ORIGINAL ARTICLE
UDC: 616.155.3:576.36:623.454.8

Arch Oncol 2005;13(2):65-8.
DOI: 10.2298/AOO0502065P

Positive correlation between micronuclei and necrosis of lymphocytes in medical personnel occupationally exposed to ionizing radiation

Sandra Petrović, Andreja Leskovac, Gordana Joksić

ABSTRACT

BACKGROUND: *Current radiation protection standards are based on premise that any radiation dose may result in detrimental health effects. The aim of this study was to evaluate extent of the DNA damages (measured by induction of micronuclei) and interphase cell death in circulating lymphocytes of medical personnel exposed to ionizing radiation.*

METHODS: *Baseline micronuclei were assessed using the cytokinesis-block micronucleus test. Cytotoxicity was analyzed by flow cytometry for human white blood cells to identify cells that displayed apoptosis-associated DNA condensation. Necrotic cells were analyzed simultaneously. All parameters were compared with corresponding controls.*

RESULTS: *A statistically significant difference (t = 4.54, p = 0.002) was found between exposed and control group in the yield of baseline micronuclei. The level of baseline micronuclei correlated positively with necrosis of leucocytes (r=0.09, p=0.68 in exposed group, r=0.02, p=0.97 in controls). An inverse correlation between baseline micronuclei and apoptosis was noted in both groups of examinees (r = -0.26, p = 0.27 in exposed group, r = -0.09, p=0.80 in controls). The data obtained also suggested an inverse correlation between necrosis and apoptosis (r = -0.37, p = 0.11 in exposed group, r = -0.89, p = 0.001 in controls).*

CONCLUSION: *Flow cytometry being a rapid, fast, and accurate method is strongly recommended in evaluation of radiation injuries. The integration of apoptosis and necrosis into micronucleus assay could be very important in the assessment of cumulative effects of ionizing radiation in occupationally exposed medical personnel.*

KEY WORDS: *Radiation, Ionizing; Occupational Exposure; Leukocytes; Apoptosis; Micronuclei; Necrosis; Medical Staff*

"Vinča" Institute of Nuclear Sciences, Belgrade, Address correspondence to: Gordana Joksić, biologist, PhD, "Vinča" Institute of Nuclear Sciences, POB 522, 11001 Belgrade, Serbia & Montenegro,

E-mail: gjoksic@vin.bg.ac.yu; The manuscript was received: 20.05.2005, Provisionally acepted: 14.06.2005, Accepted for publication: 15.07.2005

INTRODUCTION

The study of DNA damage at the chromosome level is an essential part of genetic toxicology because chromosomal mutation is an important event in carcinogenesis. Alternatively, the cytokinesis-block micronucleus (CBMN) assay has become one of the most commonly used methods for assessing chromosome breakage in human lymphocytes (1). It can provide the following measures of genotoxicity and cytotoxicity: chromosome breakage, chromosome loss, chromosome rearrangement (nucleoplasmic bridges), cell division inhibition, necrosis, and apoptosis (2). Since cytogenetic assays require cell division in the culture for expressing of the endpoint measured (e.g., micronuclei) there is always a concern that not all damaged cells are observed because some have undergone interpahse death via apoptosis or necrosis instead of completing nuclear division (3). Necrosis refers to a range of morphological changes resulting from the enzymatic digestion of the cell, the disruption of cellular membranes, and denaturing of proteins that accompanies cell death. Apoptosis, in contrast, is a programmed, active, highly selective mechanism of cell death allowing for the removal of cells that are excessively damaged (4). Lethal DNA injury leads to cell death via apoptosis or necrosis or cells can undergo nuclear division where micronuclei will be expressed. Recently published studies have shown that radiation response of human tissues is very complex and could be unpredictable, because many factors may influence the human response to ionizing radiation; among them are the variability within genes that affect the amount of cell damage (5-9), the ability to properly respond to and repair the damage, and the level of tolerance to DNA mutations resulting from the damage (10). In this paper we presented the results of lymphocyte micronuclei, necrosis, and apoptosis of leukocytes on the medical personnel occupationally exposed to ionizing radiation and the corresponding controls.

MATERIAL AND METHODS

Subjects

The study included 29 individuals: 20 hospital-based radiologists of mean age 46.05 ± 6.02 with 14.90 ± 5.24 years of occupational exposure to ionizing radiation and 9 male physicians of mean age 38.00 ± 5.89 from the same hospital who were occupationally unexposed to ionizing radiation (control group). Blood samples were obtained during a routine annual checkup of health. There were six smokers in the exposed group and two in the control group.

Micronucleus analysis

Micronucleus analysis was carried out on cultures of phytohemaglutinin-stimulated blood lymphocytes. Into 5 ml of RPMI-1640 medium supplemented with 15% of calf serum, 0.5 ml of whole blood was added. Lymphocytes were incubated at 37ºC. For micronucleus preparation Cytochalasin B at a final concentration of 4 μg/ml was added after 44 hours of culture incubation according to the method of Fenech and Morley (1985) (11). The lymphocyte cultures were incubated for another 24 hours. Micronucleus slides were made by fixation in methanol: acetic acid (3:1) after a brief cold hypotonic (0.056%) KCl treatment. The slides were stained in 2% alkaline Giemsa. For each subject 1000 binucleated (BN) cells were scored for each sample recording micronuclei under Zeiss microscope.

