LONG TERM EFFECTS ON MINKS OF THE RADIATION FACTORS FROM THE CHERNOBYL ACCIDENT

A.Y. BONDAR, V.P. ZAMOSTIAN
Kiev Mogilyansk Academy,
Kiev, Ukraine

V.I. RIASENKO
Scientific & Technical Centre of the RIA "Pripyat",
Chernobyl, Ukraine

1. Structural and Functional Changes in Female Reproductive Organs That Reside in the Stage of Menopause and Fetus Embryogenesis.

Introduction

The study of small radiation dose influence on human and animal reproductive functions becomes more and more topical after Chernobyl Nuclear Power Plant (ChNPP) accident. In the number of cases, animals that reside in continuos internal, as well as external exposure zone, have pregnancy interruption in its early stages (up to 30 days). This, without any doubts testifies for reproductive process disorder as a whole (hypophysis-ovary-uterus system) and also, as its separate links.

The important thing is that a break in any one of those links leads to pregnancy interruption. Hence, in order to determine any disorders in reproductive system functional state, profound and detailed morphofunctional study of the system links (accounting for radiation exposure factors) needs to be done. Because research in this field has just started, we were unable to find any material on this topic. There are, however, some references for morphofunctional changes of endocrine glands, hypophysis in particular [1], [2], [3], [4] and sex glands [5], [6], [8], refereed to small radiation doses.

It was determined by the authors on a light-optical level that small dose exposure (up to 50cGy) of part of hypophysis does not lead to any significant functional of morphofunctional changes. Only with exposure doses more than 300cGy on the anterior lobe of hypophysis, some small morphological changes took place. These are the changes in a number of secretory elements in cells with increase of acidophile and decrease of basophile cells. Out of dystrophic changes, protoplasm turbidness and nuclei piknosis in basophile cells have been reordered by the authors [4]. And only with large doses (more than 2,000cGy) significant destructive changes in hypophysis (secretory cell decay) took place [7]. The main objective of this research is morphofunctional study of structural and functional peculiarities of endocrine-reproductive animal system with low internal and external exposure doses. Also, the link of such changes with pregnant female's reproductive system disorders.
Materials and Methods:

As a part of the project we examined hypophysis, ovaries, and uterus in 10 pregnant females from Chernobyl zone, exposed to small internal, as well as external radiation doses. Also, control animal group (10 minks), with almost negligible radiation factors was studied. The examined females included the ones with normal and pathological pregnancy progression. Bioplates of hypophysis, ovary, and uterus were studied on light-optical, as well as on electron-microscopic levels. Ultrathin slices of 400-600Å were prepared on LKB and Reichert ultratoms. Stained according to Reinhold for contrast enhancement, slices were examined with EM-400T Phillips Electron Microscope. For accurate ultratoming and comprehensive pathological process estimation, half-thin slices of 1500Å were prepared from the blocks jellied in epoxide resin and stained with toluide blue-peronine for microscopic examination.

Conclusion:

Complex histochemical, electron-microscopic, and electron-enzyme-histochemical examination of hypophysis, ovary, and uterus has been carried out. Purpose of the examination was to study morphofunctional state of endocrine-reproductive system accounting for extended internal and external small exposure doses from Chernobyl radioactive factors.

The results showed different dystrophic-destructive changes in cell elements and microcirculatory vascular channels that both lead to disorder of reproductive function and pregnancy interruption in females. Morphological changes in follicle-stimulating and luteinizing cells of adenohypophysis is a very good example of this.

During normal pregnancy progression, increase in morphofunctional activity with increased number and hypertrophy of secretory granules has been observed in many honadotropocytes on a background of high protein synthesis and energy generation of these cells. Females with pregnancy interruption had definitely lower morphofunctial activity of preponderant honadotropocytes. That is because in the most follicle stimulating honadotropocytes, absence or significant decrease in secretory granules was observed. These adenocytes had quite significant dystrophic-destructive changes in intercellular organelles due to decrease in protein synthesis and energy generation levels.

