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Effect of vitamin E on preovulatory stage irradiated female mouse expressed as chromosomal abnormalities in generated embryos

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Background: The present study has been carried out to investigate the effects of preovulatory stage gamma-irradiation of female mice in the absence or presence of vitamin E on numerical chromosome abnormalities in 8-cell embryos after mating with non-irradiated males. ***Materials and Methods:*** The 8-11 weeks adult female NMRI mice were whole body irradiated at preovulatory stage (post PMSG injection and about 12-18 hours before injecting HCG) with 4 Gy gamma-rays generated from a cobalt-60 source alone or in combination with 200 IU/kg vitamin E, intraperitoneally administered one hour prior to irradiation. Soon after HCG injection super ovulated irradiated females were mated with non-irradiated males. About 68-h post coitus (p.c), 8-cell embryos were flushed from the oviducts of pregnant mice and were fixed on slides using standard methods in order to screen for metaphase spreads and numerical chromosome abnormalities. ***Results:*** In control embryos, 8% of metaphase plates were aneuploid, whereas in preovulatory stage irradiated female mice, about 50% of metaphase plates of embryos showed numerical chromosome aberrations ($P<0.001$). Administration of vitamin E one hour before the irradiation reduced chromosomal aberrations significantly ($P=0.005$). ***Conclusion:*** Results show that the effects of gamma-irradiation on preovulatory stage oocytes led to chromosomal abnormalities in subsequent embryos generated by these oocytes. Increase the frequency of numerical chromosome abnormalities -mostly aneuploidy- may be due to abnormal chromosomal non disjunctions during 2nd meiotic division. Reduction of the frequency of chromosome aberrations in the presence of vitamin E is probably due to antioxidant effects of this vitamin, and scavenging free radicals induced by gamma-rays in mice oocytes' environment. ***Iran. J. Radiat. Res., 2006; 4 (1): 35-40***

Keywords: *Preimplantation embryos, chromosomal abnormalities, mouse oocytes, gamma-rays, vitamin E.*

INTRODUCTION

Ionizing radiation can induce DNA damage in the germ cells of exposed individuals and lead to various deleterious effects in their progeny, including miscarriage, low birth weight, congenital abnormalities and perhaps cancer [1]. Irradiation may induce various types of chromosome aberrations in the germ cells. Some of them are compatible with the survival of the cells and if transmitted to the embryos, they will result in the formation of malformations in the progeny [2]. The relative sensitivity of the female germ cells to genetic anomalies leading to developmental defects has remained poorly defined. *In vivo* studies on X-irradiated mice have shown that chromosomal aberrations can be induced in female germ cells, and strongly depends on the stage of maturation reached by the oocytes at the time of irradiation [3, 4]. Chromosomal radiosensitivity of mouse oocytes irradiated *in vitro* at well defined developmental stages is reported recently [5].

Experiments performed to determine germ-line mutation induction by X-irradiation have provided evidences for an elevated germ line mutation rate in the parents directly exposed to ionizing radiation. However, the result of numerous publications suggests that radiation may also have an indirect effect on genome stability which is transmitted through the germ line of irradiated parents to their offspring [6, 7].

Genetic effects of ionizing radiation in the progeny of exposed parents could cause severe developmental disorders (fetus death, still birth, early postnatal mortality, malformation hereditary disease, sterility), increased cancer risk manifested as elevated incidence of spontaneous tumors, increased

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sensitivity to carcinogenic agents, and decreased fitness (non-carcinogenic negative health effects). The last two types are presumably due to instability and functional inferiority of cell genome in the progeny of irradiated parents [8].

Sparsely ionizing radiation such as X and Gamma rays exert their biological effects through water radiolysis and free radical formation. Free radicals with approximate life time of 10^{-5} seconds can break chemical bonds. As a result of interaction of reactive oxygen species (ROS) with macromolecules, structural damages are produced in cells leading to biochemical function disorder [9-11]. Ionizing radiation is such a potent free radical former that intracellular enzymatic antioxidant defense mechanism can not scavenge all induced free radicals [9, 10].

