AMCoR
Asahikawa Medical College Repository http://amcor.asahikawa-med.ac.jp/

MUTATION RESEARCH-FUNDAMENTAL AND MOLECULAR MECHANISMS OF MUTAGENESIS (2002) 504 1-2:183-191.

Radiation- and chemical-induced structural chromosome aberrations in human spermatozoa

Kamiguchi, Y; Tateno, H

Radiation- and chemical-induced structural chromosome aberrations in human spermatozoa

Yujiroh Kamiguchi*, Hiroyuki Tateno

Department of Biological Sciences, Asahikawa Medical College, 2-1-1-1 Midorigaoka-higashi, Asahikawa 078-8510, Japan

* Corresponding author. Tel.: +81-166-68-2729; fax: +81-166-68-2783.

E-mail address: yujiroh@asahikawa-med.ac.jp

Abstract

Previous studies on the clastogenic effects of mutagens on human sperm chromosomes were reviewed. A marked increase of structural chromosome aberrations has been reported in the spermatozoa irradiated in vitro with five kinds of ionizing radiation (^{137}Cs gamma-, ^{60}Co gamma-, X-, and ^{3}H beta-rays and ^{252}Cf neutrons). The micronucleus test with hybrid 2-cell embryos generated from human sperm and hamster oocytes was shown to be useful as a simple and rapid method for assessing the effects of radiation. Radiosensitivity of human spermatozoa was highest, being followed by golden hamster, Chinese hamster and mouse spermatozoa. Chromosome-damaging effects were also found with some chemicals (bleomycin, daunomycin, methyl methanesulfonate, triethylenemelamine, neocarzinostatin, N-methyl-N'-nitro-N-nitorosoguanidine, and mitomycin C), but not with other chemicals (urethane, nitrobenzene, dioxin, cyclophosphamide, benzo(a)pyrene, and N-nitrosodimethylamine). The clastogenicity of chemical metabolites was confirmed for cyclophosphamide and benzo(a)pyrene, by using the S9-based metabolic activation system. The results of sperm chromosome analysis from cancer patients who had undergone radio- and/or chemotherapy were contradictory among investigators and further studies are necessary. The importance of mutagenicity testing with human spermatozoa is discussed.

Keywords: Human spermatozoa; Chromosome aberration; Radiation; Chemical; In vitro exposure

1. Introduction

Since the development of an interspecific in vitro fertilization system between human spermatozoa and zona-free golden hamster oocytes [1,2,3,4], a number of cytogenetic studies with human spermatozoa have been carried out during the last two decades [5,6,7,8]. This method has been applied to chromosomal studies of spermatozoa exposed in vitro to various mutagens. The mutagenicity testing with human spermatozoa is valuable for the following reasons. First, effects of mutagens can be assessed directly in human gametes. This approach is especially important, because it has been shown that susceptibility to some mutagens is very different between somatic and germ cells, and also between laboratory animal species. Second, human spermatozoa are obtainable far more easily than human oocytes, and their in vitro treatment with mutagenic agents is also easy. Third, mature spermatozoa are thought to be highly vulnerable to mutagens, because they have no cytoplasm, and therefore, have no DNA-repair capacity. This is in marked contrast to oocytes and somatic cells. In this connection, it is noteworthy that the majority of de novo structural chromosome aberrations in fetuses and newborns are of paternal origin, that is, of sperm origin [9].

We review here the previous studies on the chromosomal abnormalities in human spermatozoa exposed to various qualities of ionizing radiation and chemicals.

2. Effects of radiation

2.1. Chromosome analysis

The effects of radiation on human sperm chromosomes have been studied with five kinds of ionizing radiation, i.e., ^{137}Cs gamma-rays [10,11], ^{60}Co gamma-rays [12,13], X-rays [14], ^{3}H beta-rays [15], and ^{252}Cf fission neutrons [12]. In our gamma-ray experiment [11], the incidence of spermatozoa with radiation-induced structural chromosome aberrations (Y) increased exponentially with increasing dosage (D), being expressed by an equation, $Y(\%)=100\ (1-e^{-0.514D})$ (Table 1, b and Fig. 1). Within the

low dose area, however, the dose-dependent increase was linear (Table 1, c). In our studies [11,12,14,15], the slope of the linear equation was similar with four kinds of radiation (Table 1, c,d,f,g and Fig. 1), showing the relative biological effectiveness (RBE) values of approximately 1. In the data of Brandriff et al. [10] and Alvarez et al. [13] (Table 1, a,e), however, the equations had a much slower gradient than ours. Although, we cannot explain why the dose-dependent increases were so different between their experiments and ours, one possible reason seems to lie in the difference of the chromosome preparation technique [14].

In the neutron experiment [12], on the other hand, the slope of the dose-effect equation was steeper, indicating that neutrons were more effective than the other four kinds of radiation in inducing chromosomal damage in human spermatozoa (Table 1, h and Fig. 1).

The dose-dependent increase of chromosomally abnormal spermatozoa was almost the same with acute (1.36 Gy/min) and chronic (1.7 cGy/min) exposures to gamma-rays (Table 1, c and d). This was in marked contrast to the results in somatic cells in which chronic exposure was shown to be far less effective in inducing structural chromosome aberrations. The possible reason for this is that spermatozoa have no cytoplasm, and therefore, have no DNA-repair enzymes which are present in the somatic cells.

