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# DESTABILIZATION OF HUMAN CELL GENOME UNDER THE COMBINED EFFECT OF RADIATION AND ASCORBIC ACID

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**The *aim* of this study was to investigate peculiarities of ascorbic acid effect on radiation-induced chromosomal aberrations frequency and range in the cultured peripheral blood lymphocytes (PBL) of healthy donors and cancer patients depending on doses of radiation and drug, as well as cells radiosensitivity (*in vitro*). *Methods*: Test system of human PBL, metaphase analysis of chromosomal aberrations. Cells were cultivated according to the standard procedures with some modifications. PBL culture was exposed to x-ray radiation in $G_0$- and $G_2$-phases of cell cycle. Immediately after the irradiation the culture was treated with ascorbic acid in concentrations of 20.0–80.0 μg/ml of blood. *Results*: Cell culture irradiation in low dose (0.3 Gy) and treatment with ascorbic acid in therapeutic concentration (20.0 μg/ml of blood) resulted in radioprotective effect, decreasing overall chromosome aberrations frequency as opposed to radiation effects. It has been established that post-irradiation effect of ascorbic acid upon the PBL culture in concentrations of 40.0 and 80.0 μg/ml, which exceeding therapeutic concentration value 2 and 4 times correspondingly, increased overall chromosome aberrations frequency 1.4 times compared with irradiation effect in a low dose (0.3 Gy). This bears evidence of ascorbic acid co-mutagenic activity in the range of concentrations exceeding therapeutic values. The peak of mitotic activity inhibition was observed at 2.0 Gy irradiation dose. Addition ascorbic acid in therapeutic concentration increased radiation effect this number ≈ 2 times (exceeding even intact control value). Compared with $G_0$-phase, co-mutagenic effect of ascorbic acid in $G_2$-phase appears earlier, starting with dose of 1.0 Gy. In the blood lymphocytes of cancer patients, the level of genetic damage was increased 1.7 times after combined treatment with low dose irradiation and ascorbic acid in comparison with irradiation alone which suggest the co-mutagenic instead of radioprotective effect of ascorbic acid. *Conclusions*: Genome destabilization enhancement of irradiated *in vitro* human somatic cells under ascorbic acid effect is due to its co-mutagenic properties. The formation of co-mutagenic effects of ascorbic acid depend on its concentration, irradiation dose and the efficiency of repair processes. Co-mutagenes may pose high carcinogenic hazard at low (above background) radiation levels.**
***Key Words:* ionizing radiation, ascorbic acid, peripheral blood lymphocytes of donors and cancer patients, chromosome aberrations, co-mutagens.**

One of the important components of primary radiogenic cancer prevention is taking into account effect of co-mutagens on humans [1]. These are compounds that, while not having mutagenic properties of their own, can significantly modify (enhance) effects of known mutagens of chemical nature. Drugs with co-mutagenic properties remain understudied, since having no own mutagenic activity, they are not detected at genotoxic screening [2, 3]. To this day, no researches are conducted, aimed at study of co-mutagens impact on radiogenic, including carcinogenic, effects formation in human cells under the impact of low radiation doses. Possible co-mutagenic effects of some common drugs, for instance, ascorbic acid (AA), which is designated as "signal molecule causing specific activity in cells", are of high scientific and practical interest [4]. In series of studies, ambiguous effect of AA on human cells has been revealed [5–10]. It has been established that as opposed to the animals, AA is not produced in human organism, and its food deficiency contributes to the development of gastric cancer, cancer of esophagus, oral cavity, cervix [6]. There is a contradictory view that vitamins, including AA, are impractical to use for carcinogenic risk [5]. Data on antitumor effect of AA at breast cancer [7], gastric cancer [8], prostate cancer [9] and other tumor localizations have been obtained. Researchers fairly state that interpretation of data received on experimental animals on radioprotective and co-mutagenic effects of some drugs, including vitamins-antioxidants, is "wrong to extrapolate to human" [11]. Data on AA impact nature is not always confirmed even in methodically close studies [2].

