic damage in the patients, whose frequencies of CA decreased as a function of time. In general, euthyroid patients presented a lower total CA rate compared with hypothyroid patients, as a function of time after ^{131}I treatment (Fig. 2), although without statistical significance. As already mentioned, a statistically significant difference was found instead between the two groups for the percentage of cells with CA at 30 days and 1 year and for the number of CA/cell at 30 days after ^{131}I administration. One year after the administration of ^{131}I, the cytogenetic values returned practically to the basal values in both groups.

The kinetics of the decline in the number of MN or CA after genotoxic exposure are not yet clear. In fact, several

Table 3 Mean frequencies ± SD (standard deviation) of cells with modal ($N = 46$) and hypomodal number ($N = 45$ and 44) of chromosomes and % of lymphocytes in different cell cycles after treatment in patients of groups A (rhTSH + ^{131}I, $n = 11$) and B (^{131}I, $n = 10$), analyzed before and after administration of ^{131}I

Group	Sample	Number of analyzed cells	Number of chromosomes (%)		Mitotic cycles (%)	
			44 e 45	46	First division	Second division
Group A	Basal	2134	7.8 ± 0.4	92.2 ± 0.8	96.9 ± 0.9	3.1 ± 0.2
	After rhTSH	1840	8.4 ± 0.6	91.6 ± 1.9	97.9 ± 2.1	2.1 ± 0.2
	24 h	1801	8.4 ± 0.7	91.6 ± 3.3	94.9 ± 3.4	5.1 ± 0.5
	7 days	2200	9.1 ± 0.5	90.9 ± 0.5	95.6 ± 0.4	4.4 ± 0.4
	30 days	1904	7.7 ± 0.6	92.3 ± 1.7	97.1 ± 1.6	2.9 ± 0.3
	1 year	1917	6.2 ± 0.4	93.8 ± 1.3	96.7 ± 1.5	3.3 ± 0.3
Group B	Basal	2000	9.4 ± 0.6	90.6 ± 0.6	96.6 ± 0.5	3.5 ± 0.5
	24 h	2000	8.5 ± 0.5	91.5 ± 0.5	97.3 ± 0.2	2.8 ± 0.1
	7 days	1785	10.2 ± 0.7	89.8 ± 2.5	93.1 ± 2.4	6.9 ± 0.7
	30 days	2100	8.6 ± 0.6	91.4 ± 1.8	96.3 ± 1.7	3.7 ± 0.5
	1 year	1781	6.0 ± 0.4	94.0 ± 3.4	97.9 ± 3.1	2.1 ± 0.3

Table 4 Mean frequencies ± SE (standard error) of structural chromosome aberration (CA) observed in vitro in healthy donors from group C ($n = 5$), analyzed under different concentrations of ^{131}I for 24 h

Group	Radioactive concentration (MBq/mL)	Number of analyzed cells	Structural CA						Cells with aberration (%)	Number of aberration/cell	Frequency of dicentric/cell
			Dic	Ring	Ace	Dm	Gap	Break			
Group C	0.000	1385	0	0	0	10	6	2	1.4 ± 1.3	0.015 ± 0.012	0.0000 ± 0.0000
	0.074	962	13	1	14	15	7	3	7.5 ± 5.0	0.075 ± 0.050	0.0156 ± 0.0193
	0.185	980	20	1	13	11	11	9	7.9 ± 4.0	0.079 ± 0.040	0.0200 ± 0.0176
	0.370	926	26	6	23	13	4	3	10.1 ± 4.8	0.101 ± 0.052	0.0382 ± 0.0339
	0.555	591	28	3	23	13	10	3	15.8 ± 7.5	0.157 ± 0.075	0.0450 ± 0.0304
	0.740	336	28	4	21	16	18	0	28.1 ± 14.9	0.285 ± 0.144	0.0920 ± 0.0534

Dic = dicentric, Ring = centric and acentric ring, Ace = acentric fragment, Dm = double minute, Gap = chromatic and chromosomic gaps, Break = chromatic and chromosomic breaks

