KAPITEL 14 / *CHAPTER 14* [14]
GENETIC DATA OF MELATONIN'S ADIOPROTECTIVE EFFECT ON NON-MALIGNANT CELLS OF ONCOGYNECOLOGICAL PATIENTS

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Introduction.

The emergence and development of carcinogenesis is associated with the accumulation of a number of genetic changes registered not only in tumour cells but also in non-malignant cells of cancer patients from the tumour environment [1]. Their increased genetic instability is caused by mutations in genes responsible for the processes of error-free DNA repair, and can also be a consequence of cells getting into extreme conditions, for example, into the zone of hypoxia [2], therapeutic irradiation [3] and others. Thus, when cells get into the zone of hypoxia, there is a decrease in the expression of DNA repair genes. This leads to disorders of nucleotide excision repair, mismatch repair, and homologous recombination [1, 2, 4, 5].

It is a well-known fact that effective radiotherapy (RT), which affects the tumour, is inevitably associated with the risk of developing early and distant radiation complications. These effects of RT differ in specific radiobiological characteristics.

Irradiated cells from the tumour surroundings are already significantly different from the original normal tissue [6,7]. Molecular, chromosomal, biochemical and other abnormalities in healthy cells of primary cancer patients change the functional state of these cells, in particular, their radiosensitivity, and therefore give grounds to consider them only conditionally normal. The risk of long-term radiation reactions on their part can be quite high, given the stochastic, including carcinogenic, nature of their formation. Additional radiation-induced damage in these cells as a result of therapeutic irradiation can contribute to a high risk of radiation complications from healthy organs and tissues surrounding the tumour, including highly radiosensitive cells in the circulating blood pool. It should be noted that international organizations IAEA, WHO, UNSCEAR have recognized that genetic aberrations in human peripheral blood

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lymphocytes (PBL) are an optimal model for studying the mechanisms of early cancer diagnosis and prognosis.

Let's briefly consider the features of early radiation complications on the example of patients with gynecological oncology, the incidence of which has been rapidly increasing in recent years [8]. The interest of researchers in this pathology is due to the risk of developing radiotherapy-related complications in critical pelvic organs (rectum, bladder, vagina) [9].

The data currently available in publications mainly relate to early radiation complications that occur in the case of cancer of the uterine body and cervical cancer, both during concurrent radiotherapy and in the first three months after treatment. The main reason for their development, according to researchers, is the excessive tolerance of the irradiated tissues. Favourable factors for their development may include an increased individual human radiation sensitivity (IRS), previous inflammatory processes, biological characteristics of the tumour, etc. [1]. Unlike early complications, the development of distant side effects is irreversible and progressive. The risk of late radiation complications remains present throughout the life of the irradiated patient [10]. Early and distant radiation complications differ in their pathogenesis. Moreover, researchers believe that the risk of late postradiation complications cannot be predicted based on the degree of early effects [11]. We consider this statement controversial, since it does not initially take into account the IRS of the patient's body [12].

It is important to note that the mechanism of development of distant radiation damage is based on disorders of more radioresistant structures. In general, it should be noted that scientific studies on the treatment of distant radiation damage to critical organs and tissues, including the pelvis, are mainly descriptive. The knowledge of the pathogenesis and biological basis for the development of RT side effects in healthy tissue cells (in particular, in lymphocytes of the circulating blood pool) is the basis for the development of selective radiobiologically based protective drugs [9, 11, 12].

Researchers are interested in cervical cancer (CC) due to the risk of developing radiation complications in critical pelvic organs as a result of RT. There is an urgent need to develop nontoxic radioprotectors that would be selective only to healthy

tissues, which would not require additional accompanying therapy and would definitely improve the quality of life of treated patients.

Researchers at R. E. Kavetsky Institute of Experimental pathology, Oncology and Radiobiology of National Academy of Sciences of Ukraine, taking into account literature data and the findings of previous experiments, carry out research to assess the feasibility and features of prescribing the drug melatonin (MT) as a radioprotector to protect healthy cells from the surroundings of the irradiated tumour or its bed in patients with cervical cancer from complications of radiation therapy. Our choice is due to the fact that MT is a powerful antioxidant, which has immunomodulatory and detoxification effects. Studies in recent decades indicate that MT has numerous oncostatic properties. MT is involved in cell cycle modulation, induction of apoptosis, stimulation of cell differentiation, and inhibition of metastasis [13].

Materials and methods.

When performing RT (external beam and brachytherapy), the negative effect on normal tissues of patients from the tumour environment is quite powerful. There is no doubt that the most vulnerable structure when tissues are irradiated is the DNA of their cells. It should also be taken into account that some non-malignant cells of primary cancer patients are only conditionally normal, as they already contain genetic damage and altered functional state.

We assessed DNA double-strand breaks (DSBs) by single-cell electrophoresis (Comet assay) [14,15] under neutral conditions [16]. Isolated cells on agarose were lysed to remove membranes and DNA bound to proteins (while maintaining the DNA connection to the nuclear matrix), and then electrophoresis was performed under neutral conditions. Under the influence of an electric current, damaged DNA leaves the cell and forms a zone resembling the tail of a comet (Fig. 1). The comet parameters were used to quantify the damage to DNA integrity [17].

