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Abstract

To investigate the chromosomal effects of topoisomerase II-interactive drugs on mammalian primary oocytes, female Chinese hamsters were treated with etoposide (VP-16) at various intervals pre- and post-human chorionic gonadotropin (hCG) injections. Chromosome analysis of oocytes at metaphase II showed that treatment with VP-16 at 50 h pre-hCG had no effect, but the treatments between 24 h pre-hCG and 2 h post-hCG often caused structural chromosome aberrations. Although treatment at 4 h post-hCG had no effect, subsequent treatments at 6 h and 8 h post-hCG produced a significant increase in structural chromosome aberrations. No effect was found following treatment at 10 h post-hCG. The incidence of aneuploidy following exposure to VP-16 was also dependent on the time of hCG injection. Taking the time course of meiotic progression in primary oocytes following hCG injection and pharmacokinetics of VP-16 into consideration, it is likely that meiotic stages from late dictyate to diakinesis are highly sensitive to VP-16, while stages at dictyate and from metaphase I to telophase I are relatively insensitive to the drug. Moreover, the effect of VP-16 on structural chromosome aberrations and aneuploidy was dose-dependent.

Chromosome analysis at metaphase I detected a frequent occurrence of structural chromosome aberrations in treated oocytes. This suggests that structural aberrations may be caused by disruption of cleavable complexes during chromosome condensation. Detection of chromosome bridges during anaphase I/telopahse I may

support the hypothesis that induction of aneuploidy by VP-16 is due to failure in decatenation of recombinant homologous chromosomes.

Key words: topoisomerase II inhibitor, etoposide, oocytes, chromosome aberrations, Chinese hamsters

1. Introduction

Aneuploidy has a critical impact on human health. Chromosome studies of human gametes clearly demonstrated that most of aneuploidy are attributable to meiotic segregation errors in oocytes [1-3]. To decrease risk of aneuploid production, detection of aneugens and understanding of the causal mechanism of meiotic errors in oocytes are essential.

Cytogenetic assays using murine oocytes have demonstrated that meiotic segregation errors can be induced by spindle inhibitors including benomyl [4], carbendazim [5, 6], colchicine [7-10], griseofulvin [11], nocodazole [12], podophyllotoxin [13] and vinblastine [13-15]. The similar meiotic errors have been caused by spindle stabilizing agent taxol [16]. These findings indicate that chemical agents that can interact with meiotic spindle have potential risk of inducing aneuploidy.

In addition to these spindle-interactive agents, topoisomerase II inhibitor etoposide has shown to induce both aneuploidy and structural chromosome aberrations in mouse oocytes [17, 18]. The studies showed that frequencies of chromosome aberrations in treated oocytes varied at different times relative to human chorionic gonadotropin injection. This suggests that chromosomal sensitivity of oocytes to the drug may be dependent on oocyte maturation. Since topoisomerase II is a ubiquitous nuclear enzyme which is involved in chromosomal dynamics such as segregation, condensation and decondensation of chromosomes and entanglement of

DNA strands in mitosis and meiosis [19], Mailhes et al. [17] proposed that etoposide interferes with decatenation of recombinant homologous chromosomes during anaphase I, thus resulting in aneuploidy. Furthermore, topoisomerase II-DNA complex formed by the drug, also known as a cleavable complex [20], may serve as an intermediate in the generation of structural chromosome aberrations. It is possible that mechanical stresses imposed on anaphase chromosomes by meiotic spindle force may cause the separation of cleavable complex. Some of topoisomerase II inhibitors are promising as drugs for the treatment of human cancers [20], and some of the agricultural, industrial and pharmaceutical chemicals may potentially act as topoisomerase II inhibitors. Therefore, further evaluation of cytogenetic effects of the inhibitors on mammalian oocytes and understanding of their mutagenic properties should be addressed.

In the present study, we examined whether chromosomal sensitivity of primary oocytes to etoposide is dependent on meiotic stages. Furthermore, we analyzed chromosomes of primary oocytes at metaphase I and anaphase I/telophase I in order to investigate whether induction of chromosome aberrations by the drug is due to failure in decatenation of recombinant homologous chromosomes. To tackle with these problems, we used Chinese hamsters because their oocyte meiotic maturation proceeds synchronously. The species has a low number of chromosomes (n=11) that are morphologically distinguishable from each other. This is advantageous to detect a false haploidy that is a combination of disomy and nullisomy [13].