Dose-effect equations for the induction of breakage-type and exchange-type chromosome aberrations were also compared among five kinds of radiation (Table 2 and Fig. 2). Breaks occurred far more frequently than exchanges, and showed a linear increase with dose, whereas the exchanges showed a quadratic increase. RBE values estimated from these data were similar to the preceding RBE value obtained from the incidence of chromosomally abnormal spermatozoa.

In relation to the high radiosensitivity of human spermatozoa, it is noteworthy that spermatozoa retained a high fertilizing ability even after a high dose of irradiation, such as 4.23 Gy of gamma-rays. This suggests that radiation-induced DNA damage

in spermatozoa may be transmitted to the next generation without being selected out at fertilization.

We have also studied the effects of microwaves [16] and extremely low frequency electromagnetic fields [17], and found that these agents have no clastogenic effect on human sperm chromosomes.

2.2. Micronucleus test

The chromosomal study of human spermatozoa is very time-consuming and demands considerable skill. On the other hand, the micronucleus (MN) test has been shown to be a simple and rapid method to measure chromosomal damage in mutagen-exposed somatic cells [18,19]. This method was applied to the sperm chromosome study for assessing clastogenic effects of radiation [20, 21]. The frequency of MN was examined at the 2-cell stage after in vitro fertilization of hamster oocytes with gamma-irradiated human spermatozoa. The incidence of 2-cell embryos with MN was consistent well with the incidence of spermatozoa with chromosomal breaks and fragments, showing nearly the same dose-effect equations (Table 3). This indicates that the MN test is useful as a substitute for sperm chromosome analysis. Nevertheless, the radiosensitivity of human spermatozoa measured by the MN test or by chromosome analysis showed a great difference between the report of Tusell et al. [21] and ours [20]. The reason of this is puzzling, although some methodological differences are present between the two studies, such as chromosome preparation technique, radiation source and dose rate.

Later, the MN test became more sophisticated by incorporating FISH technique with human and hamster genomic DNA probes, making it possible to distinguish betweem MN originated from human sperm chromosomes and MN from hamster oocyte chromosomes, and to distinguish between MN originated from acentric fragments and MN from whole chromosomes [22,23].

2.3. Comparative radiosensitivity in mammalian spermatozoa

The dose-response relationship for the induction of structural chromosome aberrations in X-irradiated spermatozoa was compared among four mammalian species (Table 4). Chromosome analyses were performed in mouse, golden hamster and Chinese hamster spermatozoa after intraspecific fertilization, and in human spermatozoa after interspecific fertilization with golden hamster oocytes. The slope of the dose-effect equation was steepest in human spermatozoa, followed by golden hamster, Chinese hamster, and mouse spermatozoa. The slope of the equation was considerably slower in the last two species, showing 2.4- and 3.7-fold lower radiosensitivity than human spermatozoa. The high incidence of radiation-induced chromosome aberrations in human and golden hamster spermatozoa may reflect a low DNA-repair capacity of golden hamster oocytes [25].

3. Effects of chemicals

3.1. Direct effects of chemicals

The direct effects of various chemicals on human sperm chromosomes were studied in vitro, using the interspecific fertilization system with hamster oocytes (Table 5).

A significant increase of structural chromosome aberrations was found in spermatozoa treated with antineoplastic agents such as bleomycin [26], daunomycin [26], methyl methanesulfonate [26], triethylenemelamine [26], neocarzinostatin [27] and mitomycin C (MMC) [28], and also with a carcinogen, N-methyl-N'-nitro-N-nitrosoguanidine [29]. Generally speaking, the incidences of cells with chromosome aberrations were higher in spermatozoa than in somatic cells, in spite of the much shorter duration of in vitro exposure to chemicals in the former [26]. This seems to reflect the fact that spermatozoa have no capacity to repair DNA damage, as mentioned earlier. Furthermore, the motility and the fertilizing ability of spermatozoa treated with chemicals was not reduced at all, suggesting that no selection of spermatozoa with chromosome aberrations takes place at fertilization, at

least as far as the above-mentioned chemicals are concerned.

In contrast, cyclophosphamide (CP) [31] had no clastogenic effect in spermatozoa, although a positive effect was found in somatic cells. However, this is not surprising, because it has been shown that the metabolite of CP, rather than CP itself, induce aberrations, as will be mentioned later. A negative effect was also found with urethane [29], nitrobenzene[29], 2,3,7,8-tetrachlorodibenzo-p-dioxin [30], benzo(a)-pyrene (BP) [31], and N-nitrosodimethylamine (NDMA) [31].

3.2. Effects of chemical metabolites

It was shown in somatic cells that chromosome-damaging effects of chemical metabolites are detectable in vitro by using a microsomal fraction (S9) which is prepared from rat liver tissue [32,33]. This metabolic activation system was applied later to human sperm chromosome studies for assessing clastogenic effects of chemical metabolites [28,31]. Spermatozoa were exposed in vitro to MMC, CP, BP or NDMA for 2 hours in the presence (+S9) or absence (-S9) of S9, and the results of chromosome analysis were compared between the two groups (Table 5).