Detected changes need to be considered as the ones that depend upon radiation, because during normal pregnancy progression we have found follicle-stimulating and luteinizing cells with low secretory activity. Although they did not have significant dystrophic-destructive changes that cause cell morphofunctional state disorders. Not only honadotropocytes, but also other adenohypophysis' cells, dominating somatotropocytes in particular had destructive changes. This is an important model of adenohypophysis radioactive damage. Significant example of radiation effect on adenohypophysis ultrastructure is partial damage of its microcircular system. This results in blooding and expansion of intrabrainal capillaries accompanied by capillarostasis and destructive changes in endothelial bedding. In particular, in marginal part of endotheliocytes were most active metabolic processes between microvessels and adenohypophyse cells take place. Changes in endothelial cells were
most specific for radiation effect presented as flating of marginal part of endotheliocytes, plasmocytosis, and absence of micropinocytotic activity. As for morphofunctional changes in uterus with normal pregnancy progression and its interruption in females that reside in continuos radiation exposure zone, we were unable to find any specific changes due to radiation. We can only assume that revealed dystrophic-destructive changes in endometrium glandular epithelium cells during pregnancy interruption were more significant than during normal pregnancy progression. Changes in microcircular vessels in both cases were the same. In ovary follicular epithelium it was almost impossible to differentiate changes due to follicle grow and differentiation from radioactive influence processes. In order to separate these processes, utilization of specific immunohistological and radioautographic methods of examination would probably be necessary.

Therefore, the conducted research had shown that most distinctive dystrophic-destructive changes in endocrine-reproductive function of Chernobyl zone pregnant females (with pregnancy interruptions involvement) have been observed in adenohypophysis' gonadotropic cells. According to the obtained morphological data, their structural-functional changes due to reproductive function damage, as well as radiation factor influence were detected.

References:

3. Embriogenesis' Peculiarities During long-time External and Internal Animal Irradiation.

Introduction:

The experiments on teratogenic and embriotoxic properties' detection of four generations of animals that for a long time resided in external and internal small dose irradiation zone have been carried out. For comparison purposes, a group of animals that resided in 'clean' areas was used.

The total number of animals used in experiment equals to 14. It includes 11 animals that resided in Chernobyl zone (9 females and 2 males) and 3 control animals. Mating was made one month before the experiment. Average Chernobyl zone female mass recorded to be 967+/-32.5 g. At the same time average control female mass was 1132+/-45 g. Out of 9 examined females, one had no sign of pregnancy: ovarian yellow bodies and fetal sac were absent. This female had macroscopic pathological changes in internal organs such as lung and liver hemorrhage, bad heart, and a number of highly enlarged vessels in stomach. The rest of the animals (8 minks) generally had 90 ovarian yellow bodies and 39 fetal sacs that had 25 viable and 5 non-viable fetuses, rest of the sacs contained hemorrhagic fluid. Blood coagulation in all examined females was speeded up, giving the average of 12.43+/-3.8 sec. At the same time males had 135 sec. and control females - 160 sec. coagulation time. Pre-implantational fetus mortality of Chernobyl zone females was 56.7% and post-implantational - 35.9%. Control minks had pre-implantational fetus mortality equal to 33% which is quite normal for this group; post-implantational mortality was not observed at all: only 1 out of 30 embryos, which gives 3%.

Therefore, embryonic mortality analysis shows that female minks resided in Chernobyl zone had increased pre-implantational and post-implantational mortality. This proves for neuroendocrine regulation damage in female minks, accounting for environment factors. Pregnant inability in female is due to hormonal disbalance: hypophysis and hypothalamus primarily. Pre-implantational death can be caused by insufficiency of hypophys' gonadotropic hormone lutropine, as well as progesterone. Post-implantational death is primarily due to progesterone deficient.

The average embryo number of studied group equals to 3.125+/-1.02. At the same time control animals had 9.7+/-0.33, that is three times less. The average embryo mass of studied group gives 6.8+/-1.07 and the mass of control embryos - 8.13+/-0.49. Embryos of both studied and control groups were deviled into two subgroups. First subgroup embryos were fixed in Byene fluid; after complete fixation 9 cuts were made for brain and intraorgan structure examination. Second group embryos were fixed in alcohol, lightened in KOH, and stained (bone tissue) by alizarin - red.

The results of the examination showed that ossification in studied and control group occurred similar. Although brain and intraorgan structures in studied group significantly differed from the controls. Big number of examined animals had brain, eye, liver, kidneys, lungs, and stomach hemorrhage along with epidural hematomas; sometimes, pleural cavity and intramuscular hemorrhage. Hence, the conducted research show a number of changes in animals that resided in superior environmental factor areas.

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Environmental factor influence of mink embryonic mortality

Female minks that resided in Chernobyl zone

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<td>39</td>
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Female minks that resided in "clean" areas.

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These are:
• pregnancy absence
• 1/3 had pre-implantational embryo deaths
• implanted embryos had big macroscopic changes such as hemorrhage and bleeding.