2. Materials and methods

2.1. Chemicals

Etoposide (VP-16; Cas No. 33419-42-0) was purchased from Sigma Chemical Co. (St. Louis, MO). Immediately before use, the drug was dissolved in 0.1 ml of dimethyl sulfoxide (DMSO, Kanto Chemicals, Tokyo) at predetermined concentrations.

2.2. Animals

Mature female Chinese hamsters, 3-4 months of age, were obtained from our CHA colony, which was raised and maintained under optimum laboratory conditions (temperature at 23 ± 2 °C, humidity 40-60%, 14 h light between 5 a.m. to 7 p.m.). Superovulation was induced according to the method of Iizawa et al. [21]. In brief, female hamsters were subcutaneously injected with 12 iu pregnant mare's serum gonadotropin (PMSG) on the day of ovulation followed 43 h later with a booster injection of 16 iu PMSG. Seven hours later, 48 iu human chorionic gonadotropin (hCG) was injected subcutaneously. The animals were used according to our institutional regulations, and ethical approval was obtained prior to experimentation.

2.3. Meiotic maturation of oocytes after hCG injection

To examine the time-course of meiotic progression in primary oocytes following hCG injection, cytological preparations of oocytes were established every 2

h between 0 h and 14 h after hCG injection. Preovulatory follicles were punctured by a fine needle to recover primary oocytes. The oocytes surrounded by follicle cells were treated with 1.0% (w/v) trypsin (1: 250, Difco, Detroit, MI) at 37 ˚C for 10 min to eliminate the zona pellucida. Then they were transferred into a solution consisting of the same quantity of 1% sodium citrate and 30 % fetal calf serum, and aspirated in and out of a fine glass pipette to mechanically remove the follicle cells. The denuded oocytes were fixed and placed on microscopic glass slides by gradual-fixation/air-drying method [22]. The slides were stained with 2 % of Giemsa (Merck, Darmstadt, Germany) in phosphate buffered solution (pH 6.8) for 8 min.

2.4. Treatment with VP-16 and chromosome analysis at metaphase II

Female hamsters were treated with a single intraperitoneal (i.p.) injection of 80 mg/kg of VP-16 at different times relative to hCG injection in order to investigate chromosomal sensitivity of primary oocytes to the drug with a special reference to meiotic maturation. The treatment protocol was as follows; 50 h, 24 h, 8 h and 4 h pre-hCG injection (–50 h, –24, –8 h and –4 h, respectively), immediately after hCG injection (0 h), and 2 h, 4 h, 6 h, 8 h and 10 h post-hCG injection (+2 h, +4 h, +6 h, +8 h and +10 h, respectively). Control females were treated with an i.p. injection of 0.1 ml of DMSO at +2 h (the most sensitive time). To examine the dose-response relationship between VP-16 and induction of chromosome aberrations, hamsters were treated with 20 mg/kg or 40 mg/kg of VP-16 at +2 h.

Seventeen to eighteen hours after hCG injection (2-3 h post-ovulation), oocytes were collected from the oviducts and freed from cumulus cells by treatment with 0.1% (w/v) bovine hyaluronidase (290 units/ml, Sigma). The cumulus-free oocytes were treated with 1.0% trypsin to eliminate the zona pellucida. They were then placed in hypotonic solution (30% of fetal bovine serum) for 1 h at 37 °C. Chromosome preparations were prepared by the gradual-fixation/air-drying method. The slides were stained with 2% Giemsa solution for 8 min.