In MMC experiments, dose-dependent increases of structural chromosome aberrations per spermatozoon were evident in both groups, but a much steeper slope was found in the presence of S9 [28]. This indicates that both MMC itself and its metabolites are clastogenic, but the latter has a much stronger effect.

When the spermatozoa were treated with CP, BP and NDMA in the absence of S9, none of these chemicals induced structural chromosome aberrations. With S9, however, a significant increase of aberration frequency was observed for CP and BP [31]. Thus, clastogenicity of CP and BP metabolites, which was shown in somatic cells, was also confirmed in human spermatozoa. In contrast, NDMA did not induce chromosome aberrations in the presence of S9, although positive results were reported in somatic cells. This suggests that NDMA metabolite(s) may not access DNA in spermatozoa, or that NDMA may need the cytoplasmic pathway(s) involved in the

production of DNA-damaging metabolites which are not available in spermatozoa.

4. In vivo studies in cancer patients

Some investigators carried out cytogenetic studies of spermatozoa obtained from cancer patients who had undergone radio- and/or chemotherapy. In these studies, spermatozoa were exposed to the therapeutic agents at their spermatogonial stage, except in a few studies [40,43,44] in which the agents were given at later stages of spermatogenesis (spermatocytes to mature spermatozoa).

As shown in Table 6, inconsistent results were obtained. Some investigators found significant increases in both numerical and structural chromosome aberrations [34,41], other groups found increases only in structural aberrations [36,37,38], and others found no effect in either aberrations [35,39,42,45]. In these studies, therapeutic treatments exposed spermatogonial stem cells to risk, so that the spermatozoa carrying structural chromosome aberrations are the descendants of affected spermatogonia which survived subsequent mitotic and meiotic divisions to form mature spermatozoa. Thus, the majority to abnormal spermatozoa are expected to have structural chromosome aberrations of the stable type such as inversions and reciprocal translocations. However, a considerable number of abnormal spermatozoa did have unstable aberrations. Positive results of aneuploidy induction were found by using sperm nuclear FISH [43,44, 46], but this method is not available for detection of structural chromosome aberrations. Thus, existing results are insufficient and controversial. Further studies are necessary in order to draw conclusions.

5. Conclusion

The clastogenic effects of radiation and chemicals on human sperm chromosomes have been studied in vitro by many investigators, using a human sperm-hamster oocyte fertilization system and a metabolic activation system with S9. These studies

revealed that human sperm chromosomes are highly vulnerable to radiation and some chemicals or their metabolites. Moreover, it was found that human spermatozoa retain fertilizing ability even after a high doses of radiation and chemicals, suggesting that chromosome aberrations in spermatozoa may not be selected but transmitted to the next generation. Therefore, the in vitro assay with human spermatozoa is thought to be valuable as a sensitive system to assess potential genetic (transgenerational) effects of physical and chemical mutagens. In future, this assay has to be extended to various suspicious agents such as medical drugs, food additives, and pesticides, for which information on mutagenic potency in humans is still insufficient.

Acknowledgements

This study was supported by Grants-in-Aid for Scientific Research (B), No. 10470339 and (C), No. 13680615 from the Ministry of Education, Culture, Sports, Science and Technology of Japan, and a grant from the Japanese Atomic Energy Research Institute.

References

[1] R. Yanagimachi, H. Yanagimachi, B.J. Rogers, The use of zona-free animal ova as a test-system for the assessment of the fertilizing capacity of human spermatozoa, Biol. Reprod. 15 (1976) 471-476.

[2] E. Rudak, P.A. Jacobs, R. Yanagimachi, Direct analysis of the chromosome constitution of human spermatozoa, Nature 274 (1978) 911-913.

[3] R.H. Martin, A detailed method for obtaining preparations of human sperm chromosomes, Cytogenet. Cell Genet. 35 (1983) 252-256.

[4] Y. Kamiguchi, K. Mikamo, An improved, efficient method for analyzing human sperm chromosomes using zona-free hamster ova, Am. J. Hum. Genet. 38 (1986) 724-740.

[5] B. Brandriff, L. Gordon, Human sperm cytogenetics and the one-cell zygote, in: J.W. Allen, B.A. Bridges, M.F. Lyon, M.J. Moses, L.B. Russell (Eds.), Biology of Mammalian Germ Cell Mutagenesis, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, 1990, pp. 183-194.

[6] R.H. Martin, E. Ko, A. Rademaker, Distribution of aneuploidy in human gametes: Comparison between human sperm and oocytes, Am. J. Med. Genet. 39 (1991) 321-331.

[7] F. Pellestor, Differential distribution of aneuploidy in human gametes according to their sex, Hum. Reprod. 6 (1991) 1252-1258.

[8] Y. Kamiguchi, H. Tateno, K. Mikamo, Chromosomally abnormal gametes as a cause of developmental and congenital anomalies in humans, Cong. Anom. 34 (1994) 1-12.

[9] S.B. Olson, R.E. Magenis, Preferential paternal origin of *de novo* structural chromosome rearrangements, in: A. Daniels (Ed.), Progress and Topics in Cytogenetics, Vol. 8, The Cytogenetics of Mammalian Autosomal Rearrangements, Alan R. Liss, Inc., New York, 1988, pp. 583-599.