Apoptosis

For the apoptosis assay, 0.5 ml blood aliquots from each subject were incubated in medium RPMI-1640 supplemented with 15% of calf serum without phytohemaglutinin (PHA) in CO_2 incubator for 24 hours. After incubation, cells were gently washed with physiological saline (0.9% NaCl) at 37ºC and fixed in methanol: acetic acid (3:1). Afterwards, the pellet was fixed in 96% ethanol. Apoptosis was assessed by flow cytometric identification of cells displaying apoptosis-associated DNA condensation. Three parameters were measured: cell size, granularity and DNA content. DNA content was assessed by measuring the UV fluorescence of propidium iodide stained DNA. Cytotoxicity was defined as the population of cells with reduced DNA fluorescence (12). Each sample was analyzed using 10,000 events.

Statistics

Statistical analysis was carried out using statistical software package Statistic for Windows 98, version 5.5. The *t* test and correlation analysis were performed. Correlation coefficients (r) were calculated at a significant level of $p < 0.05$.

RESULTS

Micronuclei findings and measurement of apoptosis and necrosis in exposed group are presented on Table 1, in control group on Table 2. Statistics regarding parameters under consideration are presented on Table 3.

Table 1. Micronuclei, apoptosis, and necrosis data in exposed group

No	Age	Percent of apoptotic cells	Percent of necrotic cells	Baseline micronuclei
1	36	55.00	4.30	32
2	47	18.70	2.00	29
3	45	26.10	4.90	19
4	54	59.30	0.50	40
5	49	53.50	6.60	41
6	46	29.70	0.70	37
7	51	27.60	3.50	34
8	53	52.90	5.50	32
9	47	70.70	2.50	9
10	38	33.80	4.20	13
11	50	62.60	4.20	14
12	52	10.90	4.10	18
13	40	96.50	0.10	5
14	39	91.00	0.50	26
15	46	80.20	0.30	27
16	39	73.20	3.50	14
17	48	44.70	3.00	20
18	52	67.20	1.10	20
19	36	68.80	5.00	26
20	53	72.70	3.40	25
X	46.05 ± 6.02	54.76 ± 24.07	2.99 ± 1.94	24.06 ± 10.22
R		-0.26[a]	0.09[b]	
			-0.37[c]	

R-correlation coefficient
a- baseline MN related to baseline apoptosis, negative correlation, statistically insignificant ($p = 0.27$)
b- baseline MN related to baseline necrosis, positive correlation, statistically insignificant ($p = 0.68$)
c- baseline apoptosis related to baseline necrosis, negative correlation, statistically insignificant ($p = 0.11$)

Table 2. Micronuclei, apoptosis and necrosis data in control group

No	Age	Percent of apoptotic cells	Percent of necrotic cells	Baseline micronuclei
1	39	80.60	1.30	23
2	38	55.10	9.30	12
3	47	54.05	7.50	14
4	37	79.70	0.50	14
5	33	95.00	0.70	9
6	38	92.30	0.20	12
7	30	86.80	3.80	18
8	47	77.80	1.60	12
9	33	92.10	0.70	11
X	38.00 ± 5.89	79.27± 15.26	2.84 ± 3.35	13.89 ± 4.23
R		-0.09[a]	0.02[b]	
			-0.89[c]	

R-correlation coefficient
a- baseline MN related to baseline apoptosis, statistically insignificant correlation ($p = 0.80$)
b- baseline MN related to baseline necrosis, positive correlation, statistically insignificant ($p = 0.97$)
c- baseline apoptosis related to baseline necrosis, negative correlation, statistically significant ($p = 0.001$)

Table 3. Statistics regarding biological endpoints under consideration

	Baseline micronuclei	Baseline apoptosis	Baseline necrosis
Exposed Control	4.54[a]	-6.69[b]	0.42[c]

a- $p = 0.002$ (baseline MN in exposed group compared to control) statistically significant
b- $p = 0.0002$ (baseline apoptosis in exposed group compared to control) statistically significant
c- $p = 0.68$ (baseline necrosis in exposed group compared to control) statistically insignificant

Exposed group. Mean value of baseline micronuclei in exposed group was 24.06 ± 10.22, mean percent of apoptotic leucocytes was 54.76 ± 24.07, whereas mean percent of necrotic cells was 2.99 ± 1.94.

Control group. In the control group, mean value of baseline micronuclei was 13.89 ± 4.23, mean percent of apoptotic leucocytes as 79.27 ± 15.26, and mean percent of necrotic cells was 2.84 ± 3.35.

