ORIGINAL CONTRIBUTION

Low Dosage of $^{131}$Iodine Effects on Chromosomes

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The aim of this study was to evaluate the effect on human lymphocyte chromosomes of the $^{131}$I dosage used in scintigraphy on thyroid patients. Until now, there has been as absence of conclusive reports on the effects produced by such dosage. Samples were obtained from 21 patients, and the blood was collected in two occasions: Twenty-four hours prior (control) and after administration of the radionuclide (test). Cells were placed in 1640 RPMI medium with bovine calf serum and incubated with phytohaemagglutinin for 48 and 72 hr at 37°C. Chromosomes were stained with Giemsa Gurr (2 percent, pH = 6.8), and analyzed by two independent investigators by optical microscopy. Of the 6,300 metaphases analyzed from the 48- and 72-hr cultures, 1,146 and 216 gaps and 682 and 52 breaks were found in the test group, respectively. Of the 6,300 metaphases analyzed from the control group, 291 gaps and 119 breaks were observed in the 48-hr cultures whereas in the 72-hr cultures, 10 gaps, and no breaks were found. Our results show that $^{131}$I is responsible for the observed chromosome alterations (paired t-test, p < .05). We suggest re-evaluating the use of $^{131}$I and replacing it with the $^{123}$I, mainly on those patients at fertile age.

INTRODUCTION

Chromosomes are long strands of deoxyribonucleic acid (DNA) in a double-stranded helical configuration. Each human lymphocyte has 46 chromosomes in 23 pairs. A chromosome contains about 100,000 genes, and each gene about 1000 nucleotides. Chromosomes, with their large molecular structure, undergo complex reactions during their duplication, segregation, and differentiation. This structure can be altered by many physical and chemical reactions [1].

Two types of chromosomal abnormalities are described: (a) changes in structural and (b) changes in the chromosome number. Both of these cause genetic disturbances in the cell. Agents that cause chromosomal breakage are clastogens and...
those causing numerical changes are mitotic poisons [2].

There are four types of alterations due to chromosome breakage: acentric fragments, minutes, rings, and dicentrics. Acentric fragments result from simple breaks across the chromosome, while minutes are usually produced from two breaks close together. Rings are the result of exchange between two breaks on the chromosomes. Dicentrics result from an exchange between two broken chromosomes, yielding a chromosome with two centromeres and an acentric fragment. The first three kinds of aberrations involve changes within a single chromosome and result in the loss of a chromosome fragment at mitosis. If the loss is large, it will be lethal. If the loss is small, however, the cell may be viable and transmit the deficiency to the daughter cell. Dicentric aberrations, involving two chromosomes, are usually lethal. Since DNA dominates the growth and differentiation of the cell, alterations in DNA strands may be serious and can lead to mutations or oncogenesis. Spontaneous aberrations have been found in all normal populations. The incidence varies with age, geographic location, and smoking habits [1].

According to Schwartz, 1998 [3], there are many variables that can influence radiation sensitivity and that might account for inter-individual differences in radiation sensitivity. These include variations in sensitivity to induction of DNA damage, changes in DNA repair, in cell growth and in proportions of cells in different phases of the cell cycle, in cell cycle checkpoint response, and in DNA content. Each parameter has been shown to vary from cell to cell, and in some cases rough correlations between one or more variables and radiation sensitivity have been reported. For example, diploid cells tend to be more radiosensitive than aneuploid cells, and more radiosensitive cells have sometimes been shown to grow more slowly than more resistant cells. There is, however, a growing body of evidence suggesting that alterations in the rate, fidelity, and total fraction of DNA double-strand breaks repaired in a cell is the primary factor underlying the differences in sensitivity, both histology-based and inter-individual. Furthermore, these variations in repair may be related to alterations in chromosome structure or organization [3].

Radioactive iodine scintigraphy is commonly used for diagnostic of thyroid diseases. $^{131}$I is a $\beta$ emitter with a mixture of $\gamma$-rays, which has a half-life of approximately eight days. It has been postulated that around 90 percent of the radiation effects are a result of the $\beta$-radiation [4].

It is difficult to establish an accurate relationship between low doses of radiation and biological effects [4]. Thus, in this paper we show the results of cytogenetic analysis of patients that were submitted to thyroid scintigraphy with $^{131}$I.

PATIENTS AND METHODS

Twenty-one patients with suspicion of thyroid diseases aged 21 to 79 years, from Serviço de Endocrinologia, Hospital Universitário Clementino Fraga Filho, Rio de Janeiro, Brazil, were submitted to thyroid scintigraphy with $^{131}$I. The indications for examination were hyperthyroidism, nodular goiter, Grave's disease, and multinodular goiter. The institutional review board approved the study protocol. Fully-informed written consent was obtained from all patients. The patients answered a questionnaire including information on smoking habits, drug intake, exposure to ionizing radiation, alcoholic intake, and clastogenic drugs. Those subjects who had been taking drugs or had been exposed to radiation for diagnostic or therapeutical purposes were excluded.

The radionuclide $^{131}$I, with a mean activity of 3.7 MBq was orally administered to these patients.
The same individual provided the control and the test samples. For this, two blood samples were withdrawn in sterile heparinized tubes from each subject in a follow-up study. The first sample was obtained before the $^{131}$I administration and was used as control. The test sample was taken 24 hr after the $^{131}$I administration.

Cells in metaphases were obtained by stimulating blood cells with phytohaemagglutinin in RPMI 1640 medium with 20 percent calf serum. Cultures from both test and control samples were incubated for 48 and 72 hr at 37°C. All the cultures and slides for the chromosome aberration assay were prepared following a modification of the technique described by Moorehead, 1960 [5].