Due to existing environmental situation in post-Chernobyl period, probabilistic development of carcinogenic effects of low dose ionizing radiation (IR) and oncogenic hazard of increased level of chromosome changes in cell population, study of AA effect on formation of radiation-induced instability of human somatic cells genome is relevant.

Application of test-system of human peripheral blood lymphocytes (PBL), which are the most radiosensitive somatic cells [12], allows to model hypothetic situations under combined irradiation and drugs effect with possible co-mutagenic activity on chromosome level.

It is expected that the formation of AA co-mutagenic effects may be specific, not only depending on its concentration or radiation dose, but differ significantly in healthy donors and cancer patients cells due to the inhibitory effect of the pathological process on repair processes efficiency.

In presented study, peculiarities of AA effect on radiation-induced chromosomal aberrations frequency and range in PBL culture of donors and cancer patients depending on radiation dose and drug concentration, as well as cells radiosensitivity, have been studied (research *in vitro*).

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*Abbreviations used*: AA – ascorbic acid; IR – ionizing radiation; PBL – peripheral blood lymphocytes.

## MATERIALS AND METHODS

Healthy donors and cancer patients (endometrial cancer) (20 observations) venous blood has been cultivated by semi-micro method with some modifications during 52 h [13]. This study was guided by regulations of Helsinki Declaration of the World Medical Association (2008), which provides informed consent of donors for participation in study. Study algorithm is represented in Fig. 1. Cells were incubated in RPMI 1640 medium ("Biowest", France), containing 0.1 μg/ml PHA (M form, "Gibco-Invitrogen", USA) for 52 h (last 3 h with colcemid ("Biowest", France). PBL culture was exposed to radiation in $G_0$- and $G_2$-phases of cell cycle (for 0 and 46 h of cells incubation correspondingly) on X-ray unit "RUM-17". The irradiation conditions: current was 10 mA, voltage — 200 kV, Cu filter (0.5 mm), dose rate — 0.89 Gy/min, studied doses range 0.3–2.0 Gy. Introduced in PBL culture right after irradiation at concentrations 20.0–80.0 μg/ml of blood that corresponded with therapeutic concentration, and also 2 and 4 times exceeding it, AA has been used as radiation effect modifier. To arrest dividing lymphocytes in metaphase, colcemid was added 3 h prior to the harvest. Preparations were made according to the standard procedure. Slides were stained with 5% Giemsa solution (Gibco, USA). All slides were coded and scored blindly at 100 × magnification under oil immersion. Metaphase analysis of cells was carried out in the first postradiation mitosis [14]. On each observation, an average 200–300 metaphases have been analyzed. Value mitotic index has been used as index of lymphocytes proliferative activity for which the amount of cells at mitosis stage was determined. Statistical analysis of obtained results was performed using of descriptive methods, and Student's *t*-criterion (program Excel) [15].

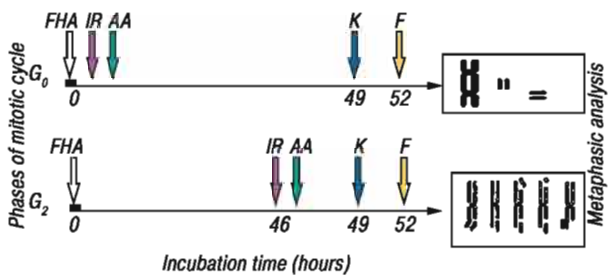


**Fig. 1.** A study algorithm of AA effect on the irradiated PBL of donors and cancer patients chromosomal level. FHA — mitogen; K — colcemid; F — cell fixation; IR — ionizing radiation; AA — ascorbic acid

## RESULTS AND DISCUSSION

AA in the studied concentrations range (20.0–80.0 μg/ml of blood) does not impact of chromosome aberrations spontaneous level in lymphocytes of donors and corresponds to mean population values (2.0 ± 0.86 per 100 metaphases). Results fit the data of study [16], in which lack of vitamins-antioxidants impact on spontaneous mutation process in human lymphocytes has been demonstrated.