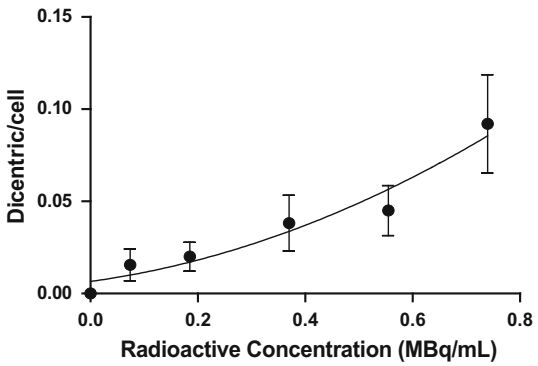

Fig. 3 Dose–response curve obtained in vitro for peripheral lymphocytes of healthy donors (Group C), submitted to different levels of radioactivity of ^{131}I for 24 h, adjusted according to the model ($Y = C + \alpha A + \beta A^2$) for the induction of dicentrics. *Each data point* represents the mean ± SE (standard error) of 5 independent experiments

Table 5 Values of absorbed dose as a function of the radioactive concentration of ^{131}I for 24 h of exposure, obtained in vitro from peripheral lymphocytes of healthy individuals, according to Monte Carlo simulation

Activity (MBq/mL)	Absorbed dose (Gy)
0.074	0.17
0.185	0.42
0.370	0.83
0.555	1.25
0.740	1.67

studies have reported results that differ somewhat from our data. Gutiérrez et al. (1999) and M'Kacher et al. (1997) found a gradual decrease in CA and MN frequencies with time after ^{131}I treatment, but the cytogenetic values in the samples taken 1 year after therapy were still above the

baseline values. Ballardin et al. (2002) showed a peak of MN frequency 7 days after ^{131}I treatment that declined thereafter, reaching the baseline levels after 6 months. Other authors (Gil et al. 2000) reported a significant and persistent increase in chromosome damage induced by ^{131}I treatment in DTC patients. On the other hand, using the comet assay, Gutiérrez et al. (1998) did not find any significant difference 1 week after therapeutic radioiodine administration in relation to the basal values of DTC patients. The gradual decline with time of the presence of cells with genetic damage in the peripheral circulation of ^{131}I-receiving patients may be in part a consequence of various integrated mechanisms, such as the selective death of lymphocytes damaged by radioiodine, the normal turnover of cells, repair mechanisms, capture by the reticuloendothelial system and the renal clearance of ^{131}I, among others factors (Gutiérrez et al. 1999; Erselcan et al. 2004).

Many studies have reported a faster renal clearance of ^{131}I (approximately 50 %) in patients submitted to rhTSH therapy in relation to THW therapy (Luster et al. 2005; Hänscheid et al. 2006), accounting for a faster excretion of radioiodine activity with a consequently lower retention of whole-body ^{131}I. Thus, the higher cytogenetic values observed in hypothyroid patients in relation to euthyroid patients in the present study may be related to the status of the former patients at the time of ^{131}I administration, a condition that decreases renal clearance of radioiodine and thus increases whole-body retention of ^{131}I, consequently resulting in a higher blood radiation dose. Furthermore, when a specific type of CA, i.e., the dicentric chromosome, is employed as a biodosimeter (Pinto and Amaral 2014), its frequency was found to be higher in hypothyroid patients than in euthyroid patients, although not significantly. Considering that the maximum ^{131}I thyroid uptake occurs 24 h after the administration (Zanzonico 1997), this period of time permits the estimation of the maximum value of radiation absorbed dose received by the patients.

Assuming that the blood cells (lymphocytes) of the patients were exposed homogeneously to radiation (Serna et al. 2008), the activity–response calibration curve obtained in vitro can be used to approximately determine the equivalent absorbed dose for the whole body, since blood and bone marrow are considered to be the critical organs in this approach (Catena et al. 2000; Serna et al. 2008; Lassmann et al. 2010; Reiners et al. 2012). Because the blood stream receives radiation from the radioiodine distributed in the body, it can be assumed that the radiation dose affecting lymphocytes is representative of the average whole-body or bone marrow doses (Catena et al. 2000; Serna et al. 2008), which are mainly responsible for the short- or long-term effects of ionizing radiation, including radionuclide therapy.