Three hundred well-spread metaphases were analyzed for each incubation time and for each subject. Chromosome aberrations were scored according to Bender et al., 1988 [6], by two independent investigators; disagreement was resolved by a third reviewer and consensus. Slides were coded during analysis in a blind study, without knowledge whether the slides were from test or control groups.

Chromosomes were analyzed by light microscopy, and photomicrographs were taken when needed. Data were statistically analyzed by paired t-test.

**RESULTS**

Samples from 21 patients with different underlying thyroid dysfunctions were analyzed with regard to chromosomal abnormalities following $^{131}$I treatment, using as controls their own blood samples taken before treatment.

A total of 25,200 metaphases were scored, corresponding to 300 metaphases per patient, per point. Of the 6,300 metaphases analyzed from the control samples, after 48 hr in culture, 5,890 were normal, and only 410 were abnormal, with 291 gaps and 119 breaks observed. Of the 6,300 metaphases analyzed from the test samples 1828 were abnormal with 1,146 gaps and 682 breaks while the other 4,472 metaphases were considered as normal. In the 72-hr cultures, only 10 metaphases of the 6,300 analyzed from the control samples were abnormal. On the other hand, in test samples, 268 metaphases were abnormal with 216 gaps and 52 breaks. The other 6,032 metaphases were considered as normal. The 48- and 72-hr distribution of normal and abnormal metaphases from control and test groups can be observed in Figure 1.

Table 1 shows the distribution of gaps and breaks found in abnormal metaphases in control and test samples after 48- and

![Figure 1. Distribution of normal and abnormal metaphases.](image-url)
72-hr cultures. Figures 2 and 3 show chromosomal studies with a gap and a break, respectively.

**DISCUSSION**

There are a number of studies correlating biological dosimetry and cytogenetic analysis, including studies relative to: clinical exposure [7-10], nuclear accidents [11, 12] and occupational exposure [13-16]. All these reports involve people that have received high dosages of ionizing radiation.

The importance of the present study refers to the paucity of studies on the cytogenetic effects produced by low ionizing radiation dosages (3.7 MBq of $^{131}$I). Only one similar study using cytogenetic analysis of low ionizing radiation dosages of $^{99}$mTc in vitro was described [17].

In our study, a number of abnormal metaphases, significantly different from those seen in control samples, were seen in lymphocytes obtained from patients 24 hr after they had been exposed to $^{131}$I. The chromosomal alterations observed were gaps and breaks. It has been described that the incubation period can affect the frequency of chromosomal aberrations [16, 18]. We observed that the frequency of chromosomal alterations decreased with the increase of the incubation time, from 48 to 72 hr. This could be explained by anaphases loss of abnormal chromosomes during mitosis.

**Table 1. Abnormal metaphases results in 48 and 72-hr cultures.**

|        | Test group | Control |
|--------|------------|---------|
|        | 48 hr | 72 hr | 48 hr | 72 hr | Total |
| Gaps   | 1146  | 216   | 291   | 10    | 1663  |
| Breaks | 682   | 52    | 119   | 0     | 853   |
| Total  | 1828  | 268   | 410   | 10    | 2516  |
Chromosomes are extremely thin at the gap region. This may facilitate the occurrence of breaks. Despite being a serious chromosomal aberration, DNA repair can occur at the gap region and the capacity to repair will depend on individual differences in radiation sensitivity [14]. In the case of induced aberrations, as described here, there was a significant increase of gap and this probably can saturate the natural repair mechanism [7, 17, 19, 20].

Breaks are chromosomal alterations where complete separation of a chromosomal region occurs. Since DNA is completely interrupted in that region, there is no possibility of repair, facilitating the occurrence of deletions and translocations. Due to their stability, translocations are not only important for biodosimetry, but are implicated in cancer and a variety of genetic diseases and, thus, represent a risk to health [21]. Like gaps, the breaks were significantly increased after the $^{131}$I administration, at both incubation periods of time [15, 22], as shown in Table 1.

Although in a lesser amount, the same types of chromosomal alterations were also found in control samples (48 and 72 hr), however, there were no chromosomal breaks at 72 hr. The decrease in the number of gaps and breaks in 72-hr cultures, both in test and control samples, might be explained by the loss of abnormal chromosomes after 48-hr cultures and also by the cells natural repair mechanism.

A large proportion of the radiation damage to chromosomes is repaired spontaneously within 30 to 90 min. Nevertheless, some chromosomal aberrations may persist for many years. For example, one group of spondylitic patients who had received 1500 rad of partial-body irradiation (whole spine and sacroiliac joints) was followed for 20 years by Buckton et al. The cytogenetic changes in blood lymphocytes persisted during the entire period but with a progressively decreasing frequency. The cytogenetic damage was probably induced in the stem cells of the irradiated marrow. These authors considered the relationship between the cell damage and increased frequency of malignancies an enigma [1].

Early investigators of human chromosomes thought that aberrations were initiating events of the neoplastic process. Recently, however, some authors concluded that most chromosomal disturbances do not lead to cancer. Nowell believed that it was impossible to identify which individuals will eventually develop leukemia on the basis of chromosomal abnormalities [23]. Furthermore, he regarded aberrations in a single analysis as insufficient evidence that an individual is at risk. Finally he considered chromosomal aberrations produced by radiation, viruses, or chemicals as relatively crude indicators of genetic and carcinogenic consequences. Of course, no aberrant chromosomes are desirable, but their incidence is ubiquitous [1].

More studies are needed on the fate of irradiated peripheral-blood lymphocytes. However, our results indicate that low dosages of $^{131}$I (3.7 MBq), administrated to patients for scintigraphy of the thyroid, may cause chromosomal aberrations. We suggest re-evaluating the use of $^{131}$I, replacing it with $^{123}$I, mainly on patients of fertile age.

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