When analyzing frequency of chromosome damages, induced by PBL irradiation in $G_0$-phase of cell cycle (in dose range 0.3–2.0 Gy), and post-radiation impact of AA (in concentrations range of 20.0–80.0 μg/ml), modifying effect of this drug was ambiguous (Fig. 2).

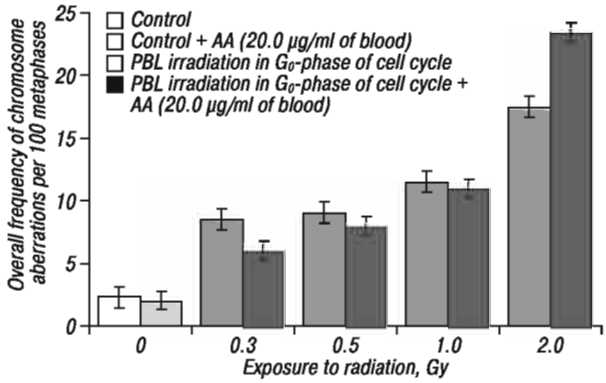


**Fig. 2.** Overall frequency of chromosome aberrations in PBL culture at combined effect of radiation and AA in therapeutic concentration of 20.0 μg/ml of blood

Combined with IR in low dose (0.3 Gy), AA in therapeutic concentration (20.0 μg/ml of blood) shows radioprotective effect, which manifests in overall chromosome aberrations frequency decrease compared to effect of radiation alone (see Fig. 2). However, PBL irradiation in relatively high dose (2.0 Gy), under the impact of AA in the same concentration, shows potentiating of this effect — increase of chromosome aberrations overall frequency in ≈ 1.4 times that point to the co-mutagenic properties of the drug. Observed potentiating of radiation-induced cytogenetic effect occurs due to radial markers — dicentric chromosomes (10/100 metaphases compared with irradiation — 5/100 metaphases) (Fig. 3).

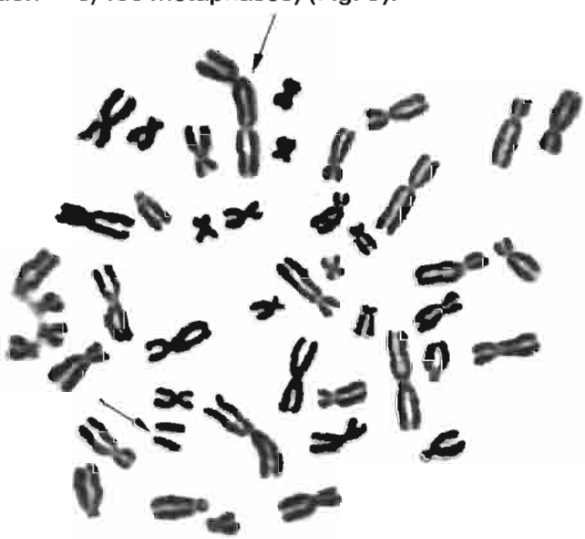

**Fig. 3.** Microphoto of a metaphase plate with dicentric chromosome and pair fragment specified by the arrow (× 100)

Since formation of exchange aberrations — dicentrics, requires local double breaks of chromosomes, resulted due to irradiation, increased aberrations of such type output under additional AA effect can be interpreted as evidence of primary radial damages realization enhancement under the effect of studied drug.