2.5. Chromosome analysis at metaphase I and anaphase I/telophase I

Females were treated with a single i.p. injection of 80 mg/kg of VP-16 at +2 h (the most sensitive time). Based on the time-course of meiotic progression in primary oocytes following hCG injection (*see* Table 1), oocytes at metaphase I and anaphase I/telophase I were recovered from preovulatory follicles of the ovaries at +4 h and +12 h, respectively. As described earlier, the zona pellucida and follicle cells were enzymatically and mechanically removed. The denuded metaphase I oocytes were placed in hypotonic solution for 30 min at 25 ˚C. However, hypotonic treatment of anaphase I/telophase I oocytes was omitted to avoid chromosomal scattering. After that, oocytes were fixed and mounted on microscopic glass slides by the gradual-fixation/air-drying method for chromosome examination. Control females were given an i.p. injection of 0.1 ml DMSO at +2 h.

2.6. Statistical analysis

Results were analyzed using Fisher's exact probability test or chi-square test. Differences at $P < 0.05$ were considered significant.

3. Results

3.1. Time-course of meiotic progression in primary oocytes

Meiotic stages of primary oocytes at various intervals after hCG injection are shown in Table 1. At the time of hCG injection (0 h), all oocytes had a germinal vesicle at dictyate (dit). Transition to metaphase I (M I) was completed between +2 h and +4 h. Oocytes passed through M I between +4 h and +8 h, and anaphase I (ana I) to telophase I (telo I) between +10 h and +12 h. They entered metaphase II (M II) at +14 h. At this time, oocytes were still present in preovulatory follicles of the ovaries. At +16 h, oocytes were found in the ovarian sacs and oviducts, indicating that ovulation had occurred between +15 h and +16 h.

3.2. Chromosome analysis at M II

Irrespective of the time of VP-16 injection, all ovulated oocytes contained M II chromosomes. As shown in Table 2, there was no significant increase in structural chromosome aberrations in oocytes from hamsters treated with VP-16 at –50 h. However, there was a significant increase in the incidence of oocytes with structural

chromosome aberrations in treatments between –24 h and +2 h. Surprisingly, the incidence of aberrant oocytes in the treatment at +4 h was low and similar to that noted at the control level. In subsequent treatments at +6 h and +8 h, the incidences of aberrant oocytes were significantly higher than that of the control, though they tended to be less than those observed in treatments between –8 and +2 h. The incidence of aberrant oocytes in the treatment at +10 h was high compared to the control, but the difference was not statistically significant.

The observed structural chromosome aberrations were classified into non-centromeric aberrations and centromeric aberrations. The former consisted of interstitial chromatid break, partial chromatid deletion, chromatid acentric fragment and chromatid exchange, while the latter consisted of centromeric break, whole chromatid deletion, and precocious division of sister chromatids (Table 2). Some examples of aberrant chromosomes are shown in Figure 1. In all treatment groups, the frequency of non-centromeric aberrations was higher than that of centromeric aberrations.

The incidences of oocytes with aneuploidy, and types of aneuploidy and their frequencies are shown in Table 3. Gain and loss of dyads (Figure 2a) were observed in all groups, whilst gain and/or loss of monads (Figure 2b) were observed in treatments between –50 h and +4 h. In treatments at –4 h and 0 h, false haploidy was observed. As shown in Tateno et al. [13], false haploidy is a combination of disomy and nullisomy induced by double nondisjunction. It was also counted as an aneuploidy in the present study. There was a significant increase in the incidence of

gain/loss of dyads in treatments at 0 h and +2 h, while treatments between –24 h and +2 h were associated with a significant increase in incidence of gain/loss of monads. Overall, treatments between –24 h and +2 h were associated with a significant increase in incidence of oocytes with aneuploidy.

Further analysis showed a dose-response relationship between VP-16 and induction of structural chromosome aberrations as well as aneuploidy (Figure 3). The incidences of structural chromosome aberrations and aneuploidy in control oocytes and oocytes treated with 80 mg/kg of VP-16 are summarized in Tables 2 and 3. In treatment with 20 mg/kg, 305 oocytes were collected from 6 females, and karyoanalyzed. The incidences of oocytes with structural chromosome aberrations and aneuploidy were 15.7% and 3.6%, respectively. The incidence of structural chromosome aberrations was significantly higher than that of the control, while there was no significant increase of aneuploidy. In treatment with 40 mg/kg, 57 (22.5%) of 253 oocytes collected from 10 females had structural chromosome aberrations, and 17 (6.7%) showed aneuploidy. These incidences were significantly higher than those of the control. Thus, it was evident that VP-16 induced both structural chromosome aberrations and aneuploidy in a dose-dependent manner.