[10] B.F. Brandriff, L.A. Gordon, L.K. Ashworth, A.V. Carrano, Chromosomal aberrations induced by in vitro irradiation: Comparison between human sperm and lymphocytes, Environ. Mol. Mutagen. 12 (1988) 167-177.

[11] Y. Kamiguchi, H. Tateno, S. Watanabe, K. Mikamo, Chromosome abnormality in human gametes by exposure to mutagens, Environ. Mutagen Res. 18 (1996) 83-86.

[12] H. Tateno, Y. Kamiguchi, S. Watanabe, K. Mikamo, S. Sawada, Relative biological effectiveness (RBE) of ^{252}Cf fission neutrons for the induction of chromosome damage in human spermatozoa, Int. J. Radiat. Biol. 70 (1996) 229-235.

[13] R. Alvarez, L. Tusell, R. Miro, A. Genesca, M.R. Caballin, M. Ribas, J.F. Barquinero, J. Egozcue, Dose-response relationship for the induction of chromosomal abnormalities in gamma-irradiated human spermatozoa, Environ. Mol. Mutagen. 29 (1997) 357-366.

[14] Y. Kamiguchi, H. Tateno, K. Mikamo, Types of structural chromosome aberrations and their incidences in human spermatozoa X-irradiated in vitro, Mutat. Res. 228 (1990) 133-140.

[15] Y. Kamiguchi, H. Tateno, K. Mikamo, Dose-response relationship for the induction of structural chromosome aberrations in human spermatozoa after in vitro exposure to tritium β-rays, Mutat. Res. 228 (1990) 125-131.

[16] Y. Kamiguchi, H. Tateno, S. Watanabe, Y. Tonomura, Effects of microwave irradiation on human sperm chromosomes, Sci. Rept. Tokyo Woman's Christian Univ. 45 (1995) 1297-1303.

[17] H. Tateno, S. Iijima, Y. Nakanishi, Y. Kamiguchi, A. Asaka, No induction of chromosome aberrations in human spermatozoa exposed to extremely low frequency electromagnetic fields, Mutat. Res. 414 (1998) 31-35.

[18] J.A. Heddle, M. Hite, B. Kirkhart, K. Mavournin, J.T. MacGregor, G.W. Newell, M.F. Salamone, The induction of micronuclei as a measure of genotoxicity, A

report of the U.S. Environmental Protection Agency Gene-Tox Program, Mutat. Res. 123 (1983) 61-118.

[19] J.T. MacGregor, J.A. Heddle, M. Hite, B.H. Margolin, C. Ramel, M.F. Salamone, R.R. Tice, D. Wild, Guidelines for the conduct of micronucleus assays in mammalian bone marrow erythrocytes, Mutat. Res. 189 (1987) 103-112.

[20] Y. Kamiguchi, H. Tateno, K. Mikamo, Micronucleus test in 2-cell embryos as a simple assay system for human sperm chromosome aberrations, Mutat. Res. 252 (1991) 297-303.

[21] L. Tusell, R. Alvarez, M.R. Caballin, A. Genesca, R. Miro, M. Ribas, J. Egozcue, Induction of micronuclei in human sperm-hamster egg hybrids at the two-cell stage after in vitro gamma-irradiation of human spermatozoa, Environ. Mol. Mutagen. 26 (1995) 315-323.

[22] L. Tusell, R. Alvarez, A. Genesca, M.R. Caballin, R. Miro, L. Barrios, J. Egozcue, Human origin of micronuclei in human x hamster two-cell embryos, Cytogenet. Cell Genet. 70 (1995) 41-44.

[23] L. Tusell, R. Alvarez, A. Genesca, M.R. Caballin, R. Miro, J. Egozcue, Analysis of radiation-induced micronuclei in two-cell human-hamster embryos using telomeric and centromeric FISH probes, Cytogenet. Cell Genet. 74 (1996) 102-106.

[24] Y. Matsuda, T. Yamada, I. Tobari, Studies on chromosome aberrations induced in the eggs of mice fertilized in vitro after irradiation. I. Chromosome aberrations induced in sperm after X-irradiation, Mutat. Res. 148 (1985) 113-117.

[25] H. Tateno, Y. Kamiguchi, M. Shimada, K. Mikamo, Difference in types of radiation-induced structural chromosome aberrations and their incidences between Chinese and Syrian hamster spermatozoa, Mutat. Res. 350 (1996) 339-348.

[26] Y. Kamiguchi, H. Tateno, Y. Iizawa, K. Mikamo, Chromosome analysis of human spermatozoa exposed to antineoplastic agents in vitro, Mutat. Res. 326 (1995)

185-192.

[27] H. Kusakabe, K. Yamakage, N. Tanaka, Detection of neocarzinostatin-induced translocations in human sperm chromosomes using fluorescence in situ hybridization of chromosome 2, Mutat. Res. 369 (1996) 51-58.

[28] S. Watanabe, Y. Kamiguchi, Clastogenic effects of mitomycin C (MMC) on human sperm chromosomes, Chrom. Sci. 3 (1999) 157.