Numerous studies have examined the radioprotective effects of antioxidant substances, known as free radical scavengers, which protect the cell and its organic constituent molecules from free radical damage. Scavenger substances are compounds that contain vitamins, Beta carotene and Thiol. Vitamin E prevents lipid peroxidation chain reactions in the cell membrane. It has been suggested that vitamin E does this in two ways: by interaction with unsaturated fatty acids, and by protecting the polypeptide chain of proteins [12]. Vitamin E can scavenge molecular oxygen, proxide and hydroxyl radicals and atomic oxygen radicals [13, 14] therefore; vitamin E exerts its protective effects in different regions of the cell [15].

It was shown that administration of vitamin E 2 hour before gamma-irradiation reduces gamma-ray-induced chromosomal damages in bone marrow cells in mice. Administration of vitamin E 2 hour prior to gamma irradiation or immediately post irradiation produced similar radioprotective effects [16]. The radioprotective effect of vitamin E was also shown on the human hepatocarcinoma (HCC) cell line [17], on radiation-induced cataract [18], on mice spermatogenesis[19] and other cellular systems and cytogenetic end points [20].

In the present study the effects of maternal exposure to gamma-irradiation in the presence of 200 IU/kg vitamin E was investigated on the frequency of numerical chromosome abnormalities in preimplantation stage embryos generated from irradiated female mice at the preovulatory stage.

MATERIALS AND METHODS

Animals

Adult 8-11 male and female albino NMRI mice with a mean weight of 30 ± 5 gr (Razi institute, Karaj, Iran) were used. The animals were housed in a room kept in mesh cages at 23 ± 2°C with a cycle of 10 hours darkness and 14 hours light and fed with standard mouse pellets and water ad libitum.

Super ovulation and gamma irradiation

To superovulate the female mice, 10 IU PMSG (Intervet, Holand) was injected intrapretoneally followed by injection of 10 IU HCG with a 42-48 h intervals (Organon, Holand). About 12-18 hours before HCG injection, mice were whole body irradiated with 4 Gy gamma-rays generated from a cobalt-60 source (Teratron II, 78°C Canada) at a dose rate of 1.32 Gy/min, with SSD = 82 cm, field size: 20×20 at room temprature (23 ± 2°C).

Vitamin E treatment

Vitamin E (Darupakhsh, Iran) was administered intraperitoneally one hour before irradiation, at a dose of 200 IU/kg. Vitamin E was dissolved in a sufficient amount of olive oil before injection. Also a control group receiving only vitamin E was studied for possible genotoxic effect of the dose used in this study.

Coupling and embryo recovery

Two irradiated female mice with a non-irradiated male were transferred to a cage for an overnight to mate. The next morning female mice were checked for vaginal plauge (VP) and a VP positive female was considered

as a pregnant mouse.

About 68 h p.c. the pregnant females were sacrificed by servical dislocation and their oviducts were flushed out using a special flushing syringe (Supa, Iran) filled with 37°C incubated T6 medium under a sterio microscope (Hund-Wetzlar, Germany) to obtain 4-8 cells embryos.

Culture medium

The collected morphologically normal embryos were transferred to fresh T6 medium (ingrediants purchased from Sigma USA, Seromed USA and Merck Germany), supplemented with 15 mg/ml Bovin Serum Albumin, (BSA, Sigma USA) containing 0.2 µg/ml colcemid (Gibco BRL, Lifetech UK) incubated in a humidified CO_2 incubator at 37°C for 16-20 hours.

Cytogenetic analysis

For cytogenetic analysis, Dyban method, which is a suitable method for analysing chromosomes of embryo cells, was used with some modifications [21, 22]. Briefly, the zona pellucida was removed by the use of tyrode's acid. This process was followed under a steriomicroscope to avoid damage to the blastomers. Then embryos were transferred to a watch glass containing 1% sodium cytrate (Sigma) as a hypotonic solution for 30 minutes. Embryos were placed on a pre-cleaned slide and fixed with a drop of fixative consisting of methanol and acetic acid (3:1) (Merck). After leaving overnight at room temperature, slides were stained in 3% Giemsa (Merck) for 3 minutes and cells were analyzed under a light microscope (Nikon) at ×1000 magnification to screen numerical chromosome abnormalities (Figure 1). The numerical chromosome abnormalities were divided into hyperdiploidy (addition of more than 7 chromosomes to the mouse normal diploid chromosome set; i.e. 40), aneuploidy (addition or loss of 1 to 7 chromosomes), hypodiploidy (loss of more than 7 chromosomes of the normal diploid chromosome set) and polyploidy (addition of 1 or more whole set of chromosomes).

