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The Study of the Transgeneration Genotoxic Effect of Drugs Based on the Analysis of Synaptonemal Complexes in Mouse Spermatocytes

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***Abstract*— The research employs immmunocytochemical methods to study the synaptonemal complexes (SC), and it targets the primary spermatocytes of mice from the offspring of males who received antimicrobials**

The males in the offspring of fathers received bacteriophage, nitrofural and cifran. When comparing the total number of damaged cells, they did not show a significant difference with the control animals. However, when comparing the spectrum of affects, they presented a significantly increased number of cells having ring SC-bivalents in the offspring of males treated with cifran. This may indicate an increase in chromosome fragility, leading to the deletion of telomere regions of chromosomes, which in turn causes the formation of ring bivalents.

Keywords*— *synaptonemal complex, meiosis, transgeneration effect, drugs, genotoxicity

I. INTRODUCTION

The study of the genotoxic effect of drugs on human germ cells is one of the urgent problems of modern genetics. The relevance of this topic results from the risk of hereditary pathology, neoplasms, male and female infertility, miscarriage in the offspring caused by the formation of chromosomal disorders in the germ cells of their parents. This problem has become noticeable with the presentation of the data on the gradual decline in male fertility in different regions of the world.

Numerous research prove that the emerging sex cells are highly sensitive to environmental factors. Previous studies established that drugs, primarily anti-tumour preparations, can cause the breaks in meiotic chromosomes of animals and the disorders of desynapsis of chromosomes [24, 25], and this can lead to persistent and even irreversible damage of fertility in men [23, 8]. Antimicrobials also have a high metabolic activity. However, their genotoxicity to germ cells are rather contradictory [22, 2]. This is due to the diversity of the chemical structure of drugs of different pharmacological groups, the difference in the mechanisms of their biological action, as well as the use of different experimental models and methods of analysis of spermatogenesis disorders.

One of the most informative methods for the identification of the disorders of the structure and behaviour of chromosomes in prophase I of meiosis is the analysis of total SC preparations in spread nuclei of primary spermatocytes [22, 17, 11]. SC is a specific three-lateral structure formed between two homologous chromosomes in representatives of all eukaryotic groups. SC is involved in the meiotic conjugation, recombination and desynapsis of homologous

chromosomes. The method of SC analysis gives the possibility to identify the nature of chromosome synapsis disorders and all types of chromosomal aberrations. The application of methods of immunocytochemical analysis of proteins makes it possible to identify the sites of chromosome inactivation, recombination and synapsis disorders and, accordingly, the risk of aneuploidy of germ cells [6, 14].

Drugs and other xenobiotics can have a genotoxic effect both directly on the organism exposed to them, and indirectly on its future offspring. Researchers called this phenomenon the transgeneration effect. For example, Zeh [3] with the research team showed that tetracycline, a broad-spectrum antibiotic, has a negative impact on the quality of the ejaculate in pseudoscorpions (Cordylochernes scorpioides), reducing the viability and number of sperm in males having undergone the treatment, as well as in their offspring. Transgeneration effect appeared only in the first generation, in subsequent generations it disappeared. In male offspring of females treated with tetracycline, the observations marked no changes in the quantity and quality of sperm.

Ionizing radiation and some chemical mutagens not only lead to the induction of mutations in the germ cells directly exposed to parents influence, but can also destabilize the genome of their offspring [19, 20, 15, 16, 10, 18, 4, 5]. Until now, there was an opinion that the risk of radiation therapy is mutations that can sustain in the next generation. However, new experimental data show that in the offspring of irradiated parents, the frequency of mutations remains elevated for at least two generations.

The studies note that despite the results of animal studies, experimental evidence of transgenerative instability in humans remains a highly controversial issue. For example, a recent study showed an increase in the frequency of chromosomal aberrations in children born from fathers exposed to radiation in the Chernobyl accident zone (Aghajanyan et al., 2011). On the other hand, no significant changes were in children of fathers who underwent radiation therapy for cancer before birth (before conception) (Tawn et al., 2005). The manifestation of transgenerative genomic instability was found in the study of offspring of male mice exposed to doses exceeding 1Sv [19, 20, 4, 5]. It is relevant that cancer patients receive a much smaller dose of radiation. The fact is that high doses of anticancer drugs can also destabilize the genome in the first generation of descendants [5, 18].