Modification cytogenetic effects of low dose IR under the AA effect in concentrations exceeding therapeutic values deserves special attention. It was estab-

lished that post-radial effect of AA on the PBL culture in concentrations of 40.0 and 80.0 μg/ml, exceeding the value of therapeutic concentration in 2 and 4 times correspondingly, increases overall chromosome aberrations frequency as opposed to effect of irradiation in low dose (0.3 Gy) in 1.4 times (Fig. 4). This may point to co-mutagenic activity of AA in the concentrations range exceeding therapeutic values. Since irradiation in low doses along with chromosome breaks may induce pre-mutative potential changes in the latter, additional effect of co-mutagens in high concentration may contribute to their realization in structural rearrangement of chromosomes, due to suppression of reparation enzymes included [17, 18]. Thus, AA antioxidant depending on concentration at cells irradiation in low doses can manifest both radioprotective and co-mutagenic effects.

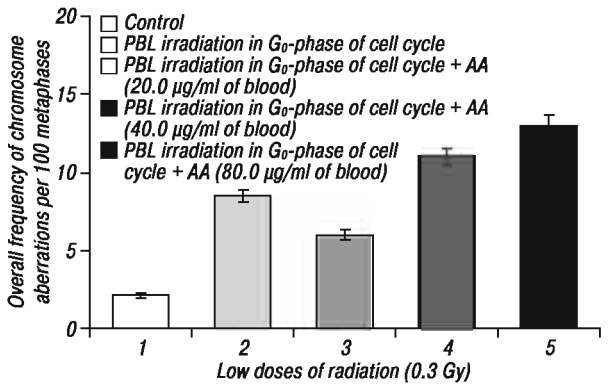


**Fig. 4.** Overall frequency of chromosome aberrations in PBL culture under combined effect of low doses of radiation (0.3 Gy) and AA in the range of concentrations (20.0–80.0 μg/ml of blood)

Thus it can be generalized that formation of some drugs co-mutagenic effects in irradiated human cells depends on both the concentration and radiation dose. Considering carcinogenic hazard of low radiation doses the greatest threat co-mutagenesis can pose is at radiation levels effect without background. Formation of AA co-mutagenic effect depending on irradiation dose is showed on Fig 5. Applying AA in concentration of 20.0 μg/ml after cell irradiation in dose of 0.3 Gy, led to decrease of chromosome aberrations frequency at culture irradiation in low doses to 6.0 ± 1.4 per 100 metaphases as opposed to 9.0 ± 0.86 dose effect, which confirms radioprotective properties of AA. Using AA in concentrations exceeding therapeutic dose value to in 4 times (40.0–80.0 μg/ml), shows co-mutagenic properties, increasing overall chromosome aberrations frequency compared with 0.3 Gy dose ≈ 1.4 times. Upon irradiating cells, co-mutagenic properties of AA are manifested at its concentrations exceeding therapeutic value. At exposure of cells to radiation in relatively high dose (2.0 Gy), co-mutagenic effect of AA is kept regadles of changes in concentrations, increasing on average frequency of chromosomal aberrations 1.4 times due to chromosome type aberrations. This results are confirmed by the study [19] data, according to which in certain conditions of experiment, AA shows prooxidant activity and in such way provides oxidative cell damage.

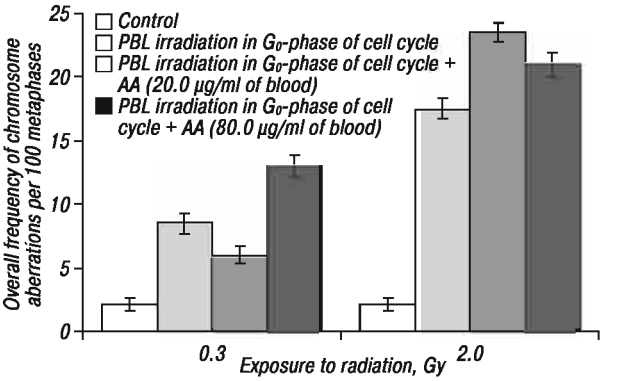


**Fig. 5.** Overall frequency of chromosome aberrations in PBL culture under combined effect of radiation (0.3–2.0 Gy) and AA in the range of concentrations (20.0–80.0 μg/ml of blood)