3.3. Chromosome analysis at M I

At +4 h, a total of 213 primary oocytes were collected from the ovaries of 7 hamsters exposed to 80 mg/kg of VP-16 at +2 h. Among them, 208 (97.7%) were at M I and 5 (2.3%) were at germinal vesicle (GV) stage. As a control, a total of 176

oocytes were collected from 5 hamsters exposed to 0.1 ml of DMSO, and there were 175 M I oocytes (99.4%) and 1 GV oocyte (0.6%). The proportions of M I and GV oocytes in both experimental and control groups were not significantly different from those in oocytes collected from non-treated females at the same time (*see* Table 1), indicating that neither VP-16 nor DMSO affects meiotic progression up to M I.

Out of 208 M I oocytes collected from treated females, 187 were successfully karyoanalyzed, and 78 (41.7%) showed structural chromosome aberrations (Table 4). This incidence was evidently higher than that (0.6%) of the control. The observed aberrations included achromatic lesion, isoachromatic lesion, chromatid break and isochromatid break (Figure 4). The broken sites of chromatid arms did not necessarily correspond with the chiasma sites of homologous chromosomes. Although a certain number of centromeric breaks and precocious divisions of sister chromatids were found in M II chromosome analysis (Table 2), no supportive evidence for these aberrations was seen in M I chromosome analysis. Even though centromeric breaks and precocious divisions of sister centromeres were present, it was difficult to detect them because of sister-chromatid cohesion in M I chromosomes. In both experimental and control groups, no oocyte showed unequivocal univalent formation.

3.4. Chromosomal behaviors during ana I/telo I

At +12 h, a total of 226 primary oocytes were collected from hamsters exposed to 80 mg/kg of VP-16 at +2 h, while a total of 188 primary oocytes were from controls exposed to 0.1 ml DMSO at the same time. The frequencies of oocytes at various meiotic stages are shown in Table 5. In VP-16 group, the frequencies of oocytes at M I and ana I were significantly high, while that of oocytes at telo I was significantly low, compared to those of the control. There was no significant difference in the frequency of M II oocytes between experimental and control groups. The finding suggests that VP-16 may delay to some extent the exit from M I.

A total of 165 and 150 oocytes during ana I/telo I were chromosomally analyzed in control and VP-16 groups, respectively (Table 5). The incidence of oocytes showing chromosome bridges (Figure 5) was significantly higher in VP-16 group (33.3%) than in control group (0.6%).

4. Discussion

The present study demonstrated that VP-16 induced both structural chromosome aberrations and aneuploidy in Chinese hamster primary oocytes, supporting the results of studies on mouse oocytes [17, 18]. Although the frequencies of both types of chromosome aberrations were not significantly high in oocytes from hamsters exposed to VP-16 at –50 h, they significantly increased in oocytes from hamsters treated between –24 h and + 2 h. Interestingly, the incidence of chromosome aberrations abruptly decreased to the control level in oocytes from

hamsters exposed to the drug at +4 h. A relatively low incidence of chromosome aberrations was also found in oocytes from hamsters treated between +6 h and +10 h (Tables 2 and 3). These results strongly suggest that chromosomal sensitivity of primary oocytes to VP-16 is dependent on meiotic stage. Although we did not examine the pharmacokinetics of VP-16 in Chinese hamster ovaries, we found that structural chromosome aberrations frequently occurred in M I oocytes collected at +4 h from hamsters exposed to VP-16 at +2 h (Table 4). This evidence indicates that VP-16 can reach the ovaries and affect oocyte chromosomes within 2 h of the injection. Therefore, VP-16 injected at –50 h, between –24 h and + 2 h and between +4 h and +10 h probably affected oocytes at dictyate, oocytes at late dictyate to diakinesis, and oocytes at M I to telo I, respectively. It is likely that meiotic stages from late dictyate to diakinesis are highly sensitive to VP-16, while stages at dictyate and from M I to telo I are relatively insensitive to the drug. It is known that topo-II plays an important role in chromosome condensation [19] and it functions as a major component of scaffold proteins of metaphase chromosomes [23-26]. It seems that treatment with VP-16 in the stages from late dictyate to diakinesis disorders chromosome condensation and organization of metaphase chromosomes. This may be the reason why stages from late dictyate to diakinesis are highly sensitive to VP-16.

