PECULIARITIES OF DNA DAMAGE CAUSED BY EXOGENOUS NITRIC OXIDE COMBINED WITH FRACTIONATED LOW DOSE IONIZING RADIATION IN NORMAL AND TUMOR CELLS

I.I. Muzalov*, V.M. Mikhailenko
R.E. Kavetsky Institute of Experimental Pathology, Oncology and Radiobiology, NAS of Ukraine, Kyiv 03022, Ukraine

The aim of this study was to investigate the reaction of normal and tumor cells to genotoxic effect of widespread environmental factors — exogenous nitric oxides and ionizing radiation. Methods: The animals were treated with NO (125 mg/m²) and low dose ionizing radiation (10 acute exposures with 0.1 Gy each). Genotoxicity was estimated in vivo in rats peripheral blood lymphocytes, bone marrow cells and tumor cells of Guerin carcinoma. DNA damages were assessed by alkaline single-cell gel electrophoresis. Results: Exogenous nitric oxides as well as irradiation caused significant increase of DNA damage in all types of investigated cells. The genotoxic effect increased in the order: peripheral blood lymphocytes < bone marrow cells < Guerin carcinoma cells. The greatest genotoxic effect was registered in Guerin carcinoma cells on terminal phase of tumor growth in rats exposed to NO and low dose ionizing radiation. Conclusions: Long-term exposure to common environmental factors (exogenous nitric oxides and ionizing radiation) capable to induce DNA damage in different cells. Severity of the genotoxic effect depends on cell type and nature of impacting factors. NO caused more significant DNA damage than low dose ionizing radiation but the highest level of DNA damage was observed after their joint action. Obtained results confirm the real threat of cancer risk increase under combined action of common environmental factors of different nature.

Key Words: nitric oxide, nitrosative stress, ionizing radiation, tumor cells, DNA damage.

According to data of the International Agency for Research on Cancer (IARC), a primary cause of human cancers is environmental pollution, especially — air pollution. Emissions from motor vehicles, power plants, domestic combustion of solid fuels are the main sources of air pollution worldwide [1].

Exogenous factors cause 75–80% of all cancer incidents. Moreover, the chemical carcinogens are responsible for the occurrence of 80–90% of all human malignant tumors. Simultaneous action of harmful factors with carcinogenic or co-carcinogenic activity increase the tumor development probability [2].

One of the main environmental air pollutants worldwide are nitrogen oxides (NOx) [3]. Anthropogenic pollution of environment with radionuclides, as well as expanding of X-ray use and radiographic methods in medical research have led to increased external and internal exposure of humans to ionizing radiation [4].

Exposure to low doses of ionizing radiation (LDIR) increases the probability of cancer as well as other types of diseases. Without causing noticeable immediate response in the body, a LDIR lead to numerous delayed negative consequences. Genetic status, overall health and additional effect of environmental factors are modifying the nature and extent of the biomedical consequences of radiation, chemical and combined influences [5].

Final target for the direct or indirect effects of NO and ionizing radiation is genetic material of cells, which leads to implementation of their acute and prolonged biological effects [6]. The reaction of cells to environmental factors of different nature and their combination depend on cells type, epigenetic status, level of energy metabolism. Proliferative activity and phase of the cell cycle determines the state of the genetic material and the activity of the cell repair system as well.

Data of literature on the impact of prolonged (chronic) combined treatment with environmental factors of low intensity, in particular NO and LDIR on the development of genetic instability and as a result increase of cancer risk are mainly absent [7].

We investigated the dynamics of DNA breaks formation in peripheral blood lymphocytes (PBL), bone marrow cells (BMC) and solid tumor Guerin carcinoma (GC) cells.

MATERIALS AND METHODS

Animals and cell lines. Adult random-bred male rats (120–150 g, 48 animals) were obtained from the vivarium of R.E. Kavetsky Institute of Experimental Pathology, Oncology and Radiobiology, NAS of Ukraine (Kyiv, Ukraine) and kept at steady state conditions with a constant temperature and natural light. The work with animals was performed according to the rules of local Ethic Committee [8–10]. The animals were divided into four groups: 1) intact control (12 animals); 2) animals that inhaled NOx for 1 month (16 h per day, 12 animals); 3) animals were regularly 10 times irradiated at a dose 0.1 Gy over the period of 1 month (total dose was 1 Gy, 12 animals); 4) animals received combined treatment of NO and LDIR (12 animals).

The study was performed on PBL, BMC and GC cells isolated from rats.
BMC were isolated according [11]. Fractionation of the obtained cells was not conducted [12]. Modeling of cancer process was performed by inoculation of rats with 0.5 ml of GC suspension (5×10^6 cells/ml) in saline. Viability of obtained cells was estimated using trypan blue according to [13].

**Isolation of PBL.** Whole blood was diluted in an equal volume of PBS and stratified on Histopaque 1077 (“Sigma”, St. Louis, MO) for lymphocyte separation according to the manufacturer’s instruction. After isolation, lymphocytes were washed in PBS, diluted in 1 ml culture medium. The amount of the isolated cells was counted after trypan blue staining (“Euroclone”, Pero, IT) with a Go
day — in 3.3-fold, rate of investigated value was in 2.9-fold higher compared to control and in 1.7-fold higher than on the 12th day. Those data indicate the intensification of genetic instability in time after NO inhalation, most likely due to malfunction of cell repair system. LDIR irradiation also caused an increase and persistence of DNA damage in PBL. On the 12th day of GC growth the level of DNA damage exceeded control level in 2.7-fold, and on the 18th day — in 3.3-fold, revealing significant correlation with tumor growth long term after LDIR influence.