It is known that low capability of lymphocytes to stimulation by different mutagens may be the evidence of presence and/or induction of genetic disorders in cells, depression of T-lymphocytes, changes in their functional state, development of malignant neoplasms in individuals exposed to radiation [20]. Analysis of PBL mitotic activity under the conditions of combined irradiation (0.3–2.0 Gy) and AA (20.0 μg/ml) effect in $G_0$-phase of cell cycle showed the following (Fig. 6). Most pronounced suppression of cells mitotic activity was noted at irradiation in 2.0 Gy dose, and additional AA effect in therapeutic concentration, on the contrary, increased it ≈ 2 times (exceeding even intact control value). We suppose that this effect is due to "removal" of radiation-induced block (delay of mitosis) under the effect of this drug which shortens time of primary damages reparation. It is confirmed by increase of total chromosome aberrations frequency in these experimental conditions (see Fig. 2).

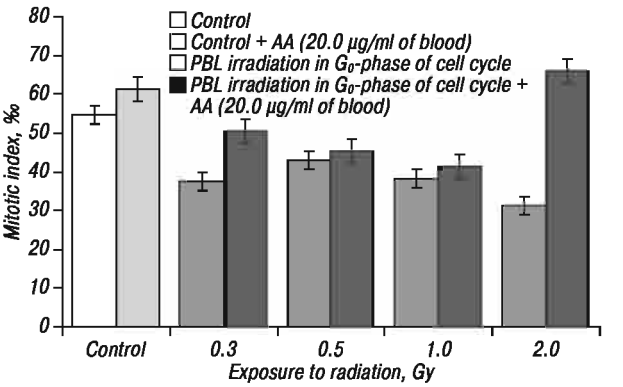


**Fig. 6.** Combined effect of IR (0.3–2.0 Gy) and AA (20.0 μg/ml) upon PBL mitotic activity

According to the paradigms of radiobiology, radiosensitivity of chromosomes is differentiated depending on phase of mitotic cycle owing to the different effectiveness of repair processes [21]. Study of this drug co-mutagenic activity depending on cells radiosensitivity presents certain scientific interest. In this regard similar experiments in the most radiosensitive $G_2$-phase of lymphocytes cell cycle were conducted in culture (see Fig. 1). Analysis of registered chromosome damage at PBL irradiation during radiosensitive $G_2$-phase showed that chromatic type aberrations, represented by deletions and exchanges, prevailed in the range (Fig. 7).

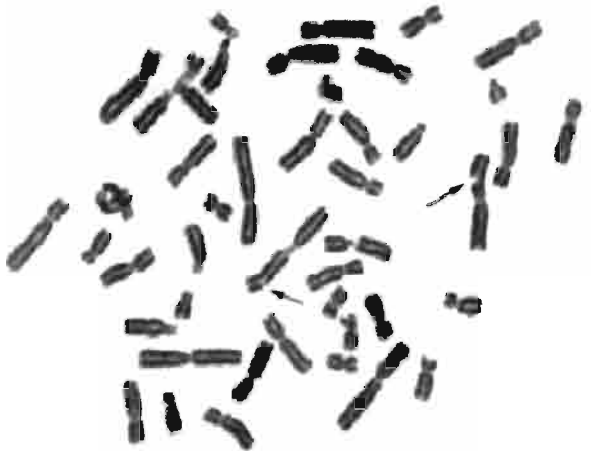

**Fig. 7.** Microphoto of a metaphase plate with deletion, indicated by the arrow (× 100)

Cytogenetic analysis showed that at PBL irradiation with a dose of 0.3 Gy during $G_2$-phase of the cell cycle the AA drug has insignificant radioprotective effect in therapeutic concentrations (Fig. 8).