Some drugs can cause epigenetic chromatin modifications associated with DNA methylation, histone modification, or non-coding RNA that can persist for several generations. There are reports saying that mitochondria play an important role in such changes [8].

II. Methods and materials

The study was conducted on males from F1 offspring, obtained from crossing intact females with males divided into 5 groups, receiving antimicrobial drugs for 10 days, including the following preparations: furacilin (nitrofural) (antiseptic from the group of nitrofuran preparations), cifran (antibiotic of the group of fluoroquinolones) and sextofag (pyobacteriophage polyvalent). In order to compare this with the experimental group, the groups of males after the introduction of water and males after the introduction of antitumour cytostatic cyclophosphane took part in the study. Single doses of drugs were calculated by the formula: D = 16Mm × Dh / Mh, where D is the dose of the drug for a mouse , Mm is the mass of the mouse body, Dh is the therapeutic dose for humans, and Mh is the mass of the human body

Males from the offspring of fathers receiving the above drugs were disposed upon reaching the age of 3 months.

Total preparations of the spread of synaptonemal complexes (SC) were by means of spreading the nuclei of spermatocytes on the surface of the hypotonic solution using the Navarro et al method [9] with modifications [12].

SC and axial elements of the chromosomes were immunostained using rabbit antibodies against the protein SCP3 (Abcam, United Kingdom); histone γH2AX was detected using murine antibodies against histone γH2AX (Abcam, United Kingdom); centromeres were identified using human IgG against protein kinetochores (ACA) (Antibody Incorporated, USA) (all at a dilution of 1: 200). Goat antibodies to rabbit IgG conjugated with FITS (Jackson, USA), goat Antibodies to human IgG conjugated with AlexaFluor 546 were used as secondary antibodies. (Invitrogen); equine IgG against mouse immunoglobulins conjugated with FITS (Jackson, USA). (dilution 1: (500-800).

The drugs washed in a phosphate buffer were placed in a Vectashield with DAPI.

The preparations were imaged using a universal fluorescence microscope Axio Imager D1 (Carl Zeiss, Germany) with IC2S–optics standard equipped with PLAN-NEOFLUAR lenses, HBO mercury lamp, black-and-white CCD signal accumulation camera AxioCam HRm Rev.3 (Carl Zeiss, Germany), a set of combined filters for fluorochromes with access to Siemens computer (Fujitsu Technology Solutions, Munich, Germany). We saved the image obtained with the Axiocam HRm camera in the Axiovision rel program. 4.6. The images were processed in Adobe Photoshop CS3 Extended (Adobe Systems, San Jose, CA, USA). The computer is equipped with Axiovision Release 4.6.3 software (Carl Zeiss, Germany) for video production.

Statistical data processing was carried out in Microsoft Excel and WINPEPI. The homogeneity of the samples was checked using the Chi-square method. The Fisher accurate test was used to assess the validity (materiality) or randomness of the differences between the study results in the control group and in the group after exposure to drugs. A section that describes the techniques and materials is in this article.

III. Results

SC analysis in offspring of control animals

Immunofluorescent analysis of the spread nuclei of primary spermatocytes of control animals obtained from males injected with water revealed cells at all stages of prophase I of meiosis from leptotene to late diplotene. At the stage of pachytene, it detected 19 autosomal SC and a typical sex bivalent with a synapsis in the pseudoautosomal area located

between X and Y chromosomes (Figure 1 a). In the early stages of prophase I of meiosis, histone γH2AX was detected in the chromatin of those chromosome regions that have not yet entered the synapsis. Starting from the stage of pachytene, the signal was localized only in the chromatin of the XY bivalent (Figure 1 b). In most nuclei, the abnormalities in the structure of SC were not found. However, in single nuclei (10.4%) there were disorders in the structure and behaviour of SC. The data are shown in Figure 3