A comparison of the frequency of the CA or MN among lymphocytes irradiated in vivo and in vitro by ^{131}I may allow a cytological assessment of internal radiation absorbed dose (Watanabe et al. 1998). According to the International Atomic Energy Agency (2011) and Serna et al. (2008), the estimate of absorbed dose by the whole body via biological methods is possible even in the case of internal incorporation of radionuclides, since these disperse fairly uniformly through the body. The most commonly used tool for performing quantitative predictions in biological dosimetry is the elaboration of a dose–response curve (activity vs. aberration yield) in which lymphocytes have been irradiated in vitro with a particular radionuclide: This may allow a prediction of the in vivo absorbed dose by the patient's circulating lymphocytes (IAEA 2011).

Using the formalism of the Medical International Radiation Dose (MIRD), Hänscheid et al. (2009) estimated that about 14 % of the administered activity of ^{131}I (3.7 GBq, in general) is found in the whole blood 1 or 2 days after administration, i.e., the mean activity concentration during the first 24 h after 3.7 GBq ^{131}I administration is lower than 0.1 MBq/mL in vivo. In order to elaborate a calibration curve in our study, the activity of 3.7 GBq of ^{131}I, traditionally administered in DTC patients, was used as reference starting point for the radioactive concentration range applied in the in vitro curve, starting from a maximum value of 0.74 MBq/mL down to a value 10 times lower (0.074 MBq/mL—equivalent to absorbed dose of 0.17–1.67 Gy). This range was apparently adequate to include the level of radioinduced damage (dicentrics/cell) obtained for both groups of patients 24 h after ^{131}I administration. Using a similar approach, other authors elaborated a calibration curve with similar dose range to estimate the internal radiation absorbed dose due to ^{131}I. M'Kacher et al. (1996) determined in vitro activity–response curves (from 0.1 to 3 Gy) for lymphocytes exposed to ^{131}I and ^{60}Co and compared the frequency of CA obtained for the DTC patients treated with 3.7 GBq ^{131}I to in vitro data. Curves obtained in vitro after irradiations were fitted using the linear-quadratic model for frequencies of translocations (via fluorescence in situ hybridization FISH) and dicentrics (via conventional analysis). Using the MN technique, Watanabe et al. also calculated the internal radiation absorbed dose of ^{131}I in DTC patients, through the construction of an activity–response curve in vitro from 0.25 to 1 Gy (1998) and from 0.5 to 2.0 Gy (2004) for external irradiation by electron beams, fitted by a linear function ($Y = c + \alpha D$). Serna et al. (2008) used a higher dose range (from 0.3 to 4.5 Gy) for the construction of a calibration curve for MN induction, fitted by a linear-quadratic model. According to the IAEA (2011), the lowest in vitro dose detected by

conventional chromosomal aberration technique is 0.1 Gy (100 mSv) for low LET radiation.

In the present study, we adopted a linear-quadratic model based on the Akaike information criterion (AICc) and on IAEA (2011) recommendations for dicentric induction by low LET radiation, taking into consideration also the biophysical background for chromosome damage and the mechanism of dicentric formation at the cellular level induced by ionizing radiation (Hall 2000).

Literature data have shown that the estimated radiation absorbed dose due to ^{131}I therapy, calculated using the biological parameter, may vary depending on the time period analyzed after treatment, number of individuals analyzed, the conditions of treatment, besides the procedure and parameter used for its determinations. Using the MN technique, Monsieurs et al. (1999) showed that the mean equivalent absorbed dose was 0.32 Gy, 1 week after iodine administration. M'Kacher et al. (1996) estimated the absorbed dose to be 0.54 Gy by conventional CA and 0.48 Gy by chromosome 4 painting, 4 days after the administration of 3.7 GBq of ^{131}I in peripheral blood samples of DTC patients. Watanabe et al. (2004) determined that the cytological radiation damage to B lymphocytes in vivo, 1 week after ^{131}I therapy with 3.7 GBq in DTC patients, was equivalent to the damage observed after a mean external irradiation absorbed dose of 0.45 Gy in vitro. Serna et al. (2008) showed that the estimated blood dose after 3 days of ^{131}I exposure to 3.7 GBq was 0.75 Gy (0.197 mGy/MBq) based on the MN assay in thyroid cancer patients. Using the chromosomal translocations technique, Frigo et al. (2009) showed that the red marrow dose per unit activity was lower in rhTSH-treated patients (0.22 Gy, 0.06 ± 0.01 mGy/MBq) compared with hypothyroid DTC patients (0.37 Gy, 0.10 ± 0.03 mGy/MBq), 45 days after ^{131}I treatment with 3.7 GBq. By physical dosimetry, Hänscheid et al. (2006) obtained mean blood absorbed dose values of 0.40 Gy (0.109 mGy/MBq) in a group of euthyroid patients and of 0.62 Gy (0.167 mGy/MBq) in a THW group, after 3.7 GBq of ^{131}I administration.

