Studies on the sensitivity of female germ cells to X-irradiation, with special emphasis on chromosome aberrations leading to congenital anomalies in the progeny

Dr. P. JACQUET

CEN/SCK
Laboratory of Radiobiology

Introduction

Quantitative human data on the risks of genetically transmitted diseases and anomalies following exposure to ionising radiation are very scanty. No clearly significant increase of genetic diseases or malformations has been observed in the progeny of irradiated persons, even in the large population of survivors of Hiroshima-Nagasaki, due to the relatively high spontaneous incidence of such damage and the difficulty of ascertaining it in an inhomogeneous population. Consequently, risk assessment for such damage must be based primarily on animal studies, whose extrapolation must rely on an understanding of mechanisms of action and their differences among species.

We have shown that the guinea pig is probably one of the best models for evaluating the genetic hazard of ionising radiation in women. Our project concentrated, therefore, on this species. Its aims were essentially

- to characterise the sensitivity of guinea-pig germ cells to induction by radiation of chromosome translocations,
- to determine the extent to which these chromosomal aberrations can be transmitted to the progeny,
- to evaluate their potential role in the induction of congenital anomalies.

Our cytogenetic investigations were performed on oocytes irradiated with various doses of X-rays at the immature stage or at different stages of follicular growth. Studies conducted by others on the mouse have shown that the sensitivity of the oocyte to radiation-induced chromosome aberrations can vary greatly according to the stage of oogenesis reached at the time of irradiation.

Transmission of translocations to the progeny and their potential role in inducing congenital anomalies were studied following irradiation with a relatively high dose at the stage which, in our studies, proved the most sensitive to induction of chromosome aberrations.

Altogether, our data and those obtained by others on the mouse should allow better evaluation of potential risks, for the progeny, of exposure of maternal germ cells to radiation at the different stages of oogenesis.

Materials and methods

Structural aberrations of chromosomes constitute a significant portion of the genetic damage produced in the germ cells by ionising radiation. Among the structural chromosomal aberrations, nonhomologous reciprocal translocations are the most relevant. These are stable aberrations which can be transmitted to the progeny with a high efficiency and which may lead to severe mental defects or congenital anomalies. Translocations and other structural chromosome aberrations can be visualised under the stereomicroscope, in oocytes fixed in metaphase of the first meiotic division (MI).

Before starting with irradiation experiments, we spent some time developing methods allowing *in vitro* maturation of guinea-pig oocytes and their preparation for cytogenetic examination in MI.

The first experiments were then devoted to assessing the radiosensitivity of the immature oocyte. For this, adult guinea pigs (3-4 months old) were mated and their young female progeny (duration of pregnancy: 68 days) were X-irradiated on the ovaries with 1 or 2 Gy. Irradiation occurred during the first 2 days after birth, at which time a maximum of oocytes are of the immature "diplotene" type, comparable to the equivalent stage in humans. We collected the oocytes 1 year after birth, ensuring that all oocytes examined for cytogenetic damage were at the immature stage at the time of irradiation. Meiotically competent oocytes were punctured from the follicles located at the ovarian surface, on day 10 of an oestrous cycle (duration of a cycle: 17 days). They were cultured for 6 hours and fixed at MI for cytogenetic analysis.

The next experiments were devoted to assessing the radiosensitivity of the oocyte at various stages of follicular growth, i.e., 15, 8, 4, 3, 2, or 1 week(s) before ovulation, or 2 days before it. For these experiments, ovaries of adult animals were X-irradiated with 1, 2, or 4 Gy and their meiotically competent oocytes collected and cultured on day 10 of a cycle (first experiments) or on day 15 (following experiments).

For the study of the transmission of chromosome translocations to the progeny, ovaries of adult females were X-irradiated with 1 Gy 2 days before ovulation (day 15), i.e., at a stage particularly sensitive to induction of such aberrations. Females were mated with males, and oocytes of their female progeny were cytogenetically analysed when the young animals reached the age of 4 months. On the other hand, all the young (males + females) were carefully examined for the presence of congenital anomalies.

Results and conclusions

The results of our different experiments enabled us to draw the following conclusions:

. In the guinea pig, the immature "diplotene" oocyte appears very resistant to cell killing by radiation and relatively resistant to induction of chromosome translocations. In this it differs from the immature "dictyate" oocyte of the mouse, which is extremely sensitive to cell killing by radiation and, according to recent results, relatively sensitive to induction of chromosome translocations (Straume et al. *Mutation Res.* 248, 123-133, 1991).

. Growing oocytes enclosed in the small follicles are also resistant both to cell killing by radiation and to induction of chromosomal translocations. This situation persists almost unchanged as the time before ovulation decreases, up to 3-2 weeks before ovulation. From then on, the sensitivity of the oocyte increases in proportion to follicle size. Guinea-pig oocytes at stages near ovulation, and thus enclosed in the largest follicles, are most sensitive to induction of chromosomal aberrations by radiation. Yet they are also extremely sensitive to cell killing, an effect resulting indirectly from the rapid atresia (or degeneration) of the large follicles. In principle, aberrant oocytes are rapidly eliminated from the ovaries. Here also, the guinea-pig oocyte appears to differ from that of the mouse. In the latter species, the sensitivity of oocytes to induction of chromosomal aberrations seems relatively constant between weeks 4 and 2 before ovulation, decreasing strongly thereafter. Oocytes enclosed in follicles near ovulation thus display very low sensitivity to induction of chromosomal aberrations (except at the "diakinesis" stage, a few hours before ovulation). They also show very high resistance to cell killing by radiation (Brewen and Payne, *Genetics* 91, 149-161, 1979).

. None of the investigated stages of guinea-pig "postnatal" oogenesis appeared refractory to induction of translocations. However, if irradiation occurs 1 week or more before ovulation, the translocation levels induced in oocytes able to survive up to ovulation (the only ones of interest as regards genetic risks) remain low for doses up to 2 Gy. A higher potential risk exists

for higher doses (at least for weeks 2-1 before ovulation), the effect per unit dose then increasing strongly.

. The highest risk theoretically concerns oocytes irradiated near the end of a cycle, just before ovulation: these could escape atresia and be ovulated and fertilised. Since these oocytes are highly sensitive to induction of chromosomal aberrations, the risk of transmission of translocations to the progeny is non-negligible, even for doses lower than 1 Gy. Our experiments show that female guinea pigs irradiated with 1 Gy 2 days before ovulation display normal fertility (expressed by a normal number of young); this indicates that a number of their oocytes do indeed escape atresia. On the other hand, our observations fail to evidence a clear link between such treatment and the presence of translocations and/or congenital anomalies in the young. These negative results must therefore be attributed to selective elimination of the oocytes and/or embryos carrying aberrations. However, the very low number of animals used in these experiments considerably reduces the import of such conclusion.

In summary, the results of our different studies on the guinea pig suggest that the risk of congenital anomalies resulting from irradiation of the mother by moderate doses of X-rays could be low in comparison to the “natural” risks associated with pregnancy, and that participation of chromosomal translocations in this risk could also be minor. Certainty this last point, however, requires additional experiments on a very large number of animals, given the very low frequency of induction of such effects in the guinea pig.