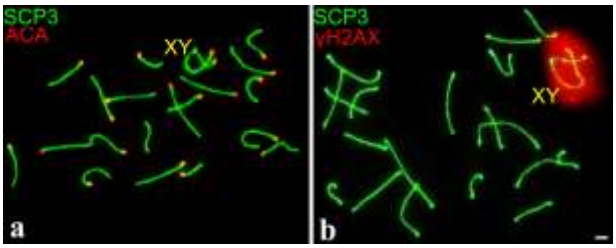

Fig. 1. The SC structure in the nuclei of male spermatocytes from F1 offspring obtained from crossing of males injected with water with intact females.. SC were immunostained with antibodies to protein SCP3 (green); centromeres are stained using polyclonal antibodies to proteins kinetochores (ACA, red); the areas of transcriptionally inactive chromatin - using antibodies against histone γH2AX (red). a. Stage of late pachytene. Autosomes are elongated. The genital corpuscle (XY) is formed and lies on the periphery of the nucleus; b. Stage of intermediate pachytene . The genital corpuscle (XY) is formed and located on the periphery of the nucleus. Antibodies to histone γH2AX (red) are associated only with sex chromosome chromatin. Bar 2 micron

The SC analysis in the offspring of males treated with sextofag.

Immunocytochemical investigation of SC of primary spermatocyteswas carried out in animals from the offspring of males who received sextofag. All animals had nuclei at all stages of prophase I meiosis. In single nuclei, the disorders in the structure and behaviour of SC were revealed, their number and range of these disorders did not differ from the control group (Figure 3).

The SC analysis in the offspring of males treated with cifran.

Using Immunocytochemical methods, SC of primary spermatocytes in animals obtained in the offspring from males who were injected with cifran were investigated. In 16% of the studied nuclei, the disorders in the structure and behaviour of SC were revealed, which was not significantly different from the control group. When comparing the spectrum of disorders with the control group, a significant increase in ring SC was revealed, specifically in 5.9% of the studied nuclei (Figure 2 a, b, e). The nuclei with the fragmentation of SC were detected in 4.2% of the investigated cells. The association of sex (XY) bivalent with an autosomal SC bivalent and the asynaptic of sex chromosomes were identified in 3.8% and 2% respectively. In 40% of the studied cells, the autosomal SC bivalents were folded into a loop (Figure 2 b, g). Nuclei with a bend on the X chromosome were found at a lower frequency (31%). This change in SC can not be clearly recognized as a disorder, since the consequences and causes of such behaviour of chromosomes are not clear, however, in the control group we have not seen these changes in the morphology of SC.

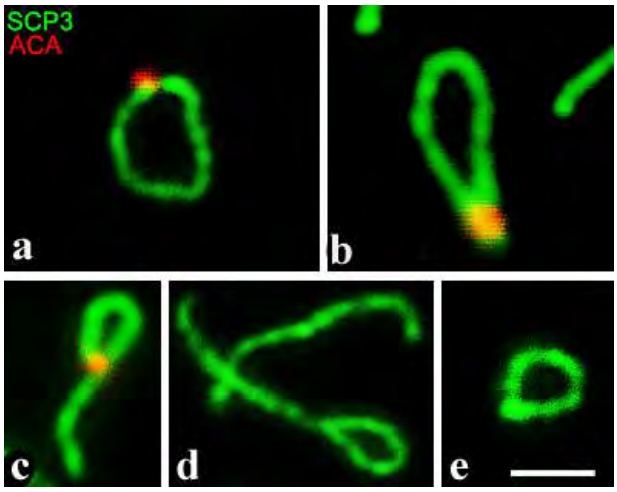

Fig. 2. Abnormalities in the structure of SC in the nuclei of spermatocytes of the descendents of the males treated with cifran. SC immunostained with antibodies to protein SCP3 (green); centromeres are identified by using polyclonal antibodies to proteins of centromeres (ACA, red). a, b, e. Ring SC bivalents. c, d, high ductility of SC, e. ring chromosome. . Bar 2 micron

The SC analysis in the offspring of males treated with sextofag.