A preferential induction of centromeric breaks by VP-16 has been observed in mouse oocytes [17]. Although centromeric breaks significantly occurred in Chinese hamster oocytes exposed to VP-16, their frequency was not greater than that of non-centrometric breaks in any treatment time and VP-16 dosage of the present study.

Explanation of this discrepancy is unknown. There may be species specific difference in sensitivity of oocyte chromosomes to VP-16. Mailhes et al. [17] proposed that formation of non-centromeric breaks by VP-16 may be due to failure in religation of DNA strand breaks at the time of decatenation of recombinant homologous chromosomes during anaphase I, while centromeric breaks may be attributable to cleavable complex at the centromere. It is possible that mechanical stresses posed on the centromere by spindle force may spur on the formation of centromeric breaks during anaphase I. If the hypothesis is correct, no chromosomal breaks should be found prior to anaphase I. However, we found a frequent occurrence of chromosomal breaks in M I oocytes (Table 4). Therefore, other mechanism might mediate VP-16-induced formation of chromosomal breaks. It is conceivable that cleavable complexes are disrupted by architectonic distortion during chromosome condensation. As mentioned above, this conclusion is based on previous findings indicating that topo-II is involved in chromosome condensation [19] and it acts as an important component of scaffold proteins of metaphase chromosomes [23-26]. Another type of topo-II inhibitors such as merbarone and ICRF-193, which cannot form cleavable complexes, may be useful for understanding of mechanism of induction of structural chromosome aberrations.

With respect to induction of aneuploidy, Angel et al. [27] considered the formation of univalents at M I and the precocious division of univalents at anaphase as a cause of aneuploidy. In the present study, however, no formation of univalents was identified even in oocytes from hamsters exposed to VP-16 at the most sensitive

time. Because VP-16 has no direct interaction with cellular organelles including kinetochores and spindle fibers [20], it is certain that mechanism of aneuploid induction by VP-16 is different from that by spindle-interactive chemicals. Mailhes et al. [17] proposed that failure in decatenation of recombinant homologs during anaphase is responsible for the induction of aneuploidy in mouse oocytes. The detection of chromosome bridges during ana I/telo I in the present study strongly supports their hypothesis. In addition, there is a possibility that disturbance of centromeric region by VP-16 may mediate chromosome segregation errors because topo-II isomer is associated with centromeric region of mammalian chromosomes [24, 28].

Recent advances have been made in our understanding of the function of chromosome-associated proteins involved in chromosomal dynamics such as chromosome condensation and decondensation, and sister-chromatid cohesion and separation [29, 30]. Further studies are important to investigate the relationship between dysfunction of chromosome-associated proteins and induction of chromosome aberrations in order to understand the mechanism of formation of chromosome aberrations in mammalian gametes.

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Figure legends

Figure 1

Examples of structural aberrations in MII chromosomes of Chinese hamster oocytes exposed to VP-16. **(A)** An interstitial chromatid break of a medium metacentric chromosome (thick arrow) and a derivative fragment (fine arrow). **(B)** A partial chromatid deletion of a large metacentric chromosome (arrow). A derivative fregment was probably lost during the first meiotic division. **(C)** A centromeric break of a small metacentric chromosome (arrow). **(D)** A precocious division of sister chromatids of a large metcentric chromosome (arrow). Bars = 10 µm.

Figure 2

Two types of aneuploidy. Normal M II complement of Chinese hamster oocyte (n=11) consists of two large metacentric, three medium metacentric, three acrocentric and three small metacentric chromosomes. **(A)** Hyperhaploidy due to gain of a large metacentric chromosome (n=12). Arrows indicate three large metacentric chromosomes. **(B)** Hypohaploidy due to loss of a monad (n=10 $^{1}/_{2}$). Arrow indicates a monad of large metacentric chromosome. Bars = 10 µm.

