RESEARCH ARTICLE

On the Stress by Photoperiod, Temperature and Noise as Possible Causes of Genomic Damaging in an Animal Model

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Abstract

Stress is a defense mechanism of an organism and is directly related to homeostasis, which is the equilibrium state of the multitude of organism systems, both among each other and with the environment. Given that stress is a major cause of cellular instability, it is directly related to its loss homeostasis. The present study assessed the changes caused by stress on the genetic material of *Rattus norvegicus* using micronuclei analysis. A total of 10 males were studied by being submitted to continuous stress from photoperiod, temperature and noise for 26 days. Peripheral blood samples were obtained immediately before submitting the animals to stress, as well as on the fifteenth and twenty-sixth days afterwards. Blood smears were stained with Giemsa and the slides were analyzed using light microscopy. Approximately 3,000 polychromatic erythrocytes from each animal were observed and classified as either normal or micronucleated. The analysis indicated a frequency of 0.0012014 for the control sample, whereas samples on days 15 and 26 had frequencies of 0.0035458 and 0.0496850, respectively. A statistical analysis revealed statistically significant differences among the studied cell samples (ANOVA $p < 0.00001$, significance of 5%). Therefore, the presence of micronuclei in the studied samples is consistent with a cause/effect relationship.

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Keywords

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Introduction

Physiological stress is a normal adaptation of an organism to various situations of tension (Tomei et al., 2008). However, several studies have demonstrated an association between physiological mechanisms related to stress and several transient changes and severe diseases (Anderson, 2001; Schneiderman, McCabe, & Baum, 1992). Considerable attention has been given to the relationship between stress and cancer (Anderson, 2001; Chen et al., 1995; Gehde & Baltrusch, 1990; Greer & Morris, 1975; Morris, Greer, Pettingale & Watson, 1981; Ramirez et al., 1989; Schneiderman et al., 1992),

because of a high incidence of the disease and the high level of stress common to contemporaneous life styles. Cancer originates genetically through mutations or the abnormal activation of genes that control cell growth, resulting in progressive modification in all activities, including proliferation, differentiation, and their interaction with each other and the extracellular medium (Cotran, Kumar, & Robbins, 2000; Louro, 2000). Cells with altered control mechanisms have a greater chance of developing genetic abnormalities and becoming unstable (Ward, 2002). Endogenous and exogenous factors might induce a lack of cell control, thus predisposing such genetic changes (Ellinger-Ziegelbauer, Aubrecht, Kleinjans & Ahr, 2009; Henderson & Feigelson, 2000; Silva, Serakides & Cassali, 2004). However, precise data on a direct relationship between stress and the generation of genetic instability still need to be effectively investigated.

Given that genomic instability, when in large-scale (chromosomal instability), can be observed by cytogenetic studies (Duesberg, Rausch, Rasnick & Hehlmann, 1998; Knudsen & Hansen, 2007; Norppa et al., 2006), the micronuclei (MN) test can contribute to the understanding of the effects of stress on the genome. MN are caused, either by being induced or spontaneously, from chromosomal fragments or entire chromosomes that are not incorporated within the nucleus of the daughter cell during mitosis. Such elements are enclosed within secondary nuclei known as MN, from which they obtained their name (Dietz, Diehl, Prolla, Furtado, & Furtado, 2000).

The goal of the present study is to assess the genotoxic potential of stress through MN analysis in an animal model, given the speed in which such estimate is increasingly becoming a rapid, cheap and effective methodological alternative.

Materials and Methods

Ten males of the species *Rattus norvegicus* and the Wistar strain, all of which 3 months of age and weighting between 500 and 600 g were raised and maintained in a restricted room at the animal care facility of the Universidade Positivo (Curitiba, PR), where they received commercial feed and filtered water. After a habituation period of a week in an environmental room (22°C) and a 12:12 h L/D photoperiod, a peripheral blood sample was obtained from them (control sample). Directly after such initial blood sample, the rats were submitted to environmental stress from the alteration of their photoperiod to 8:16 h L/D for 15 days. In the fifteenth day of stress submission, a new blood sample was obtained, followed by 11 days of additional photoperiod stress together with two additional stress sources: a random temperature oscillation between 23°C and 25°C, and sound stress in the form of uninterrupted radio static. On day 26 of the experiment, a final blood sample was obtained.