In the present study, the absorbed dose was estimated by biological dosimetry as 2.2 Gy per MBq/mL, 24 h after administration of ^{131}I by Monte Carlo simulation (Table 5), considering a correction factor of 0.9585, which corresponds to 24-h radioactive decay. Using internal physical dosimetry method (e.g., OLINDA/EXM), the expected dose after 24 h will be 2.6 Gy per MBq/mL, which is higher than that deduced in the present study. In our opinion, this difference between the values obtained by physical method and those obtained by the cytogenetic analysis via Monte Carlo simulation can be attributable to the two distinct procedures and to inter-laboratory variation mentioned. Indeed, literature data have shown that the estimated radiation dose due to ^{131}I may vary within and between laboratories, even when using the same procedure and parameter for its determination.

Although it is advisable to use care in extrapolating in vitro data to the clinical situation, our study shows that the cytogenetic damage to peripheral lymphocytes in vivo 24 h after ^{131}I therapy may be equivalent to the damage observed in vitro for mean absorbed doses of 0.65 Gy and 0.53 Gy for group A and group B, respectively, as estimated by the Monte Carlo program. The values obtained are somewhat higher than those obtained by the other authors, possibly because the estimate was carried out 24 h after treatment with a higher activity of ^{131}I (5.9 ± 0.4 GBq).

The genotoxicity data show that, within the first 24 h after ^{131}I treatment, the two groups of patients presented similar high levels of damaged lymphocytes, independent of whether they had received rhTSH or not. However, a gradual decline of CA frequency with time was seen, somewhat higher in group A than in group B, as shown in Fig. 2. No aneugenic effect mediated by radioiodine was found in the peripheral lymphocytes of the patients. 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 the blood lymphocytes of DTC patients.

Our data are in agreement with previously published reports (Remy et al. 2008; Taïeb et al. 2010; Reiners et al. 2012), showing that radioiodine therapy, as well as rhTSH administration, is safe and effective procedures for the treatment of patients with DTC. The best quality of life for the DTC patients is obviously provided by rhTSH-aided therapy (Hänscheid et al. 2006; Rosário et al. 2008). After administration of high activities of ^{131}I, the absorbed dose remained well below the 2 Gy threshold in the blood in all patients and treatments, which has long been considered to be a safe threshold level for all radioiodine therapies, avoiding serious myelotoxicity (de Keizer et al. 2004). In both the in vivo and in vitro studies, ^{131}I-irradiated lymphocytes were used under similar exposure conditions and in the same assay system. It is important to emphasize, however, that, under the in vitro conditions in our study, renal clearance was not considered: ^{131}I remained in direct contact with the blood for 24 h. A factor of 0.9585 was, moreover, applied to the result, taking into account the 24-h decay correction. There is, however, a clear need for further investigation on a much larger number of patients submitted to ^{131}I therapy, in order to increase the statistical approach and provide a more accurate evaluation of the genetic risk involved.

Conclusions

A general conclusion that can be drawn from this study is that DTC patients on euthyroid therapy presented lower frequencies of CA than those on hypothyroid therapy. Within 24 h after ^{131}I treatment, however, both groups showed similar levels of genotoxic damage, with cytogenetic values in both groups returning practically to the normal range after 1 year, with or without administration of rhTSH.

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Compliance with ethical standards

Conflict of interest The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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