DNA measurements after radiation-induced tissue structure of thyroid gland of rats

Primeira submissão em 17/01/05
Última submissão em 31/03/05
Aceito para publicação em 13/06/05
Publicado em 20/06/05

Análise do conteúdo de DNA (ploidia) em tecidos de glândula tireóide de ratos após indução por radiação

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key words

Thyroid cancer

Irradiation

DNA content (ploidy)

abstract

Introduction: Thyroid gland exposures to radiation induce nuclear chromosomal alteration. Objective: To evaluate the DNA content of thyroid gland submitted to radiation. Materials and methods: We radiated 75 rats while 25 were not radiated to be used as control group. Exposure was conducted by the use of Cobalt-60 radioactive source in the right anterior cervical region in a field of 3-30cm, comprising the second and the sixth tracheal rings with 600-centigray (cGY) doses. The DNA content (ploidy) was obtained with Feulgen-thionin stain and was quantified with CAS 200 quantitative measurement equipment. Results: Diploid pattern was obtained in 88 cases (95.7%), independently of time of exposure: on the other hand, aneuploidy was observed in four cases (4.3%) only in the group sacrificed to the 33 days. Eight cases were excluded due to technical reasons. Conclusion: The early aneuploid pattern found in our study certainly corroborated that radiation affects thyroid gland with important consequences in terms of disorders.

unitermos

Câncer tireóideo

Irradiação

Conteúdo de DNA (ploidia)

resumo

Introdução: A exposição da tireóide à irradiação está associada à alteração do componente cromossômico nessa glândula. Objetivo: Avaliar o conteúdo de DNA (ploidia) em glândula tireóide submetida à radiação. Material e métodos: Foram irradiadas tireóides de 75 ratos; 25 foram usados como grupo controle e não sofreram irradiação. A exposição à irradiação foi realizada com o uso de Cobalt-60 na região cervical anterior direita com espaço de 3-30cm, com anel traqueal de 2º e 6º e dose de 600 centigrays (cGY). O conteúdo de DNA (ploidia) foi obtido com o método de Feulgen-Thionin e quantificado com o aparelho CAS 200 de citometria estática. Resultado: Dos 92 casos, 88 (95,7%) foram diplóides, independente do tempo de exposição; aneuploidia foi observada em quatro casos (4,3%), somente no grupo sacrificado aos 33 dias. Oito casos foram excluídos por problemas técnicos. Conclusão: A irradiação afeta muito precocemente o conteúdo de DNA nuclear levando a aneuploidia.

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Introduction

Malignant thyroid cancer of differentiated type is believed to have a good prognosis. Worst behaviour is expected from those carcinomas of aggressive type such as insular or oxyphilic carcinomas[1]. Usually, and in spite of exceptions, patients' death is not directly associated to thyroid differentiated carcinomas[2]. Currently, a statistically significant increase of thyroid cancer incidence has been observed, but, conversely, mortality rates have shown a statistically significant decrease[3].

Radiation effects on thyroid tissue are well known for almost a hundred years. Hypothyroidism, thyroiditis and cancer are part of the radiation-induced disorders reported[4]. Presently, radiotherapy is commonly used for treatment of several neoplasias, including those of the head, and neck malignancies. As a consequence, non-target organs, including the thyroid, are affected by radiation[4]. The susceptibility of thyroid tissue to radiation-induced damages was demonstrated in epithelial cell culture model[5]. The thyroid is one of the organs most likely to produce clinically significant abnormalities after therapeutic external radiation. Direct or incidental thyroid irradiation increases the risk for well-differentiated, papillary, and follicular thyroid cancer from 15 to 53-fold. Thyroid cancer risk is highest following radiation at a young age. This risk decreases with the increasing age at treatment, but increases with follow-up duration[6]. Abnormal nuclear DNA content has been reported in the majority of the malignant tumours, and aneuploidy strongly correlates with malignant conditions of the thyroid gland[7, 8].