In immunocytochemical analysis of the spread nuclei of primary spermatocytes in 3 animals, in the first meiosis prophase, we did not reveal significant deviations in the number of damaged cells from the control group of mice (Figure 3).

The SC analysis in the offspring of males treated with cyclophosphan.

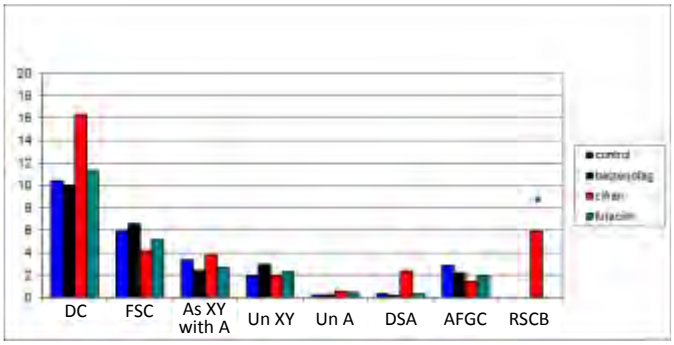

Fig. 2. The distribution of the number (%) of abnormalities of the structure and behaviour of SC of seven different types in spermatocytes in mice of offspring derived from fathers introduced to sextofag, cifran, nitrofural and in control group.

Table of symbols: DC – total number of damaged cells; FSC – fragmentation of SC; As XY with A – association of XY with Autosomal SC; Un XY – univalents of X - and Y-chromosomes; Un A – univalents of autosomes; DSA – disorders of autosomes synapsis; AFGC – abnormalities of the formation of genital corpuscle; RSCB – ring SC bivalent. * P <0.05-significant difference from control group.

The males mouse, after a ten-day intraperitoneal administration of cyclophosphan, were put together with the intact females. Within 30 days, the pregnancy did not occur, after which other females were exposed to the males to exclude the infertility of the females. After 60 days, to end the ten-day cyclophosphan administration, the animals were slaughtered. The autopsy showed the malnutrition and the signs of fatty degeneration of the tissue of the testis. Animals are not able to provide of SC drugs.

IV. CONCLUSION

The research established that the descendants of irradiated parents had not only well-known hereditary disorders (fetal death, malformations, sterility, mutations), but also an increased risk of oncogenic diseases; there were the evidence of physiological disorders, decreased immunity, genome instability even in species with normal karyotype [16].

During the irradiation of C. elegans at the stage of the late pachytene, 2 hours after the irradiation, chromatin was demodulated, short-term desynapsis of homologues, fast and less effective sister (not homologous) chromosome in the repair of DNA breaks and subsequent desynapsis of homologues. It is possible that the mutagenic effect of xenobiotics at the stage of late pachytene also shows the risk of less effective repair.

The results of the present study showed that the structure of SC in the offspring of animals injected with sextofag and nitrofural did not differ from the control animals, both when comparing the total number of damaged cells and when comparing the spectrum of these disorders.

In males in the offspring of fathers, who were administered the antibiotic cifran, when comparing the total number of damaged cells, no significant difference with the control animals was revealed. However, when comparing the spectrum of affects, they presented a significantly increased number of cells having ring SC-bivalents in the offspring of males treated with cifran. This may indicate an increase in chromosome fragility, which leads to the deletion of telomere regions of chromosomes, which in turn causes the formation of ring bivalents.

In response to the acute toxic effects of drugs, including xenobiotics, in the nuclei of spermatocytes, global chromosome fragmentation at the stage of leptotene-zygotene (meiotic catastrophe) was observed. Apparently, this mechanism provides fast total selection at mass defeat of spermatocytes. This phenomenon is revealed in nuclei after the administration of nitrofural and cyclophosphan [21]. The lack of offspring from the males that received cyclophosphan within 10 days can result from quite serious lesions of the cells of spermatogenic series, after which there was a fat degradation of the testes.

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