Micro specimens were stained with SYBR Green I, 10000x gel staining solution (Lumiprobe Corporation, USA) by applying and distributing several drops of dye on the surface of the preparation. Micro specimens were visualized using an Axio Scope A1 microscope (Carl Zeiss, Germany) with a high-sensitivity Axio Cam ICc 5 camera

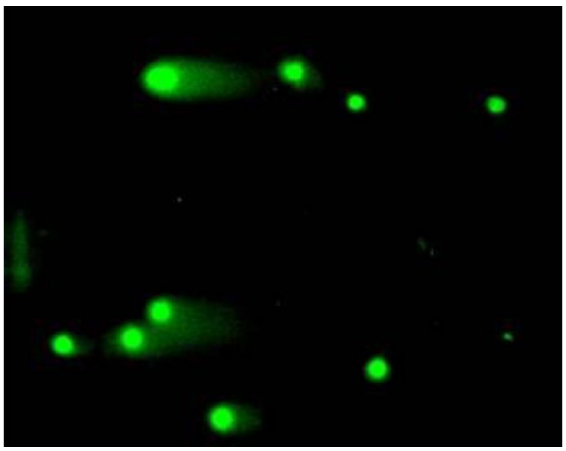

Figure 1. - DNA comets in blood lymphocytes of a CC patient (magnification x 200)

in fluorescence mode. 100 comets per observation were counted per photo. Comets were analysed using the CometScore computer image analysis system (TriTek Corp., Sumerduck, VA, USA). The percentage of DNA that escaped from the cell (% DNA in the comet tail) was used as an indicator of DNA damage.

Test irradiation of blood samples of primary 8 oncogynecological patients (study group) and 8 healthy individuals (group of conditionally healthy control - CHC) was performed on the Xstrahl X-ray irradiation machine of the National Cancer Institute of the Ministry of Health of Ukraine in the range of doses 2-6 Gray. Their value corresponds to clinical doses per one fraction of irradiation of patients with this pathology.

Results.

The spontaneous level of DSBs DNA in PBSs of CC patients (study group) and CHC was investigated before RT, as well as after test irradiation of blood samples supplemented with MT as a potential radioprotector. There was a significant decrease in spontaneous DNA DSBs levels in the blood lymphocytes of CC patients by 40 and 57% according to the MT concentrations used (0.2 and 0.4 mM) (Fig. 2. A,B). At the same time, a 37% decrease was recorded in CHC blood only at the MT concentration of 0.2 mM (Fig. 3.).

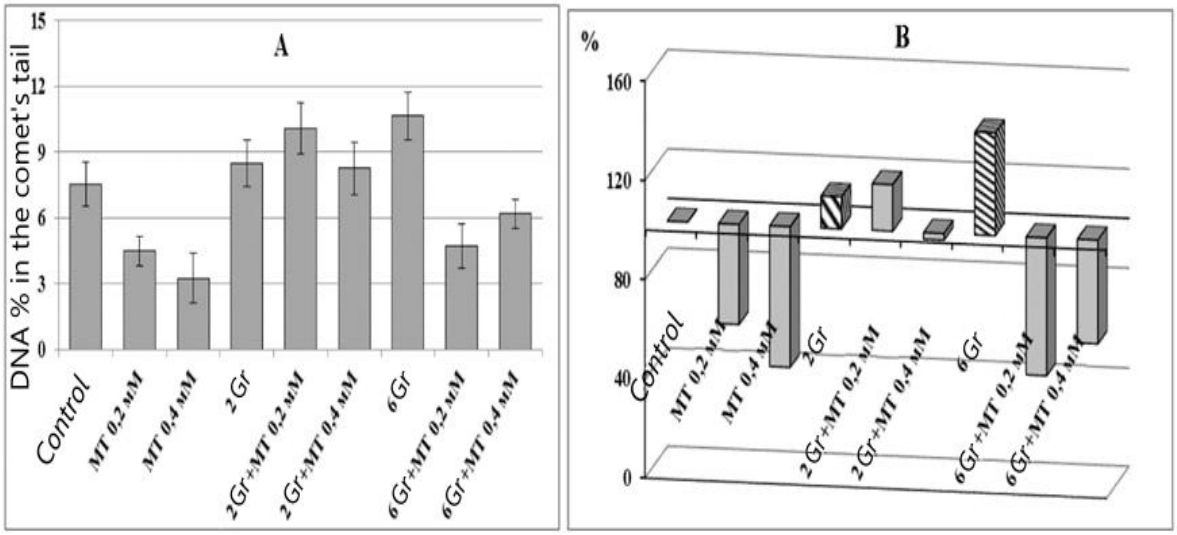

Figure 2 - DNA DSBs level in blood lymphocytes of CC patients (A) under in vitro irradiation at doses of 2 and 6 Gr and additional exposure to MT at concentrations of 0.2 and 0.4 mM and ratios of values to the corresponding control (B), %

X-ray irradiation led to a significant increase in the amount of DSBs DNA in the blood lymphocytes of healthy individuals by 44 and 69% according to the dose of 2 and 6 Gr. At the same time in the blood of patients, the revealed changes were less pronounced: the increase was 12 and 41%, respectively.