[29] H. Tateno, S. Iijima, A. Asaka, Y. Kamiguchi, Evaluation of clastogenicity of chemical agents using in vitro assay with human spermatozoa, Mutat. Res. 379 (Suppl.) (1997) S89.

[30] Y. Kamiguchi, Y. Ishii, Genetic effects of dioxin: from a viewpoint of the effects on human sperm chromosomes, Environ. Mutagen Res. 21 (1999) 201-205.

[31] S. Watanabe, Y. Kamiguchi, Chromosome analysis of human spermatozoa following in vitro exposure to cyclophosphamide, benzo(a) pyrene and N-nitrosodimethylamine in the presence of rat liver S9, Mutat. Res. 491 (2001) 57-63.

[32] D. Bimboes, H. Greim, Human lymphocytes as target cells in a metabolizing test system in vitro for detecting potential mutagens, Mutat. Res. 35 (1976) 155-160.

[33] A.T. Natarajan, A.D. Tates, P.P.W. Van Buul, M. Meijers, N. De Vogel, Cytogenetic effects of mutagens/carcinogens after activation in a microsomal system in vitro. I. Induction of chromosome aberrations and sister chromatid exchanges by diethylnitrosoamine (DEN) and dimethylnitrosoamine (DMN) in CHO cells in the presence of rat-liver microsomes, Mutat. Res. 37 (1976) 83-90.

[34] R.H. Martin, K. Hildebrand, J. Yamamoto, A. Rademaker, M. Barnes, G. Douglas, K. Arthur, T. Ringrose, I.S. Brown, An increased frequency of human sperm chromosomal abnormalities after radiotherapy, Mutat. Res. 174 (1986) 219-225.

[35] J. Jenderny, G. Rohrborn, Chromosome analysis of human sperm. I. First results with a modified method, Hum. Genet. 76 (1987) 385-388.

[36] R.H. Martin, A. Rademaker, K. Hildebrand, M. Barnes, K. Arthur, T. Ringrose I.S.

Brown, G. Douglas, A comparison of chromosomal aberrations induced by in vivo radiotherapy in human sperm and lymphocytes, Mutat. Res. 226 (1989) 21-30.

[37] A. Genesca, M.R. Caballin, R. Miro, J. Benet, X. Bonfill, J. Egozcue, Human sperm chromosomes: Long-term effect of cancer treatment, Cancer Genet. Cytogenet. 46 (1990) 251-260.

[38] A. Genesca, J. Benet, M.R. Caballin, R. Miro, J.R. Germa, J. Egozcue, Significance of structural chromosome aberrations in human sperm: analysis of induced aberrations, Hum. Genet. 85 (1990) 495-499.

[39] J. Jenderny, M.L. Jacobi, A. Ruger, G. Rohrborn, Chromosome aberrations in 450 sperm complements from eight controls and lack of increase after chemotherapy in two patients, Hum. Genet. 90 (1992) 151-154.

[40] S. Rousseaux, B. Sele, J. Cozzi, E. Chevret, Immediate rearrangements of human sperm chromosomes following in-vivo irradiation, Hum. Reprod. 8 (1993) 903-907.

[41] B.F. Brandriff, M.L. Meistrich, L.A. Gordon, A.V. Carrano, J.C. Liang, Chromosomal damage in sperm of patients surviving Hodgkin's disease following MOPP (nitrogen mustard, vincristine, procarbazine, and prednisone) therapy with and without radiotherapy, Hum. Genet. 93 (1994) 295-299.

[42] R.H. Martin, A.W. Rademaker, N.J. Leonard, Analysis of chromosomal abnormalities in human sperm after chemotherapy by karyotyping and fluorescence in situ hybridization (FISH), Cancer Genet. Cytogenet. 80 (1995) 29-32.

[43] W.A. Robbins, M.L. Meistrich, D. Moore, F.B. Hagemeister, H.-U. Weier, M.J. Cassel, G. Wilson, B. Eskenazi, A.J. Wyrobek, Chemotherapy induces transient sex chromosomal and autosomal aneuploidy in human sperm, Nature Genet. 16 (1997) 74-78.

[44] M. Monteil, S. Rousseaux, E.Chevret, R. Pelletier, J. Cozzi, B. Sele, Increased aneuploid frequency in spermatozoa from a Hodgkin's disease patient after

chemotherapy and radiotherapy, Cytogenet. Cell Genet. 76 (1997) 134-138.

[45] R.H. Martin, S. Ernst, A. Rademaker, L. Barclay, E. Ko, N. Summers, Analysis of human sperm karyotypes in testicular cancer patients before and after chemotherapy, Cytogenet. Cell Genet. 78 (1997) 120-123.

[46] R.H. Martin, S. Ernst, A. Rademaker, L. Barclay, E. Ko, N. Summers, Analysis of sperm chromosome complements before, during, and after chemotherapy, Cancer Genet. Cytogenet. 108 (1999) 133-136.

Explanation of figures

Fig. 1. Dose-response relationship for radiation-induced structural chromosome aberrations in human spermatozoa.

Fig. 2. Doe-response relationship for the induction of breakage-type and exchange-type aberrations.