Blood samples were taken after the animals had been anesthetized by ether inhalation in a sealed chamber. A volume of 0.5 mL of peripheral blood was removed from the caudal vein using a sterilized syringe. Immediately after collection, blood smears were performed on previously cleaned slides (two slides per individual). Following the blood smear, slides were left to dry at room temperature for 1 h and then stained with Giemsa diluted in a pH 6.8 phosphate buffer solution at a 20:1 ratio (40 mL of buffer solution and 2 mL of Giemsa). Slides were left on this solution for 7 min and then washed with distilled water and dry at room temperature.

The analysis of the cells from each animal was carried out in an optical microscope with an immersion objective. The analysis consisted in the observation of a total of 3,000 polychromatic erythrocytes per individual, with each cell being characterized as normal or micronucleated. The statistical analysis was done using ANOVA (significance of 5%).

This study was approved by the Ethics Committee on Research of Universidade Positivo—Curitiba/Brazil (Resolution 003/2002—National Health Council/Brazil).

Results

The results obtained from the analysis of the frequency of MN revealed a significant increase in the number of MN in the cells of the studied animals according to the increase and the continuation of the stress.

The control sample showed a frequency of MN (total number of MN/total number of analyzed cells) of 0.001266, whereas samples on days 15 and 26 had frequencies of 0.003533 and 0.004866, respectively. Table I shows the results obtained from the analysis of the numbers of normal and micronucleated cells and the frequency of micronucleated cells in relation to normal cells over the period in which the animals were exposed to stress.

Table I. Results obtained from the analysis of blood cells of the studied animals with respect to the presence of micronucleated cells over the periods of exposure to stress

Individuals	Sample 1		Sample 2		Sample 3	
	Control		15 Days		26 Days	
	Normal Cells	Micronucleated Cells	Normal Cells	Micronucleated Cells	Normal Cells	Micronucleated Cells
1	2,998	2	2,993	7	2,988	12
2	2,996	4	2,991	9	2,985	15
3	2,999	3	2,993	7	2,983	17
4	2,993	2	2,986	8	2,992	8
5	2,997	3	2,991	9	2,989	11
6	2,996	4	2,989	11	2,984	16
7	2,997	3	2,992	14	2,989	15
8	2,998	7	2,984	16	2,984	16
9	2,996	4	2,988	12	2,983	17
10	2,994	6	2,987	13	2,981	19
Total	29,962	38	29,894	106	29,854	146
Frequency*		0.001266		0.003533		0.004866

* Total number of micronuclei/total number of analyzed cells.

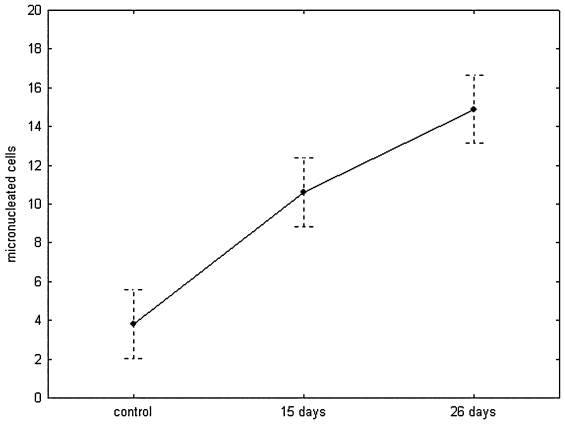

Figure 1 ANOVA test ($p < 0.00001$) showing the differences of micronucleated cells from the control group and samples after 15 and 26 days of exposure to continuous stress. The dots represent the average micronucleated cells (MNs), the continuous line indicates the increasing number of MNs among the experiments, and the dashed lines indicate the specific standard deviations observed. (The figure was generated by Statistica v.6.0.)