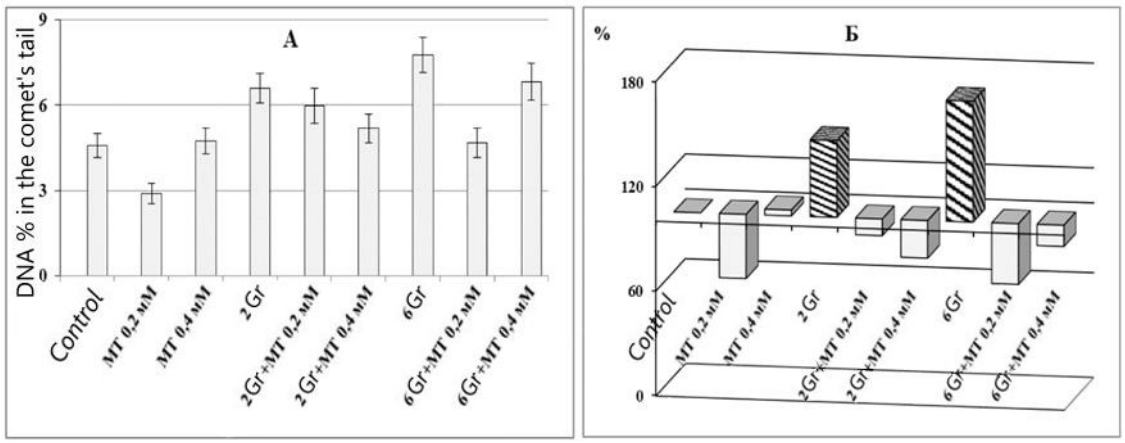

Figure 3: Level of DNA damage in blood lymphocytes of healthy individuals (A) under in vitro irradiation at a dose of 2 and 6 Gr and additional exposure to MT at a concentration of 0.2 and 0.4 mM and the ratio of values to the corresponding control (B), %.

The radioprotective effect of MT was observed in the study of the level of DNA damage in both blood samples of CHC and CC patients when irradiated at a dose of 6.0 Gr (reduction of the amount of DNA damage in CHC lymphocytes by 35 and 22%, respectively, at MT concentrations of 0.2 and 0.4 mM, and in the lymphocytes of CC patients by 56 and 42%, respectively). When blood samples were irradiated at a dose of 2.0 Gr, a slight decrease in the level of DNA damage (22%) was observed only for CHC lymphocytes at a higher MT concentration of 0.4 mM.

Thus, the radioprotective effect of MT at the genetic level of highly radiosensitive non-malignant cells of primary CC patients and healthy donors was observed. Of particular note is the radioprotective effect of the drug when irradiating blood lymphocytes of patients in a 6 Gr dose used in brachytherapy. The observed effects are recorded relatively independently of the MT concentration.

At present, several key factors involved in the development of early and late post-radiation effects can be identified: genetic defects in DNA molecules; reduced cellular repair potential; increased resistance to radiation therapy; cytokine production; increased genomic instability; modification of reactive oxygen species (ROS) formation; and a high risk of fibrogenesis. The knowledge of the pathogenesis and biological basis for the development of RT side effects in healthy tissue cells (in particular, in lymphocytes of the circulating blood pool) is the basis for the development of selective radiobiologically based protective drugs. The dose-limiting factor of RT is the tolerance of intact organs and tissues surrounding the tumour, and the delivery of tumour-inducing doses to the tumour focus is associated with a high probability of radiation damage in risk areas [18]. This requires the prescription of additional therapy and, in some cases, interruption of the course of therapeutic radiation. The following approaches to protecting normal tissues are distinguished: preventive - if performed before therapeutic radiation; attenuating - if performed at the time of radiation or immediately after it, i.e. before the onset of clinical symptoms; therapeutic - if clinical symptoms appear to treat radiation complications. Currently, the key problem in radiation oncoloG and clinical radiobiology is the search for and development of nontoxic (or low-toxic) radioprotectors that show affinity only for

healthy (nonmalignant) cells from the environment of the irradiated tumour without affecting its radiosensitivity. Adherence to such a radioprotection scenario will not only improve the quality of life of cancer patients, but also reduce the psychological and economic burden on their families and society as a whole.

Conclusions.

The obtained genetic data provide a radiobiological justification for the need to improve the tertiary prevention of radiation complications in CC patients by developing and implementing means of selective protection of healthy tissues that may be exposed to radiation.

Genetic screening of CC patients before the beginning of RT will make it possible to form groups of patients at high risk of radiation complications, which will provide a personalized strategy of their treatment.