Figure 3

Dose-response relationship between induction of chromosome aberrations and exposure of oocytes to VP-16 at the most sensitive time, 2 h post-hCG injection. *P < 0.05, **P< 0.001, compared with the control (0 mg/kg).

Figure 4

Chromosome aberration in M I oocyte collected at 4 h post-hCG injection from an animal exposed to 80 mg/kg of VP-16 at the most sensitive time, 2 h post-hCG injection. Note the isoachromatid lesion (arrow) in a tetrad resulting from paring of homologous large metacentric chromosomes. Bar = 10 µm.

Figure 5

A chromosome bridge (arrow) in oocyte during anaphase I collected at 12 h post-hCG injection from an animal exposed to 80 mg/kg of VP-16 at 2 h post-hCG injection. Bar = 10 µm.

Table 1

Meiotic stages in Chinese hamster primary oocytes at various intervals after hCG injection

Time post-hCG (h)	No. of females used	No. of oocytes analyzed	Meiotic stages (%)					
			dit [a]	dia[b]	M I[c]	ana I[d]	telo I[e]	M II[f]
0	6	104	100.0	0	0	0	0	0
2	7	126	98.4	1.6	0	0	0	0
4	5	111	0.9	0	99.1	0	0	0
6	5	145	0	0	100.0	0	0	0
8	5	139	0	0	100.0	0	0	0
10	5	168	0	0	63.7	27.4	8.9	0
12	5	185	0	0	0	14.1	66.5	19.5
14	5	153	0	0	0	0	0	100.0

[a] dictyate, [b] diakinesis, [c] metaphase I, [d] anaphase I, [e]telophase I, [f] metaphase II.

Table 2

Incidences of structural chromosome aberrations in M II oocytes from females exposed to 80 mg/kg of VP-16 at various time intervals pre- and post-hCG injection

Treatment time pre/post hCG (h)	No. of females used	No. of oocytes analyzed	No. (%) of oocytes with structural chromosome aberrations	Types of structural chromosome aberrations and their frequencies							Total
				non-centromeric aberrations				centromeric aberrations			
				interstitial chromatid break	partial chromatid deletion	chromatid acentric fragment	chromatid exchange	break at centromere	whole chromatid deletion	precocious division of sister chromatids	
Control	7	218	4 (1.8)	0	1	2	0	0	0	1	4
–50	7	152	3 (2.0)	0	3	1	0	0	0	0	4
–24	6	190	21 (11.3)[a]	7	10	3	0	3	1	3	27
–8	5	212	55 (25.9)[a]	26	4	28	0	5	1	4	68
–4	8	237	48 (20.3)[a]	8	7	18	0	9	4	7	53
0	5	244	86 (35.2)[a]	29	15	40	0	12	8	10	114
+2	6	208	112 (53.8)[a]	82	22	68	0	29	17	13	231
+4	9	370	10 (2.7)	5	1	4	1	0	0	0	11
+6	5	332	24 (7.2)[b]	18	2	5	0	2	0	0	27
+8	7	301	26 (8.6)[b]	19	0	6	0	0	0	0	25
+10	6	201	10 (5.0)	7	0	4	0	0	0	0	11

[a] $P < 0.001$
[b] $P < 0.01$

Table 3

Incidence of aneuploidy in M II oocytes from females exposed to 80 mg/kg of VP-16 at various time intervals pre- and post-hCG injection

Treatment time pre/post hCG (h)	No. of females used	No. of oocytes analyzed	No. (%) of oocytes with aneuploidy	Types of aneuploidy and their frequencies						
				dyad			monad			pseudo-haploidy (%)
				gain	loss	total (%)	gain	loss	total (%)	
Control	7	218	6 (2.8)	3	3	6 (2.8)	0	0	0	0
–50	7	152	6 (3.9)	1	4	5 (3.3)	0	1	1 (0.7)	0
–24	6	190	14 (7.4)[a]	7	3	10 (5.3)	0	4	4 (2.1)[a]	0
-8	5	212	17 (8.0)[a]	7	3	10 (4.7)	2	5	7 (3.3)[b]	0
-4	8	237	16 (6.8)[a]	4	2	6 (2.5)	2	7	9 (3.8)[b]	1 (0.4)
0	5	244	35 (14.3)[c]	14	9	23 (9.4)[b]	5	6	11 (4.5)[b]	1 (0.4)
+2	6	208	28 (13.5)[c]	8	9	17 (8.2)[a]	2	9	11 (5.3)[c]	0
+4	9	370	12 (3.2)	3	8	11 (2.9)	1	0	1 (0.3)	0
+6	5	332	10 (3.0)	6	4	10 (3.0)	0	0	0	0
+8	7	301	13 (4.3)	7	6	13 (4.3)	0	0	0	0
+10	6	201	10 (5.0)	3	7	10 (5.0)	0	0	0	0