A statistical test using an ANOVA showed highly significant differences in the observed frequencies among the groups ($p < 0.00001$), as shown in Figure 1.

Discussion

In the present study, the obtained results revealed a statistically significant difference with respect to the frequency of MN among the periods of exposure to stress. Such differences were noticed soon after (15 days) the first environmental stimulus, which already generated sufficient genomic damaging to be expressed in the form of MN, as can be observed in Table I and Figure 1. Likewise, one can observe that the association of stressing agents (photoperiod, temperature and noise) seemed to have induced an increase in the frequency of MN, which was positively associated with the continuity and intensity of the stressing stimuli.

Given that MN can only be detected in cells that underwent division (Dietz et al., 2000), and that the stressful conditions can lead to an atypical physiological condition that can indirectly alter the cell cycle (Cohen, Marshall, Cheng, Agarwal, & Wei, 2000; Setlow, 1978), the presented scenario might indicate that such events can represent a cause/effect relationship. The physiological conditions generated by prolonged, continual stress might act directly and/or indirectly at the genetic level, leading not only to genomic damaging, but also to the deregulation of the cell cycle, favoring the occurrence of MN and possibly cancer predisposition. Several previous and current researches have revealed a positive association of genomic instability (as genomic damage) with cancer predisposition (Blount et al., 1997; Bonassi et al., 2007; Norppa et al., 2006; Spitz & Bondy, 2010; Valverde & Rojas, 2009).

Given that stress could be a possible cause of such chromosome damages, the results obtained herein suggests a relationship between cell events induced by stress, which could have caused genomic instability. Such instability could have been generated by the

stimuli through a disordered cascade of events, resulting in a likely loss of control of cell division, with the genetic damage being expressed as MN. The positive relationship observed between the increased stressing conditions and the increasing frequency of MN might support the hypothesis of association of continuous altered environmental factors (temperature oscillation, noise and photoperiod) and genomic damaging. Several authors have suggested stress as an induce genotoxic damage, both at the chromosomal and the molecular levels (Adachi, Kawamura, & Takemoto, 1993; Ciaranello, Dornbusch, & Barchas, 1972; Fischman, Pero, & Kelly, 1996; Glaser, Thorn, Tarr, Kiecolt-Glaser, & D'Ambrosio, 1985; Kiecolt-Glaser, Stephens, Lipetz, Speicher, & Glaser, 1985) and the present research seems to corroborate such previous results by new environmental-based associations with a progressive chromosome damage on peripheral blood cells of *Rattus norvegicus*.

The scarcity of data with respect to the effects of stress agents on genetic material has generated interests on this topic because of the possible role of stress as a carcinogenic agent. The current study also contributes to solving this issue. If one takes into account that cancer is intimately related to genomic instability as mentioned above, the increase in MN in the studied individuals (in this case, *Rattus norvegicus*) potentially predisposes them to cancer. Thus, the blood cells analyzed herein appeared to be appropriated for assessing both DNA damage and elevated cancer risk. Yet as being circulating cells their cellular, nuclear and metabolic state can reflect the overall conditions of the body (Valverde & Rojas, 2009). Thus the present results also reinforce that the cancer predisposition could be minimized with a stimulus (stress) removing, given the damaged cells would be also removed as faster as the stimulus removing, except for a permanent damage (Heuser, de Andrade, Peres, Braga, & Chies, 2008; Matsumoto & Cólus, 2000; Schlegel, MacGregor, & Everson, 1986).

Given the complexity of the effects rising by stress at the physiological, cellular, and molecular levels continuing studies should be carried out in order to elucidate the mechanisms by which these effects act on the organism, in an attempt to minimize them for improvements on the quality of life.

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