All these can possibly cause change in blood coagulation of pregnant females. The following data shows effects of residing in Chernobyl zone, accounting for internal and external irradiation of reproductive system and also, presence of macroscopic changes, such as bleeding and hemorrhage to different organs in studied group embryos.
Introduction:

Objectives and type of the research first of all are determined by genetic-physiological peculiarities of animals. Different genotype mink cytogenetic study could possibly reveal those changes (on chromosome level) that accompany ionized radiation mutagenic influence. Such examinations could not only detect cytogenetic changes in different genotype minks, but also present material for future comparative study research of similar changes with time, depending upon the radiation dose.

Another set of problems, that need cytogenetic research methods, is due to embryonic mortality reasons. In this case it is very important to understand what is the role of lethal structure damages and chromosome number in prenatal deaths. Perhaps, study of chromosome anomalies' occurrence not only in sex, but also in somatic mink cells could possibly clear up the discussed problem [1].

It is known that normal diploid chromosome set damages are accompanied by distinct morphologic and physiological anomalies. Adequate function study of proliferative system is determination of cell distribution according to cellular cycle phases. As an experimental model we have chosen proliferative processes' activity phenomenon (aneuploidy and polyploidy) on different stages of cellular cycle. All intracellular structures get damaged due to radiation influence. Different reactions, division and DNA synthesis delays, along with membrane damages were detected in cells.

All these speaks for need in cytogenetic examinations, in particular for proliferative process activity study on different stages of cellular division. Such studying can significantly improve understanding of the process, help with radiation effect estimation.

Cytogenetic research was conducted by laser flowing cytofluorometry method and examinations of chromosome aberrations. The research has been carried out by using up-to-date methods: computers with (CellFIT-Analysis Results) software for estimation of DNA condition in bone marrow and peripheral blood lymphocytes of both humans and animals. All the equipment had undergone metrologic control in time.

Methods and Results:

For research we used minks from Chernobyl zone and Barishivka village. Dates for animal collection (May-November) on farms were the same as for their mortifying. For experimental purposes 10 clinically healthy minks were killed. The animals were mortified by injection of dose 1000 times the muscle relaxant one. Out of bone marrow cells obtained from thigh bones, chromosome preparations were made. Metaphase slides, good for cytogenetic examinations were also analyzed [2].
We examined bone marrow cells and peripheral blood lymphocytes of Letreola vision Brisson minks. Cell suspension was obtained by dispersing, later re-suspended and filtered. Cell washing and centrifuging (g=300 rev/sec. for 5 min.) were made two times in Henks medium (pH=7.4). After that cells were fixed in 70% alcohol with medium. Right before cytogenetic analysis cell suspension was re-suspended and centrifuged for the second time (300 rev/sec. for 5 min.). To obtained sediment a fixing buffer was added according to Gacrson N., along with bromic ethidium of 10 E-7 mcg/ml final concentration. Cells were analyzed by I. Lefkovits method [3], using laser flowing cytoflourometer (FACStar Plus by Dickinson, USA). Argon laser was set to 488 nm wavelength with 250 mVt power. Average cell analysis speed equaled 500 cells/sec. Total of 10,000 cells were analyzed in every sample. After triton fixing, cell membranes were loaded with bromic ethidium, a fluorescent stainer that interacted with nucleus' DNA. Later on, this cell suspension had to be injected under pressure into analyzer's optical system.

Chromosome sample preparation was done according to generally accepted methods [5]. The results of the research had to undergo different statistical analysis in order to determine validity of changes between studied and control animals with 95% assurance level. For statistical data analysis IBM PS/2 (30,60) computers with "Statgraphics" and "Foxgraph" software were used.

Conclusion:

Our data shows that minks most frequently have some deviations in big [1-6] chromosomes and that another cases of aneuploidy involve medium and small chromosomes. With a half-year research we are yet unable to make some definite conclusions as for genetic damage significance of bone marrow imunocompetent tissues and peripheral blood lymphocytes of animals that reside near ChNPP. Cytogenetic bone marrow and peripheral blood cells' analysis showed that the exposed group had less percentage of cells in S-phase. At the same time an increase in G2-phase cells, if compared to controls, was observed. In other words, under small doses of ionized radiation influence, percentage of cells with intensive biosynthesis process, were energy accumulation takes place is going up. Causing, at the same time decrease in number of those cells where DNA replication and gystone synthesis (to which every DNA cell is connected) take place. The similar situation was observed in peripheral blood lymphocytes. Therefore, according to our and other scientists' data, chronic exposure with small radiation doses that is not the same for studied tissues can cause an increase in genetic instability [7].

This shows that those animals that were exposed with small radiation doses had less intensive reparation processes in tissues if compared to animals that resided in more or less radioactively clean areas. Taking into account the obtained data on number and type of genetic damages, one can assume that basis for the future development of diagnostic test (small doses of ionized radiation organism influence) has been found.
References