RADIO 2005 (094)

Study of p53 Protein Expression Levels from Irradiated Peripheral Blood Lymphocytes for Biodosimetry

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ABSTRACT

Biodosimetry can be defined as the investigation of radioinduced biological effects in order to correlate them with the absorbed dose. Scoring of unstable chromosomal aberrations and micronuclei, from *in vitro* irradiated peripheral blood lymphocytes, is commonly used for biodosimetry based on cytogenetic analysis. However, this method of analysis is time-consuming, which may represent a pitfall when fast investigation of a possible exposure to ionizing radiation (IR) is needed. The interaction of IR with the living cell can cause injuries in the DNA molecules. However, normal cells possess mechanisms of repair that are capable to correct those damages. During the repair process of the DNA various proteins are expressed. Among these proteins, p53 plays an important role. This protein is a transcription factor that helps in the maintenance of the genomic integrity. p53 protein is found into the cytoplasm in reduced concentrations and has a short average life. However, expression of p53 protein can be induced by DNA harmful radioinduced, which increases the concentration and the average life of this protein, making possible its detection. Thus, the correlation between the increasing of p53 expression and the irradiation may constitute a fast and reliable method of individual monitoring in cases of accidental or suspected exposures to IR. In this context, the objective of this research was to evaluate the p53 protein expression levels from lymphocytes of the human peripheral blood after *in vitro* irradiation. For this, samples of peripheral blood from healthy individuals were irradiated with known doses. Lymphocytes were separated on ficoll gradient by centrifugation and re-suspended at 1x 10^6/ mL in RPMI medium enriched with fetal calf serum. Hence, lymphocytes were incubated in 5% CO_2 at 37ºC prior to the methodology of flow cytometry, using intranuclear antigens for the quantification of p53. In this report, the methodology performed and the results obtained are presented and discussed, as well as the possible use of p53 protein expression as an important tool on radiological protection.

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1. INTRODUCTION

Defined as the amount of energy imparted to matter by ionizing radiation (IR) per unit of mass, absorbed dose is the most important physical quantity to evaluate potential biological response as a result of radiation exposures. In the International System (IS) the unit of dose is gray (1 Gy = 1 $J.kg^{-1}$) [1]. Absorbed dose assessment is performed, in generally, by physical dosimetry.

However, in most cases of real or suspected exposures to IR, physical dosimetry cannot be performed for retrospective estimates. In such situations, biological indicators (bioindicators) have been proposed as an alternative method for dose assessment, being so called as through of the Biodosimetry [2, 3]. The bioindicators can be defined as biological parameters (cellular or molecular changes) resultant of the interactions of IR with the living tissues. In particular, the scoring of induced chromosomal aberration (stables and unstable) and micronuclei from lymphocytes of human peripheral blood are the most investigated bioindicators serving as biodosimetric tools for radiological protection purposes. This biodosimetry is so called cytogenetic dosimetry. In this case, the dose-effect relationship obtained after *in vitro* irradiation of blood samples can be used as a calibration to estimate biological effects of an *in vitro* irradiation [4].

However, cytogenetic dosimetry has limitations due to the methods are time consuming for cell culture and cell analysis, which can represent a pitfall when it is necessary a fast identification of a suspected exposure for the planning of therapy [5].

The IR, when interacting with the cell, can produce some types of injuries, being most important those related with the DNA, because this molecule is responsible for the transmission of genetic information. Thus, the DNA repair mechanisms are essential, while errors in the repairing process cause mutations in the proper molecule. These mutations can be transmitted for the new generation of cells originating cancer or cell death (apoptosis). These injuries in DNA induce the expression of several proteins in order to repair such damages. Among the proteins expressed during the repair process, p53 has an important role, which is to conserve the integrity of the genome.

This protein is found in the inactive form into the cell in low concentrations and has an inferior average life of 20 minutes. However, when the cells suffer the action of a variety of harmful physicochemical agents, occurs an increase of the concentration of this protein with conformational alterations. In these conditions, an increase of the average life of p53 is observed, starting to be around 24 hours, where its detection becomes possible. Thus, the correlation between the increase of p53 expression and the dose of radiation may constitute a fast and reliable method of individual monitoring in cases of accidental or suspected exposures to IR [6].