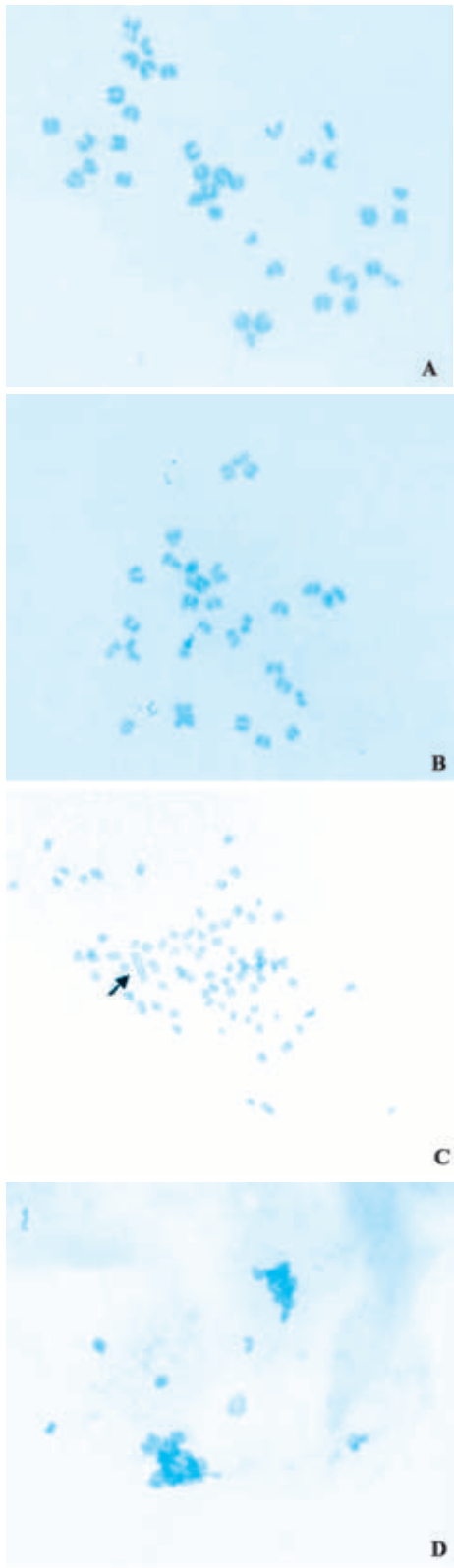

Figure 1. Photomicrographs of chromosome spreads of blstomers using Dyban Method magnification x 1000. A: Normal set of chromosomes, B; hypodiploid blastomer, C: a hyperdiploid blastomer with one dicentric chromosome, D: Dividing cells at anaphase with lagging chromosomes.

Statistical methods

χ^2 was used for statistical analysis to determine if there was any statistical difference in the rate of numerical chromosome abnormalities induced preimplantation embryos follwing by gamma-irradiation of female mice in the absence and presence of vitamin E (200 IU/kg).

RESULTS

Results are summerized in table 1 and shown in figure 2. As seen radiation dramatically increased the frequency of chromosomal aberations in pre-implantational embryos compared to embryos generated from non-irradiated female mice table 1.

About 10% of metaphase plates in the control group showed aneuploidy. As seen, The total number of embryos generated from the preovulatory stage irradiated female group was 203 embryos from which 171 embryos were 4-8 cells and morphologically normal. About 50% of observed metaphase plates in Irradiated female group showed numerical chromosome abnormalities which is significantly higher than control ($p<0.001$).

Administration of 200 IU/kg vitamin E alone did not induce statistically significant number of numerical chromosome abnormalities in comparison with the control (P=0.890), whereas in embryos generated from vitamin E treated preovulatory stage γ-irradiated female mice a significant decrease in the frequency of numerical chromosome abnormalities (30%) was seen comparing with those generated from γ-irradiated mothers in the absence of vitamin E (50%) (P=0.015) (figure 2). Therefore, administration of 200 IU/kg vitamin E led to 40% reduction in the frequency of numerical chromosome abnormalities in generated embryos (figure 2).

DISCUSSION

The teratogenic effects of ionizing radiation are well documented, but less is known about the radio sensitivity of the human oocytes [23]. The potential genetic risk to the human population of germ cell exposure to ionizing radiation has stimulated a considerable number of experimental studies in female mice to characterize the effects of irradiation on oocytes. Although studies of preimplantation stage embryos by classic cytogenetic techniques have some limitations, starting with the need for metaphase stage when only one-third of all analyzed embryos show good quality metaphase (table 1) [24, 25].

