CYTOGENETIC EFFECTS OF IN VITRO IRRADIATION OF HUMAN SPERMATOZOA

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Abstract

The effects of human mutagens, clastogens and aneugens have been studied almost exclusively in somatic tissues. However, currently there is a considerable discussion about the potential of ionizing radiation to induce heritable germ cell mutations. While the various viewpoints remain controversial, one of the aims of germ cell cytogenetic studies must be to improve the ability to identify and estimate the actual genetic risk in humans.

One way to assess the risk of transmission of genetic anomalies by men occupationally or accidentally exposed to ionizing radiation is to determine whether there is a dose-related genetic damage in human spermatozoa.Cytogenetic analysis of human spermatozoa is possible after interspecific *in vitro* fertilization between zona pellucida-free hamster oocytes and human spermatozoa.Using this assay system we have analyzed the radiation induction of structural chromosome abnormalities in sperm derived complements at the first embryo cleavage, as well as the radiation induction of micronuclei and aneuploidy in two-cell hybrid embryos.

Induction of structural chromosome abnormalities

Using the interspecific *in vitro* fertilization system, we have established a dose-effect relationship for the cytogenetic effects of gamma-rays on human spermatozoa. Semen samples from three healthy men were irradiated at doses of 0.00, 0.10, 0.25, 0.50, 1.00, 2.00 and 4.00 Gy. We observed that human spermatozoa retained a high fertilization ability even after high doses of gamma-rays. This indicates that induced DNA lesions are accumulated in male germ cells and may be transmitted to the zygote without being selected against at fertilization.

A total of 340 chromosome complements derived from no-irradiated human spermatozoa and 987 complements from irradiated spermatozoa were analyzed after sequential uniform staining-G banding. Both, the frequency of spermatozoa with structural chromosome abnormalities and the incidence of such abnormalities per cell showed strong dose-effect relationships, that were best expressed by linear-quadratic equations: $Y=0.06413(\pm0.00475)+0.1982(\pm0.00833)D-0.00763(\pm0.00204)D^2$ and $Y=0.07385(\pm0.00838)+0.23329(\pm0.03124)D+0.02317(\pm0.00955)D^2$ respectively (Fig.1).

The incidence of structural abnormalities per cell showed a linear-quadratic dose-response relationship (with a positive quadratic coefficient), where the quadratic trajectory was only visible at the highest dose. The linear dose-response relationship for the induction of spermatozoa with structural abnormalities showed a saturation effect (negative quadratic coefficient). This saturation effect was very small, and it could only be detected at the highest dose. This effect could probably be due to the fact that, at increasing doses, the probability that a cell will be affected by more than one chromosomal abnormality also increases, although this will be counted as a single abnormal spermatozoon. This saturation effect has also been reported in a study about the cytogenetic effects of *in vitro* X- and γ-radiation on Syrian hamster spermatozoa (Tateno et

al., 1996). In a previous study about the cytogenetic effect of γ-radiation on human spermatozoa (Mikamo et al., 1990), the induction of spermatozoa with structural abnormalities was 1.5 higher than the one found in the present study, and no saturation effect was detected for the induction of spermatozoa with structural abnormalities. The reason for this apparent discrepancy could be that in the study of Mikamo et al. (1990) the maximum radiation dose was 1.11 Gy. At this dose, a small saturation effect like the one found in our study, can easily go unnoticed.

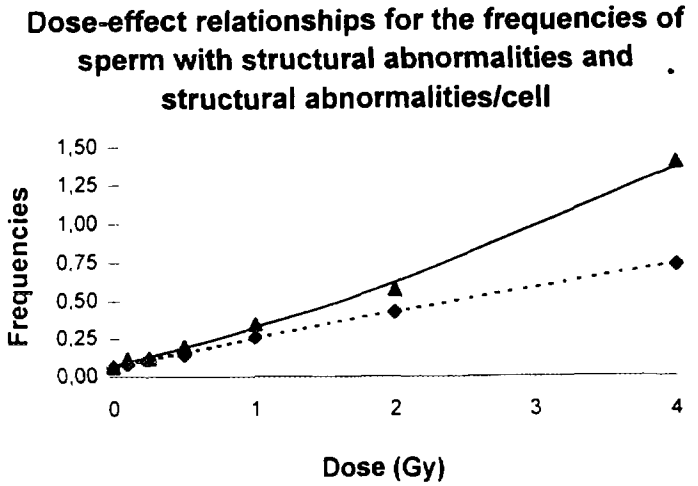

Figure1. Cytogenetic effect of gamma radiation on human sperm. Triangles and continuous line represent the frequency of human spermatozoa with structural abnormalities. Rombs and discontinuous line represent the frequency of structural aberration per cell.

Chromosomal aberrations may be categorized as to the number of breaks involved and the subsequent interactions among broken ends. Thus, structural abnormalities were classified as unrejoined and rejoined. Unrejoined structural abnormalities consist of such chromosome abnormalities as breaks, terminal deletions and acentric fragments, whereas inversions, translocations, dicentrics and rings are rejoined structural abnormalities.When analyzing separately both types of structural abnormalities, we found that the incidence of unrejoined lesions was four times higher than the incidence of rejoined anomalies. The induction of unrejoined abnormalities showed a linear, dose-dependent increase, whereas the incidence of rejoined abnormalities showed a quadratic, dose-dependent increase.