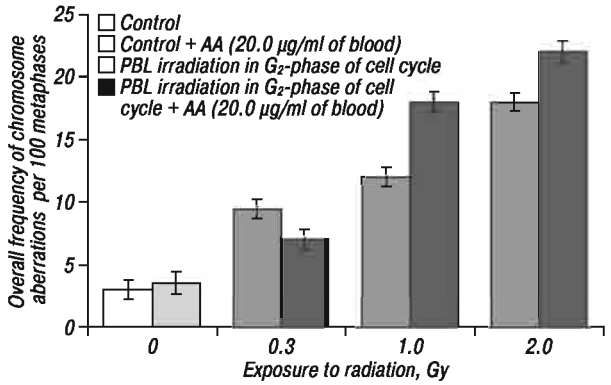


**Fig. 8.** Overall frequency of chromosome aberrations in PBL culture at combined effect of radiation and AA in therapeutic concentration of 20.0 μg/ml of blood

Compared with $G_0$-phase, co-mutagenic effect of AA in $G_2$-phase appears earlier, starting from 1.0 Gy dose (18.0 ± 1.4 and 12.0 ± 1.1, correspondingly; meaning potentiating of effect ≈ 1,5 times) due to higher cells radiosensitivity in this phase of cell cycle (see Fig. 2, 8).

Equally important are the results obtained with AA effect at the chromosomal level of cancer patients PBL (Fig. 9). It is shown that AA concentrations (20.0 and 80.0 μg/ml) did not cause any increase in the cells with chromosome aberrations as opposed to the spontaneous level of chromosome aberrations (7.0 ± 0.8) in the patients non-irradiated PBL. However, the combined action of low IR doses (0.3 Gy) and AA (20.0 and 80.0 μg/ml of blood) in cancer patients lymphocytes display different co-mutagenic effects then the results obtained in similar experimental conditions on the donor cells. Combined with irradiation AA shows co-mutagenic effect in both drug concentrations (see Fig. 4, 9).

Scientific novelty of this findings lies in the fact that at combined *in vitro* effect of low dose irradiation and AA in the therapeutic concentration in the blood lymphocytes of cancer patients there is an increase of genetic damage frequency 1.7 times as opposed to the irradiation effect (meaning the co-mutagenic instead of radioprotective effect of AA appears). The results come as solid proof of the repair processes dominant role in some drugs co-mutagenic activity display, in this case, at the combined effect of radiation (additional) radiation levels and AA.

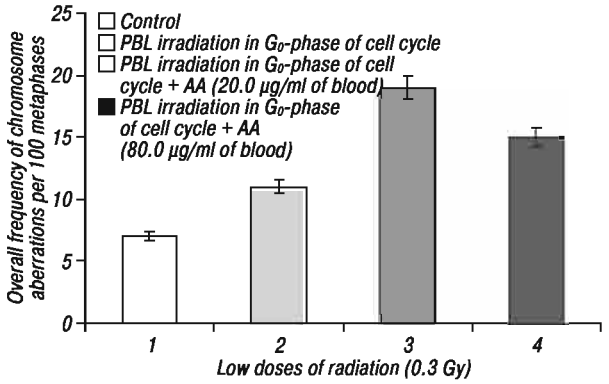


**Fig. 9.** Overall frequency of chromosome aberrations in cancer patients PBL culture under combined effect of low irradiation doses (0.3 Gy) and AA in the range of concentrations (20.0; 80.0 μg/ml of blood)

Thus, based on cytogenetic studies, it has been established that co-mutagenic properties of AA can appear in irradiated cells depending on its concentration, value of IR dose and cells radiosensetivity. High co-mutagens concentrations potentiate damaging effect of low dose IR. We believe that individuals with high sensitivity to radiation and being under contact with sources of IR need an individual approach to use drugs with co-mutagenic activity in medical purposes.

## CONCLUSIONS

Genome destabilization enhancement of irradiated *in vitro* human somatic cells under AA effect is due to its co-mutagenic properties. The formation of co-mutagenic effects of AA depend on its concentration, irradiation dose and the efficiency of repair processes. Co-mutagenes may pose high carcinogenic hazard at low (above background) radiation levels.

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