Our data indicated that figure 2 the frequency of numerical chromosome abnormalities in subsequent embryos generated from preovulatory stage gamma-irradiated female mice is significantly higher than the control group. These abnormalities may be due to translocations and other chromosomal abnormalities induced in oocytes which can lead to a generation of abnormal embryos. Induction of structural

Table 1. Frequency of the numerical chromosome abnormality in the embryos generated frrom pre-ovulatory stage gamma-irradiated female in the presence and absence of vitamin E.

	Total embryos	4-8 cells normal embryos	Observed metaphase plates	Normal metaphase	Abnormal metaphase	Aneuploidy	hyperdiploidy	hypodiploidy	polyploidy
Control	50	49	40	36	4	4	-	-	-
Vitamin control	45	43	38	35	3	3	-	-	-
Radiation alone	203	171	121	60	61	34	12	14	1
Radiation + vitamin	159	94	50	35	15	8	3	4	-

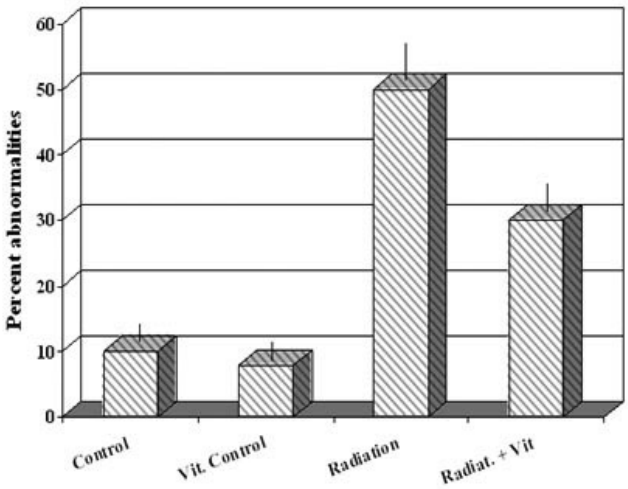

Figure 2. The frequency of numerical chromosome abnormalities in preimplantation embryos generated by gamma irradiated (4 Gy) female mice in the absence and presence of vitamin E (vit).

chromosome changes by ionizing radiation in female germ cells is reported [5]. These anomalies are often lethal for cells or embryo, and only a small proportion are transmitted to the F1 first generation [26].

The result of numerous publications suggest that radiation may also have an indirect effect on genome stability, which is transmitted through the germ line of irradiated parents to their offspring [4, 6-8]. Oocytes at different stages of maturation vary in their radio sensitivities, with those a few hours from ovulation being considerably more sensitive than maturing dictyate stages [1, 5, 27, 28]. It was shown that oocytes irradiated at the beginning of the oestrous cycle had a low frequency of chromosome aberrations, while those irradiated at the middle of the oestrous cycle (when growing Graafian follicles are clearly visible at the surface of the ovaries), exhibited heavy chromosome damage [2]. Our observation is in line with previously reported data as described and it is a proof that maternal irradiation has an important role in chromosomal abnormalities of generated embryos.

In the present study administration of 200 IU/kg vitamin E using i.p injection, an hour before gamma-irradiation of female mice significantly decreased the frequency of numerical chromosome abnormalities in subsequent embryos figure 2. This finding is also in line with other observations with different cellular systems. It was shown that administration of vitamin E, 2-hour before gamma-irradiation reduces gamma-ray-induced chromosomal damages in bone marrow cells in mice. Similar protective effects were seen when vitamin E, was administered 2 hour before irradiation or immediately after irradiation [16]. Radioprotective effects of vitamin E was shown for Chinese hamster V-79 cells exposed to ultraviolet-b light against DNA single strand breaks, chromosomal aberrations and mutation induction [20]. Recent reports indicate that vitamin E effectively protects human HCC cell line against radiation [17], radiation-induced cataract [18] and mice spermatogenesis [19].

Various protection mechanisms against these effects have been proposed for vitamin E. Vitamin E either acts as an antioxidant or scavenge free radicals produced due to indirect effect of ionizing radiation. Therefore, vitamin E can be used at supra physiological doses for protection against radiation-induced DNA alterations transferable to the next generated embryos.

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