Summarizing, the knowledge of induction of thyroid cancer in humans by ionising radiation is still limited despite the permanent efforts. Shore[9] described some problems to be solved, which include the average estimation of thyroid cancer risk following external irradiation, the effects of age on thyroid cancer induction, shape of the dose-response curve for acute irradiation, magnitude of risk at low doses, effects of dose fractionation or dose protraction, the relative effectiveness of iodine-131 (131I) in inducing thyroid cancer compared to external radiation, the temporal course of radiogenic thyroid cancer risk, mortality caused by thyroid cancer, host-susceptibility factors for radiogenic thyroid cancer, and biological factors involved in the estimative of risk calculation. The magnitude of Chernobyl reactor accident, for example, has not been properly quantified yet. Recent data exhibit marked increases in the incidence of thyroid cancer among all ages, mainly among children, in regions neighbouring the area of the accident[10]. Experimental data to assess more information on thyroid cancer risks with fractionated radiation exposures are scarce and difficult to be performed[11].

The objective of our work was to study the thyroid gland of healthy naive Wistar rats exposed to radiation injury and to evaluate the DNA content (ploidy) by computer-assisted cytometry.

Material and methods

The subjects were 100 healthy naive isogenic Wistar rats weighting around 55g from an authorised rat-breeding unit of the Medicine School of Universidade de São Paulo, São Paulo, Brazil. All animals were housed with *ad libitum* access to water and laboratory diet. The rats were randomly divided in four groups of 25 subjects each, according to the time of observation after radiation (groups I to IV).

Radiation was provided to 75 rats, whereas 25 not radiated served as a control group. The animals were anaesthetized and immobilized in a plate in dorsal decubitus position, with head arrangement positioned to expose the thyroid gland region. A luminous focus was used to determine the area to be irradiated. Exposure was conducted by the use of Cobalt-60 radioactive source in the right anterior cervical region in a field of 3-30cm, comprising the second and the sixth tracheal rings with 600 centigray (cGY) doses (build-up of point calculation).

During the follow-up period, animals from groups II, III, IV were housed in identified acrylic cages with five rats each. The animals were provided with *ad libitum* water and standard food. Daily, the animals were examined in order to observe their vital signs and comportment.

The groups

The groups were divided in different periods in order to observe eventual changes in DNA content in time.

1. Group I: The rats were killed just after radiation exposure;

2. Group II: The rats were killed 20 days after the radiation;

3. Group III: The rats were killed 33 days after the radiation;

4. Group IV: The rats were killed after 33 days but did not receive radiation. This is supposed to be a control group.

The animals were killed by individualized ethylic ether exposition. The thyroid glands were removed and fixed in buffered 10% formaldehyde solution.

DNA content evaluation

The methods of staining the samples and analysing the DNA content followed the protocol previously published by our group[12, 13]. Sections of 5µ were cut and placed in 10% neutral formaldehyde for 30 minutes and air-dried. Subsequently, hydrolysis was performed with 5mM HCl for 60 minutes at room temperature. The Feulgen-Thionin (Becton & Dickinson, USA) stain was applied, and coverslips were mounted with Entellan (Merck, Germany). The DNA content was quantified with CAS 200 quantitative measurement 3.0 program (version 8.1) (Becton & Dickinson, USA). The nuclei of thyroid follicular epithelium were examined with an image enhancement of 400x; ploidy was determined after examination of 150 to 200 cells per case, excluding the cellular overlapping. For external control and to calibrate the DNA content of cells for the diploid reference of the G0/G1 peak, we used the DNA content quantification of rat hepatocyte (slide control, Becton & Dickinson), also stained by Feulgen-Thionin. Cases where the DNA content exhibited the modal G0/G1 peak in a similar position to the observed in those showed in normal reference cells were classified as diploid. In fine-tuning, we used the DNA content quantification of rat hepatocyte (slide control, Becton & Dickinson), also stained by Feulgen-Thionin. Fifty lymphocytes were selected within the in-study slide and quantified as an internal control. The DNA index (DI) was represented by histograms with a logarithmic scale, and the nuclear DNA content (ploidy) was based on the following classification: diploid (DI = 0.90-1.1); tetraploid (DI = 1.8-2.2); aneuploid (DI = indices different from the previous ones).

Results

From the 100 rats used in this study, eight were excluded due to technical problems related to the poor representative tissue samples; the Feulgen stain did not decorate the nuclei adequately, in spite of our efforts. The DNA content evaluation from the remaining 92 cases is depicted in the **Table**. We observed a remarkable predominance of diploid pattern along the groups, with 88 cases (95.7%), and four (4.3%) of aneuploid. Considering group III, the aneuploid matched four in 24 subjects, and the aneuploid index reached 16.6%. Aneuploid histogram is represented in the **Figure**. All cases from the control group were diploid and non-changes were observed in architectural structure of the gland.