Induction of micronuclei

The analysis of human derived chromosomes at the first cleavage of the hybrid embryos, although useful, is very time consuming. Therefore in our laboratory we have developed a new assay system, which is faster and gives an estimate of the frequency of structural as well as numerical chromosome aberrations. It consists of the analysis of micronuclei in two-cell human-hamster embryos. The technique used was that described by Kamiguchi et al (1991) adapted to our human-hamster interspecific fertilization system (Genescà et al., 1990).

To ascertain whether the micronuclei present in the hybrid embryos were of human or of hamster origin, we hybridized them with either human or hamster genomic DNA probes. This experiment demonstrated that close to 99% of micronuclei were of human origin.

To establish a dose-effect curve for radiation-induced micronuclei, we performed the micronucleus test in two-cell human-hamster hybrid embryos after exposure of human spermatozoa to doses of 0, 0.10, 0.25, 0.50, 1.00, 2.00 and 4.00 Gy of γ-rays. The results were then compared to those obtained when analysing chromosome breaks and fragments in human sperm chromosomes using classical metaphase spreads.
A linear relationship between the different doses of radiation and the induction of micronuclei was demonstrated (Fig. 2), although at the highest dose (4 Gy) this relationship showed a slight saturation effect.

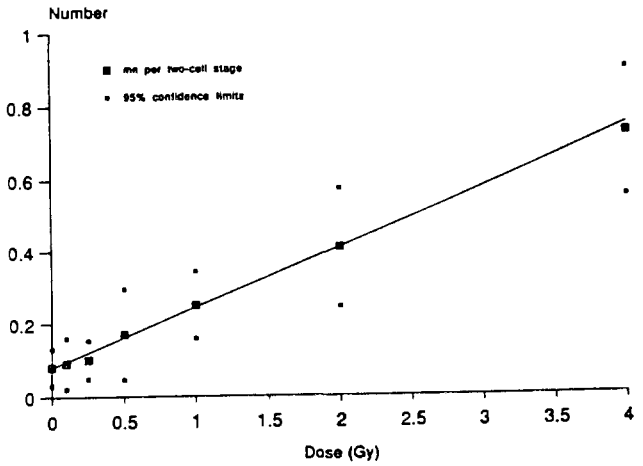

Figure 2. Dose-effect curve for the production of micronuclei (mn) by gamma-irradiation of human spermatozoa.

To determine if scoring micronuclei could be used for the quantification of chromosome damage induced in human spermatozoa, we compared the frequency of micronuclei per two-cell embryo (corrected for the estimated incidence of micronuclei of hamster origin) to the frequency of breaks and fragments in human sperm chromosome preparations, and a good coincidence was found.

Since micronuclei may contain acentric fragments (Heddle and Carrano, 1977) as well as centric fragments or whole chromosomes with damaged centromeres (Farooqi et al., 1993), and even groups of chromosomes that produce large micronuclei (Yamamoto and Kikuchi 1980), we decided to carry out a FISH study using a combination of human specific centromere probes and of unspecific telomere probes to determine the content of the micronuclei induced by the fertilization of hamster oocytes with human spermatozoa previously treated with γ-rays. In this case, human spermatozoa were irradiated under a ^{60}Co source at 2 and 4 Gy. Fluorescent in situ hybridization was carried out on fixed two-cell embryos using biotin labelled DNA probes specific for human centromeres as well as digoxygenin labelled telomere probes. Most micronuclei contain just one fragment, and the aim of the study was to determine whether the fragment was centric or acentric, and included telomeres or not either at one or both ends. Single and double FISH experiments were carried out. In this series of experiments, the frequency of micronuclei was also dose-dependent. Using double FISH, most centromere positive micronuclei were also telomere positive, indicating that they probably contained whole chromosomes; the presence of whole chromosomes in micronuclei through a lagging effect had already been demonstrated by Viaggi et al (1987) and may be related to a weak aneugenic effect of ionizing radiation. The proportion of centromere positive-telomere negative micronuclei was always under 10 %.

Induction of aneuploidy

An aneuploid individual usually arises at fertilization by the fusion of an abnormal gamete, which itself has resulted from a defect in meiosis. However, non-disjunction and the loss of mitotic chromosomes at the initial stages of embryogenesis may also result in trisomy, monosomy or chromosomal mosaicism.

Here, we describe a new assay system which has been developed by combining two techniques, the interspecific fertilization between zona-free hamster oocytes and human spermatozoa, and the fluorescent *in situ* hybridization (FISH) technique using centromere specific DNA probes. By tracing the marker chromosomes in two-cell embryos, reciprocal products of chromosome malsegregation can be easily traced. In this way, scoring of fluorescent spots in daughter nuclei and in micronuclei, gives an estimate of aneuploidy arising from meiosis, as well as the aneuploidy due to first mitotic division errors (both, non-disjunction and anaphase lag).
To determine the baseline frequency of these numerical abnormalities we have analysed 162 two-cell embryos from one normal donor with centromeric DNA probes for chromosomes 4,7 and 18.We have not taken into account those embryos showing more than one human chromosome complement because we cannot distinguish between an embryo originated by the penetration of a diploid spermatozoa from an embryo resulting from the fertilization with two normal ones. We have found a two-cell embryo with mitotic non-disjunction for chromosome 18 and some micronuclei without fluorescent signal. Therefore, accepting that all chromosomes have the same probability of being involved in these processes, the frequency of non-disjunction of the first mitotic division is 4.7%. The frequency of anaphase lag cannot be estimated until more embryos are analysed, but it seems to be lower.

This test will be used to analyse the effects of physical or chemical agents on spermatozoa during the first embryonic division.

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