Statistical analysis showed highly significant difference in yields of baseline micronuclei and apoptosis between exposed and control group ($t = 4.54$, $p = 0.002$; $t = -6.69$, $p = 0.0002$, respectively). Based on these findings, we can assume that lymphocytes from controls have a greater capability to undergo apoptosis than lymphocytes from radiation workers. Inverse correlation between baseline micronuclei and apoptosis in both study groups was observed ($r = -0.26$, $p = 0.27$ in exposed group; $r = -0.09$, $p = 0.80$ in control group). Our results also showed positive correlations between baseline micronuclei and necrosis in both study groups ($r = 0.09$, $p = 0.68$ in exposed group, $r = 0.02$, $p = 0.97$ in controls) and negative correlation between apoptosis and necrosis, ($r = -0.37$, $p = 0.11$ in exposed group, $r = -0.89$, $p = 0.001$ in controls)

DISCUSSION

Since expression of micronuclei is possible only in cells undergoing division, this study was aimed to evaluate a portion of potentially damaged cells that could be lost by interphase death before entering mitosis. For this purpose the level of cells dying via apoptosis and necrosis were evaluated. Apoptosis as principal form of cytotoxicity in lymphocytes starts with a lethally injured but otherwise normal cell, and ends in organized lysis of the cell. An important characteristic of apoptosis is that it results in the elimination of the dying cell without inflammation since the dying cell maintains the integrity of its plasma membrane (13). Apoptotic cell death is characterized by controlled autodigestion of the cell. Apoptosis has proven to be closely interlinked with other essential cell pathways and required for maintenance of normal tissue homeostasis (14). Maintenance of cell homeostasis depends on the strict control of cell proliferation and cell death through apoptosis, and many disease states result from errors in the regulation of apoptosis. If the cell cannot undergo apoptosis, it follows the route of necrosis (13). In the present study apoptosis of all lymphocytes

was examined with no PHA stimulation according to suggestion of Ozashin et al. (1997) (15). This approach avoids complication of PHA on response levels, but otherwise active cellular response is reduced. Marked variability of apoptosis among exposed individuals was observed, but in general, depressed apoptotic potential of leucocytes of occupationally exposed persons was found. Variability in portion of leucocytes dying via apoptosis could be associated with a different sensitivity of T cell subsets to low doses of ionizing radiation (16, 17) or oxidative stress that is certainly induced by low doses of ionizing radiation. Apoptotic potential of leucocytes correlates inversely with baseline micronuclei in exposed group, whereas positive correlation is observed between micronuclei and necrosis. Necrosis occurs following a wide variety of cellular injuries. Necrotic cells are unable to repair the caused damage and rapidly lose their viability (18). Pathways associated with apoptosis seem to be interconnected with those leading to necrosis. It is likely that the cascades that lead to apoptotic or necrotic mode of cell death are activated almost simultaneously and may share some common pathways. Damaged cells incapable to undergo apoptosis are mostly visible as binucleated cells carrying micronuclei. Our previous studies considering individual radiosensitivity of healthy subjects occupationally unexposed to known mutagenic agents have shown inverse relationship between individual radiosensitivity and antioxidative activity of intracellular superoxide dismutase (SOD). Numerous studies (19-21) confirm important role of antioxidative defense mechanisms in response to different agents, but relatively few evaluate influence of low radiation doses on antioxidative defense mechanisms. Chronic exposure to low doses of ionizing radiation enhances the level of free radicals, and possibly mediates the balance of intracellular oxidant/antioxidant status, the level of ATP in the cell, and the extent of induced membrane damage (22-24), which leads cells to necrosis rather than apoptosis.

Reduced apoptosis is observed in genetic diseases like ataxia, telangiectasia, and Fanconi anemia (25, 26) because of the failure of p53 activation of proteins that respond to oxidative stress. Chronic exposure to low doses of ionizing radiation certainly generates reactive oxygen species (ROS), which could inhibit caspase dependent apoptotic pathway. Although intracellular oxidation in general is a stimulus for apoptosis the optimal rate of oxidant/antioxidant status is required. Cumulative effects of chronic exposure to low doses of ionizing radiation disturb the balance between pro- and antiapoptotic molecules leading cells rather in necrosis.

There is now compelling evidence that insufficient apoptosis can result in cancer or autoimmunity. Understanding the molecular mechanism and regulation of normal apoptotic function is important for understanding the state of these diseases and how they can be best treated (27,28). Further prospective monitoring of persons with chromosome and micronuclei findings by flow cytometry will enable determination of the full significance of the exposure to the low doses of ionizing radiation and evaluation as to whether they are prognostically associated with an increased risk for adverse health consequences.

CONCLUSION

Inverse relationship between baseline micronuclei and apoptotic potential of leucocytes is the most important finding in this study; it suggests that Flow cytometry analysis can discriminate differences in radiation-induced cytotoxicity between individuals occupationally exposed to low doses of ionizing radiation.

Acknowledgement

The study has been financially supported by the Serbian Ministry of Science, Project 1991.

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