Radiation risk during prenatal development: Preimplantation period.

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The prenatal mammalian development is divided into three periods: The preimplantation period, the major organogenesis and the fetal period. Several studies have shown that the radio-sensitivity of the preimplantation mouse embryo is very high in general, but especially in the early 1-cell stage and somewhat less in the late 2-cell embryo. The major effect after irradiation of preimplantation embryos of different mammalian species is the early embryonic death. The embryo generally fails to implant into the uterus wall and organogenesis which follows the preimplantation period is lacking. We have investigated details of the radiation effect under very clear in vitro conditions (1, 2, 3).

Mouse embryos were cultivated in vitro (4) beginning from the 2-cell stage. Irradiation of 2-cell embryos either with X-rays (240 kV) or neutrons (7 MeV) was performed in the late G₂-phase. The following results were obtained:

1) Disturbances of blastocyst formation and a reduction of the number of hatched blastocysts occurred in the dose range between 0.5 and 1.0 Gy X-rays (5). In the case of neutron irradiation such damage of preimplantation development was already observed after a dose of 0.12 Gy. The RBE-values for blastocyst formation for doses of 0.12, 0.25 and 0.5 Gy were 9.0, 6.1 and 4.5 respectively.

2) Concomitant to the morphogenetic damage impairment of cell proliferation was found. At the end of the preimplantation period an X-ray dose of 1.0 Gy had caused a reduction of the average cell number to 67 cells per embryo in comparison to 100 cells in the unirradiated embryo. After neutron doses of 0.25 and 0.50 these values were 72 and 63 cells respectively (5).

3) Initially after irradiation a mitotic delay due to a G₂-block was
observed. An X-ray dose of 1.88 Gy induced a $G_2$-block of about 4 hours which was measured by cytophotometry. During the later preimplantation period after the morula stage cell death was the dominant event (5).

4) Cell death can be explained with the occurrence of chromosomal damage which led to hypoploid cells (DNA content of the cell nucleus: <diploid; cytophotometrically investigated). Hypoploid cells were found 1 and 2 cell divisions after irradiation in 4-cell and 8-cell embryos and in morulae (3 mitoses p.r.). The hypoploid cells lost their ability to divide after progression through 2-3 cell cycles post radiation (5).

5) Acentric chromosomal fragments were the reason for the formation of hypoploid cell nuclei. The fragments were lost from the cell nuclei during cell division. They were observed as micronuclei (6) in the cytoplasm of interphase cells or they were scored directly in metaphases with chromatid breaks (7). When compared with X-rays the higher frequency of cytogenetic damage after neutron irradiation explained the higher frequency of hypoploid cells and thus the stronger impairment of preimplantation cell proliferation and embryological development (8). The development of micronuclei was studied during various stages of the embryos in dependance on the radiation dose, the number of cell cycles and other factors. The micronucleus test turned out to be a good assay for radiation damage on the genome and its cellular consequences.

6) Two different types of recovery processes were observed: intercellular and intracellular mechanisms. After X-ray doses of 1.0 and 2.0 Gy a small percentage of the embryos showed cell numbers at the end of the preimplantation period which were in the range of the control values (100 cells per embryo). Transplantation of these embryos into foster female mice proved that even after an X-ray dose of 2.0 Gy a complete recovery from radiation damage was possible. Some of the embryos developed to normal newborn mice which were free of gross malformations. Concluding from autoradiographic results it is assumed that during the preimplantation period an increased cell proliferation had compensated for the radiation induced cell death (intercellular mechanisms!).
When X- or neutron-irradiation was performed either in the late or in the middle of $G_2$-phase a higher frequency of acentric fragments and other chromosome aberrations as well as the higher impairment of proliferative and morphogenetic development was found in the case of irradiation at the end of the $G_2$-phase of 2-cell embryos. Those embryonic cells which had the longer interval between irradiation in $G_2$-phase and the 1$^{st}$ mitosis post radiation had a better chance to repair radiation damage than cells with the shorter interval. Damage which remained unrepaired until the 1$^{st}$ mitosis became manifest. The frequency of acentric fragments was less in the daughters of those cells which were irradiated at the middle of the $G_2$-phase and had divided comparatively late after irradiation. Apparently an appreciable repair of chromosomal damage had taken place before cell division (8, 9).

These studies demonstrate the development of radiation damage after exposure to X-rays and fast neutrons in preimplantation mouse embryos on the cellular and embryonic basis. The transplantation experiments show that even after high radiation doses the prenatal development is either inhibited completely or occurs normally. The recovery processes can be very extensive. The conditions of such mechanisms have been studied.

References

1. UNSCEAR, 1977 Sources and Effects of Ionizing Radiation, United Nations Publication Sales No. E.77.IX.1