Table 1
Dose-response relationships for radiation-induced structural chromosome aberrations in human spermatozoa

	Radiation	Dose (Gy)	Dose rate (Gy/min)	Dose-effect equation [a]	Reference
(a)	Gamma-rays (^{137}Cs)	0.0-4.0	5.0	$Y=0.96+15.14D$ [b]	[10]
(b)	Gamma-rays (^{137}Cs)	0.0-4.23	1.36	$Y=100\ (1-e^{-0.514D})$	[11]
(c)	Gamma-rays (^{137}Cs)	0.0-2.11	1.36	$Y=3.70+32.52D$	[11]
(d)	Gamma-rays (^{60}Co)	0.0-2.0	$1.7x10^{-2}$	$Y=1.68+33.85D$	[12]
(e)	Gamma-rays (^{60}Co)	0.0-4.0	1.07-1.17	$Y=1.05+17.98D$ [b]	[13]
(f)	X-rays	0.0-1.82	0.44	$Y=0.08+34.52D$	[14]
(g)	Beta-rays (^{3}H)	0.0-1.93	$0.3-2.5x10^{-2}$	$Y=2.20+34.62D$	[15]
(h)	Neutrons (^{252}Cf)	0.0-1.0	$1.3-1.7x10^{-2}$	$Y=1.15+49.43D$	[12]

[a] Y indicates the percentile yield of spermatozoa with chromosome aberrations, and D the dose in Gy.
[b] For correct comparison, the net incidence of spermatozoa with chromosome aberrations was calculated using the formula described by us [14,15].

Table 2
Dose-response relationships for the induction of breakage-type and exchange-type chromosome aberrations

	Radiation	Dose (Gy)	Dose-effect equation [a]	Reference
(a)	Gamma-rays (^{137}Cs)	0.0-4.23	Br: Y=0.009+0.481D Ex: Y= -0.006+0.072D+0.007D ^{2}	[11]
(b)	Gamma-rays (^{60}Co)	0.0-2.0	Br: Y= -0.047+0.534D Ex: Y= 0.001+0.033D+0.023D ^{2}	[12]
(c)	X-rays	0.0-1.82	Br: Y= -0.014+0.478D Ex: Y= -0.005+0.034D+0.012D ^{2}	[14]
(d)	Beta-rays (^{3}H)	0.0-3.74	Br: Y=0.002+0.534D Ex: Y= -0.0004+0.058D+0.009D ^{2}	[15]
(e)	Neutrons (^{252}Cf)	0.0-1.0	Br: Y=0.004+0.791D Ex: Y=0.003+0.133D+0.041D ^{2}	[12]

[a] Y indicates the number of chromosome aberrations per spermatozoon, and D the dose in Gy.
Br: breakage-type aberration, Ex: exchange-type aberration

Table 3
Clastogenic effects of gamma-rays on human sperm chromosomes: comparison of micronuclei and chromosomal aberrations

Index	Radiation			Dose-effect equation [a]	Reference
	Source	Dose (Gy)	Dose rate (Gy/min)		
(a) 2-cell embyros with MN (%)	^{137}Cs	0.0-2.13	1.36	Y=1.38+38.00D	[20]
(b) Spermatozoa with SCA (%)				Y=1.70+36.39D	
(c) 2-cell embryos with MN (%)	^{60}Co	0.0-4.0	1.07-1.17	Y=0.63+12.00D [b]	[21]
(d) Spermatozoa with SCA (%)				Y=0.29+14.76D [b]	

[a] Y indicates the percentile yield of spermatozoa with MN or SCA, and D the dose in Gy.
[b] For correct comparison, the net incidence of chromosomally abnormal embryos (spermatozoa) was calculated with the formula described by us [14,15].
MN: micronuclei, SCA: structural chromosome aberrations (breaks and fragments)

Table 4
Dose-response relationships for X-ray-induced structural chromosome aberrations in spermatozoa of four mammalian species

Species	Dose (Gy)	Dose-effect equation [a]	Reference
(a) Mouse	0.0-4.0	$Y=-0.35+9.27D$	[24]
(b) Golden hamster	0.0-0.91	$Y=0.29+29.66D$	[25]
(c) Chinese hamster	0.0-3.63	$Y=0.11+14.11D$	[25]
(d) Human	0.0-1.82	$Y=0.08+34.52D$	[14]

[a] Y indicates the percentile yield of spermatozoa with chromosome aberrations, and D the dose in Gy.