Exposure rats to the combined impact of NO and LDIR resulted in significant increase of DNA damage on the 12th day of tumor growth that exceeded control level in 3.4-fold. This value was in 1.6-fold higher than after single treatment with NO and in 1.3-fold when exposed to LDIR. On the 18th day of tumor growth level of DNA damage has exceeded control level in 4-fold. The level of DNA damage in PBL of rats exposed to NO or LDIR alone was exceeded in 1.4- and in 4-fold. The level of DNA damage has exceeded control level in 2.7-fold, and on the 18th day — in 3.3-fold, revealing significant correlation with tumor growth long term after LDIR influence.

The results of DNA damage assessment in BMC of intact animals and rats with GC, both affected by NO and LDIR, are presented in Fig. 2.

**RESULTS AND DISCUSSION**

Results of in vivo DNA damage evaluation in PBL of intact animals and in rats with GC, both affected by NO and LDIR, are presented on Fig. 1.

**Fig. 1.** The level of DNA damage (%) in PBL of rats exposed to NO and/or LDIR

Spontaneous level of DNA damage in animals of the control group was 4.9 ± 0.3%. The level of the studied parameter after GC transplantation varied slightly, rising on 18th day in 1.1-fold.

NO treatment led to increase of DNA damage rate on the 12th day of tumor growth in 2.2-fold compared to intact control demonstrating real genotoxic effect of exogenous NOx due to direct and indirect molecular mechanisms. The difference increased during 18 days, rate of investigated value was in 2.9-fold higher compared to control and in 1.7-fold higher than on the 12th day. Those data indicate the intensification of genetic instability in time after NO inhalation, most likely due to malfunction of cell repair system. LDIR irradiation also caused an increase and persistence of DNA damage in PBL. On the 12th day of GC growth the level of DNA damage exceeded control level in 2.7-fold, and on the 18th day — in 3.3-fold, revealing significant correlation with tumor growth long term after LDIR influence.

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The results of DNA damage assessment in BMC of intact animals and rats with GC exposed to NO and LDIR are presented in Fig. 2.

Spontaneous level of DNA damage in BMC was 4.7 ± 0.3%. LDIR irradiation caused an increase in DNA damage in 2.6-fold, and inhalation of NO increased genotoxic effects in 3-fold. Combined effect of NO and LDIR resulted in increase of DNA damage in 4-fold compared to controls and, respectively, in 1.5- and
remains uncertain. In this regard, a study of DNA damage in GC cells exposed to separate and combined treatment with NO and LDIR was conducted and results are presented in Fig. 3.

Spontaneous levels of DNA damage in GC cells on 12th day of tumor growth exceeded values for PBL and BMC in 1.7-fold. On the 18th day of GC growth excess of DNA fragmentation reached 3.5-fold, and was in 1.6-fold higher than on 12th day indicating an intensification of the processes that leads to DNA damage and the development of genetic instability in the GC cells during tumor growth.

LDIR treatment led to an increase in the level of DNA damage after 12 days of GC development — in 2-fold, and on the 18th day — in 1.6-fold compared to controls. Prolonged NO impact caused an increase of DNA damage in 2.3-fold on 12th day and in 1.9-fold on 18th day of GC growth, compared to corresponding control values. Obtained data indicate higher sensitivity of actively proliferating GC cells to both investigated environmental factors, than normally proliferating somatic cells. Combined effect of both factors increased in 2.9-fold the level of DNA damage on 12th days of tumor growth compared to control group thus exceeding in 1.3–1.5-fold separate action of NO and LDIR.

Accordingly to obtained results and literature data [19], the response of cells to separate and combined effects of environmental factors of different nature may depend on the proliferative activity of cells. Proliferative index — dynamic parameter that reflects, as for BMC, existing level of hematopoietic cells generation. Proliferative index also directly related to the changes that occur in the BMC during cells maturation and differentiation of precursor cells to certain subtypes [20].

Genotoxicity of NO and LDIR are more pronounced in somatic cells with relatively higher proliferation rate — BMC [21] vs PBL [22]. In 3.5-times intensively proliferating cancer cells (GC cells) [23] exposed to investigated factors revealed even higher level of DNA damage than normal somatic ones, providing the basis for development of genetic instability.

Malignization leads to cell cycle deregulation and modification of proliferative activity. Creating the heterogeneity of cell populations, genetic instability provides the material for the selection of increasingly
autonomous and aggressive cells. Genetic instability provides the preconditions for tumor progression which begins in the precancerous period [24].

Thus, the assessment of DNA damage in cells exposed to NO and LDIR revealed the ability of both factors to induce notable genotoxic effects in the way of formation a single — and double strand DNA breaks. NO caused more significant DNA damage than LDIR but the highest level of DNA damage was observed after the joint action of investigated factors.

The elevated combined genotoxic effect of NO and LDIR (that cannot be reduced to the sum of their individual effects) partially can be explained by existence of common mechanisms for the implementation of genotoxic effects for both factors (formation of reactive oxygen and nitrogen species), as well as individual significant contribution of NO due to chemical DNA modification, peroxynitrite formation and inhibition of repair enzymes. Obtained results about increased level of induced DNA damage confirms the development of genotoxic lesions and real threat of cancer risk increase.

REFERENCES