No significant histological alteration was observed. In general, the structure of the glands was maintained. Few exceptions comprised small figures of fibrosis and oedematous areas. Vascular congestion was also observed. In glandular follicles, nuclear alterations represented by mild dyskariosis were seen. No adenomatous/hyperplastic alterations were identified.

Discussion

Cancer radiotherapy is a worldwide-accepted method for several neoplasias. The radiation-induced malignancies following radiotherapy for cancer have been studied, and the comparison of radiotherapy and non-radiotherapy cohorts showed an elevated relative risk for the former[14].

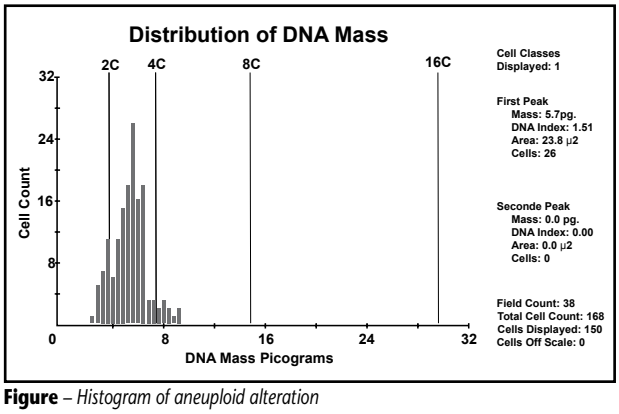

Figure – *Histogram of aneuploid alteration*

Table Cases according to the groups and DNA content evaluation

Group	Number of animals	Diploid pattern	Aneuploid pattern
I	23	23	0
II	20	20	0
III	24	20	4
IV	25	25	0
Total	92 (100%)	88 (95.7%)	4 (4.3%)*

**This value represents 4.3% in 92 subjects; considering group III this percentage is 16.6%.*

The susceptibility for DNA content alteration to occur by radiation exposure is, indeed, well documented as previously mentioned. Because of that, DNA content is assumed as an important marker of nuclear disarrangement and is closely related to thyroid neoplastic transformation[7, 8].

In our study, we irradiated thyroids of rats in order to test the hypothesis of DNA content alteration in different periods after radiation exposure.

We attempted to monitor any DNA alteration in three groups with time of radioactive effects ranging from zero to 33 days after exposure, because thyroid is recognized as a very sensitive organ to radiation-induced disorders[4]. Our results showed alterations in DNA content in the 33-day group. Four (16.6%) out of 24 cases exhibited aneuploidy. This is quite interestingly because this fact reinforces the premise that patients who receive therapeutic external radiotherapy require careful follow-up due to likelihood of consequent morbidity induced by radiation[6]. We chose an equivalent dose used in medullar transplant of children with leukemia disease[15]. Undoubtedly, we can hypothesize that higher doses could induce more aneuploid cases, but our choice was made trying to maintain a correlation with the current well-tested protocols of radiotherapy.

Despite prominent radiation sensitivity of endocrine organs, DNA aberration initiated by radiation does not necessarily correlate with DNA content measurement. As reported by Komorowski *et al.*[16], different types of thyroid carcinomas associated to radiation exposure were diploid. Conversely, an experimental study with rats conducted by Christov *et al.*[17], using normal and induced hyperplasia and benign and malignant thyroid neoplasias, showed that DNA content of control animals were diploid for all subjects, such as the cases of hyperplasia (except for few cells), but adenomas and carcinomas scattered hyperdiploid cells suggesting that an increased number of cells have entered the cell cycle. Our previous report with this subject has shown that immunohistochemical markers of the cell cycle are significantly expressed in post-radiated thyroids[18]. The early aneuploid pattern found in our study positively corroborates that radiation affects thyroid gland importantly, with plausible consequences in terms of disorders. If this DNA content alteration is directly involved in thyroid neoplasms it is not so clear if this fact is the cause or the effect of neoplastic transformation[18]. The prognostic value of DNA content measurement is more reliable for prognostic purposes than diagnostic[17]. As stated by Shore[9], there are several questions to be answered in radiation-induced lesions of the thyroid. Further experimental studies might be designed to this goal.

Conclusion

The thyroid gland exposition to radiation can induce aneuploid pattern as we observed in our study. The aneuploid cases occurred independently of the morphological alterations, which were faintly and not significant.

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