Table 5
Clastogenic effects of various chemicals on human sperm chromosomes

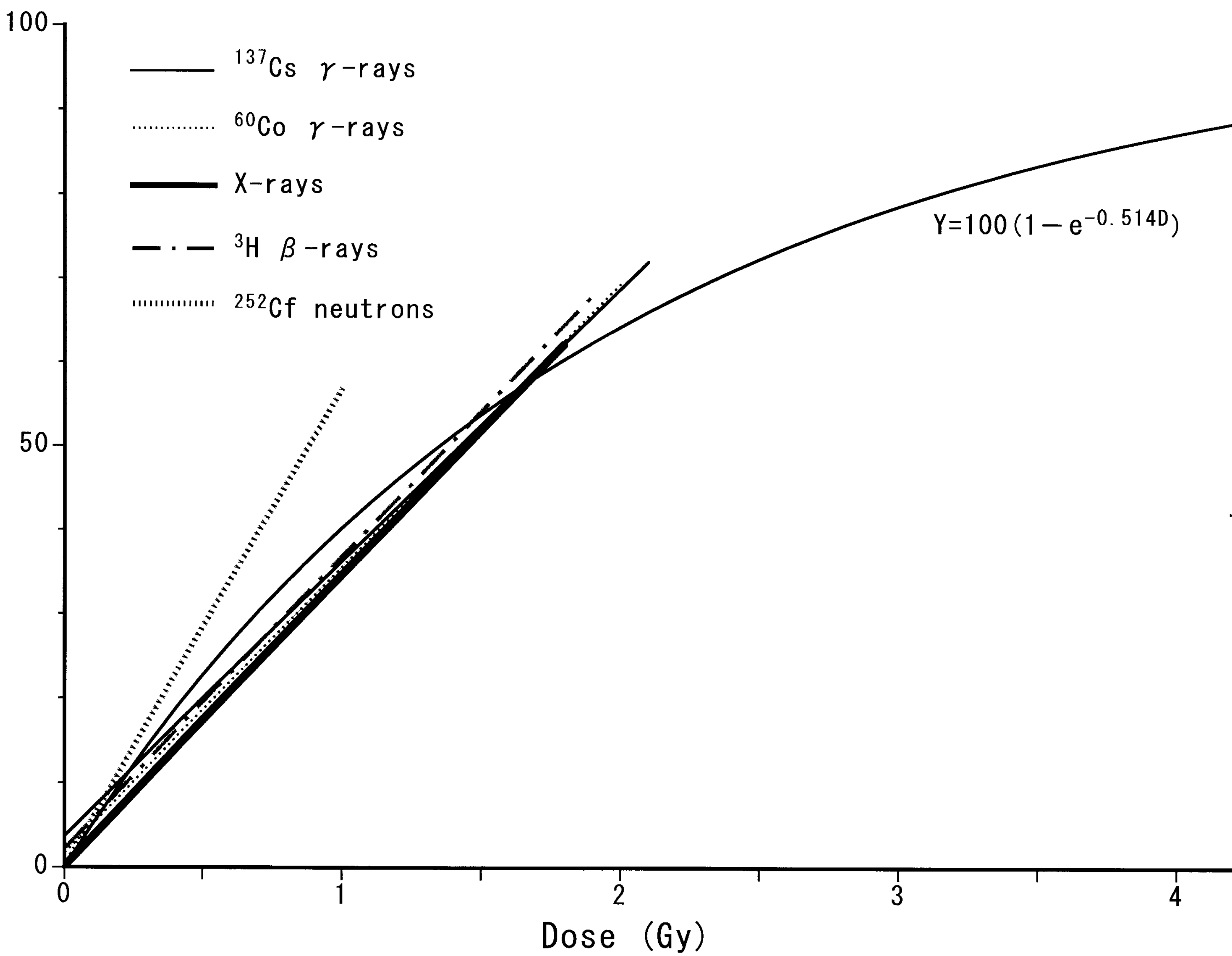
Chemicals	Dose		Duration of exposure (min)	Induction of chromosome aberrations	Reference
Bleomycin	50 ug/ml	(-S9) [a]	90	+	[26]
Daunomycin	0.1 ug/ml	(-S9)	90	+	[26]
Methyl methanesulfonate	100 ug/ml	(-S9)	120	+	[26]
Triethylenemelamine	0.1 ug/ml	(-S9)	120	+	[26]
Neocarzinostatin	0.5-2.0 ug/ml	(-S9)	120	+	[27]
N-methyl-N'-nitro-N-nitrosoguanidine	1.0 ug/ml	(-S9)	120	+	[29]
Urethane	1.0 mg/ml	(-S9)	120	-	[29]
Nitrobenzene	500 ug/ml	(-S9)	120	-	[29]
2,3,7,8-tetrachlorodibenzo-p-dioxin	1.0-5.0 ug/ml	(-S9)	120	-	[30]
Mitomycin C	5.0 ug/ml	(+S9)	120	+	[28]
	5.0 ug/ml	(-S9)	120	+	
Cyclophosphamide	20 ug/ml	(+S9)	120	+	[31]
	20 ug/ml	(-S9)	120	-	
Benzo(a)pyrene	200 ug/ml	(+S9)	120	+	[31]
	200 ug/ml	(-S9)	120	-	
N-nitrosodimethylamine	20 mg/ml	(+S9)	120	-	[31]
	20 mg/ml	(-S9)	120	-	