The flow cytometry (FC) is a modern method for the study of cells. Through this method, multiple physical and biological properties of the cells can be determined, such as: size, graininess, complexity and intensity of fluorescence of each cell. The FC is a method of detection of surface antigen expression, intracitoplasmatic and intranuclear ones. It is a half-automatized technique, if it can process and analyze, jointly, a great

number of samples in a low period of time [7]. In this context, the objective of this research was to evaluate the p53 protein expression levels from lymphocytes of the human peripheral blood by flow cytometry after *in vitro* irradiation.

2. MATERIALS AND METHODS

2.1. Subjects and sampling

The samples analyzed were human peripheral blood collected in sterile and heparinized syringes. Before blood collection, all participants signed an informed consent form. 16 samples of peripheral blood, from 04 healthy donors (1 female and 3 male), were analyzed. Besides this, 4 non-irradiated samples were studied as control, and 12 samples were submitted to *in vitro* irradiation. The samples had been classified with the letters **A**, **B**, **C** and **D**

2.2. Irradiated samples

The samples were exposed to gamma radiation from ^{60}Co source (dose rate 18.47 $cGy.min^{-1}$) with doses of 50, 200 and 400 cGy.

2.3. Cell culture

After irradiation, the samples were transported for the Cytogenetic Laboratory (HEMOPE, Recife, BR). The human mononuclear cells were separated by gradient density (ficoll-hypaque) and by centrifugation. The cells were resuspended in RPMI-1640 medium supplemented with 10% fetal calf serum. Cell suspension was adjusted to 1×10^6 cel/mL/well plates, with 6µg/mL phytohemagglutinin (Cultilab, Campinas, BR). The plates were incubated in 5% CO_2/air humidified atmosphere at 37°C for different time.

2.4 Flow cytometry

2.4.1 Preparation of samples

For this methodology, the samples were transported to Immunophenotyping Laboratory (HEMOPE, Recife, BR), centrifuged and the cells resuspended with permeabilized solution per 10 min and centrifuged again. This step is repeated with Tween 20 to wash the cells.

After the last centrifugation, it was added to the medium the monoclonal anti-p53 antibody conjugated with phycoerythrin (PE) (Clone: G59-12; BD-Biosciences Pharmingen™ Tchnical Data Sheet, USA). Before the analysis, the cells were incubated

in the dark for 20 minutes at room temperature. Then, all samples were centrifuged and the cells were resuspended with paraformaldehyde before analyses.

2.4.2 Sample analyses

Cells were analyzed in FACScalibur Flow Cytometer (Becton Dickinson, San-Jose, Ca, USA) using CellsQuest software version 3.1 (Becton Dickinson immunocytometry systems, San Jose, CA, USA) with acquire of 10.000 events.

3. RESULTS AND DISCUSSION

The p53 protein expression levels of the irradiated and no-irradiated (control) samples of individuals **A**, **B**, **C** and **D** are summarized in Table 1.

Sample	Expression Levels (%)			
	Control	50 cGy	200 cGy	400 cGy
A	0.05	0.27	1.19	3.05
B	0.23	1.05	2.45	2.69
C	0.74	8.23	18.22	26.17
D	0.16	1.97	4.82	5.36

The p53 protein expression levels in the samples of individuals **A**, **B**, **C** and **D** have increased with the absorbed dose. This increase was proportional to the extension of damage in the DNA molecule, then, the higher absorbed dose the higher the cellular damage.

It was observed that the expression levels in the samples of subject **C** after exposure to IR presented higher values when compared with the other samples. Those values are probably related to his individual radiosensibility.

4. CONCLUSION

The analyses performed in this research have shown an increase in the p53 protein expression levels with the absorbed dose, suggesting their measurement as an important bioindicator to individual exposures to IR. One sample presented a higher values of p53 expression levels, which suggests that this test is related with the interindividual radiosensibility.

ACKNOWLEDGMENTS

Fundação de Hematologia e Hemoterapia de Pernambuco (HEMOPE); Centro de Radioterapia de Pernambuco (CERAPE); Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES).

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