[a] S9: microsomal fraction of rat liver homogenate

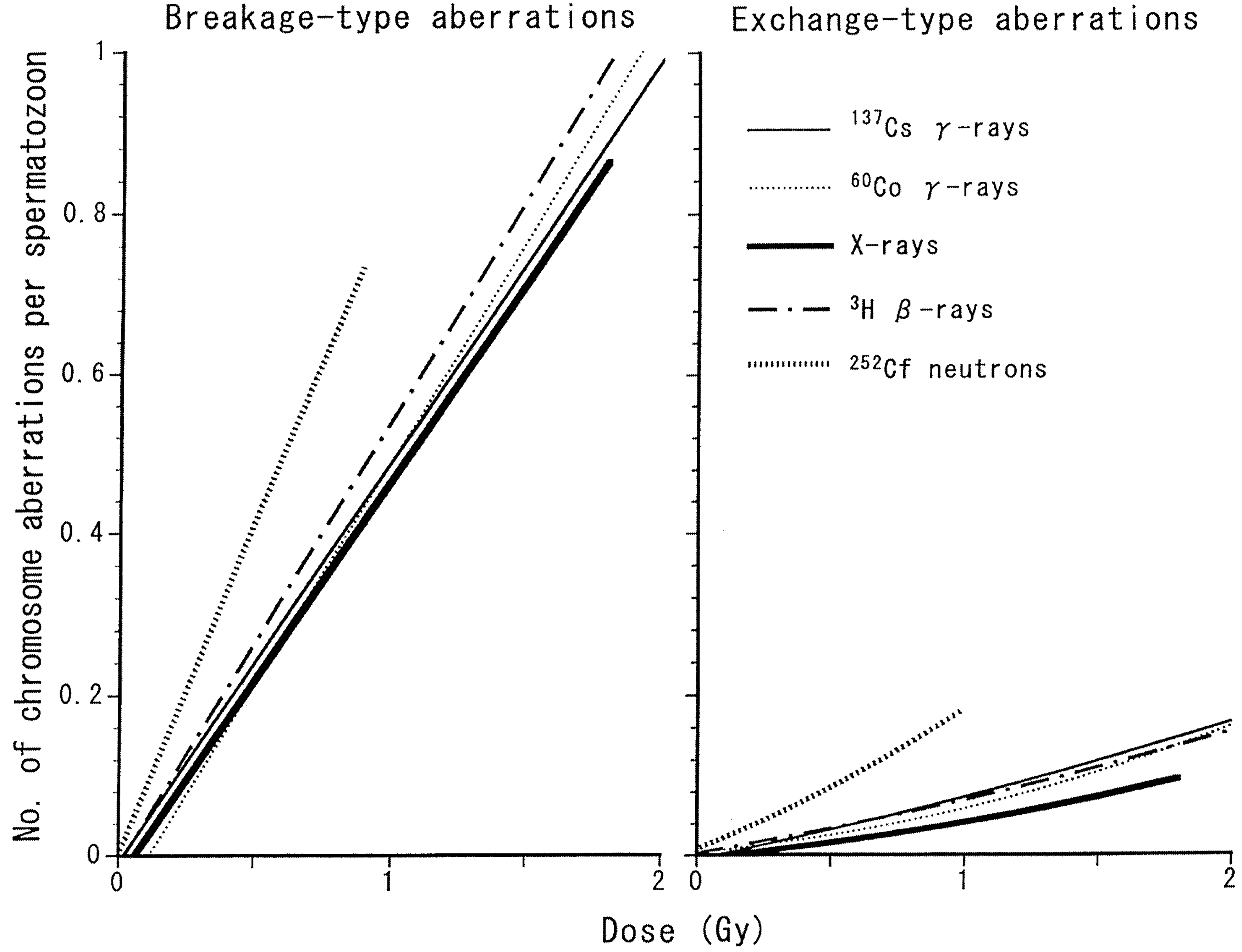
Table 6
Chromosome aberrations in the spermatozoa of cancer patients

Author	Disease	Treatment	Chromosome aberrations	
			Aneuploidy	Structural anomaly
Martin et al. [34]	seminoma etc.	radiotherapy	+	+
Jenderny & Rohrborn [35]	?	radiotherapy	-	-
Martin et al. [36]	seminoma etc.	radiotherapy	- ?	+
Genesca et al. [37, 38]	Wilms' tumor etc.	radio- and/or chemotherapy	-	+
Jenderny et al. [39]	seminoma etc.	chemotherapy	-	-
Rousseaux et al. [40]	Hodgkin's disease	radio- and/or chemotherapy	- ?	+
Brandriff et al. [41]	Hodgkin's disease	radio- and/or chemotherapy	+	+
Martin et al. [42]	lymphoma	chemotherapy	-	-
Robbins et al. [43] [a]	Hodgkin's disease	chemotherapy	+	?
Monteil et al. [44] [a]	Hodgkin's disease	radio- and/or chemotherapy	+	?
Martin et al. [45]	testicular cancer	chemotherapy	-	-
Martin et al. [46] [a]	testicular cancer	chemotherapy	+	?

[a] FISH study with sperm nuclei



Incidence of human spermatozoa with structural chromosome aberrations (%)
100
50
0
0
1
2
3
4
Dose (Gy)
137Cs γ-rays
60Co γ-rays
X-rays
3H β-rays
252Cf neutrons
Y=100(1−e−0.514D)


Breakage-type aberrations
Exchange-type aberrations
No. of chromosome aberrations per spermatozoon
0
0.2
0.4
0.6
0.8
1
0
1
2
Dose (Gy)
137Cs γ-rays
60Co γ-rays
X-rays
3H β-rays
252Cf neutrons