[a] $P < 0.05$
[b] $P < 0.01$
[c] $P < 0.001$

Table 4

Chromosome analysis of M I oocytes collected from females exposed to 80 mg/kg of VP-16 at +2 h

Treatment	No. of females used	No. of oocytes analyzed	No. (%) of aberrant oocytes	Types and frequencies of aberrations			
				achromatic lesion	isoachromatic lesion	chromatid break	isochromatid break
Control	5	166	1 (0.6)	0	0	1	0
VP-16	7	187	78 (41.7)[a]	6	41	44	29

[a] $P < 0.001$

Table 5

Meiotic stages in oocytes collected at +12 h from females exposed to 80 mg/kg VP-16 at +2 h and incidence of ana I/telo I oocytes showing chromosome bridges

Treatment	No. of females used	No. of oocytes collected	Meiotic stages (%)				ana-I/telo-I oocytes showing bridges (% ana-I/telo-I)
			M I	ana-I	telo-I	M II	
control	4	188	1 (0.5)	18 (9.6)	147 (78.2)	22 (11.7)	1 (0.6)
VP-16	5	226	47 (20.8)[a]	81 (35.8)[a]	69 (30.5)[a]	29 (12.8)	50 (33.3)[a]

[a] $P < 0.001$

Figure 1

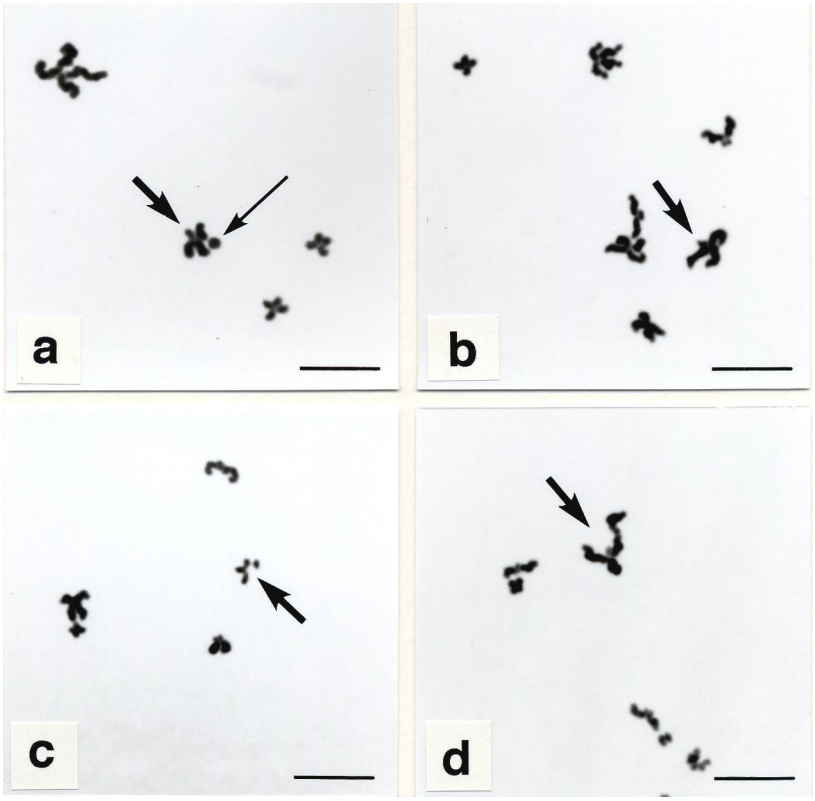

Figure 2

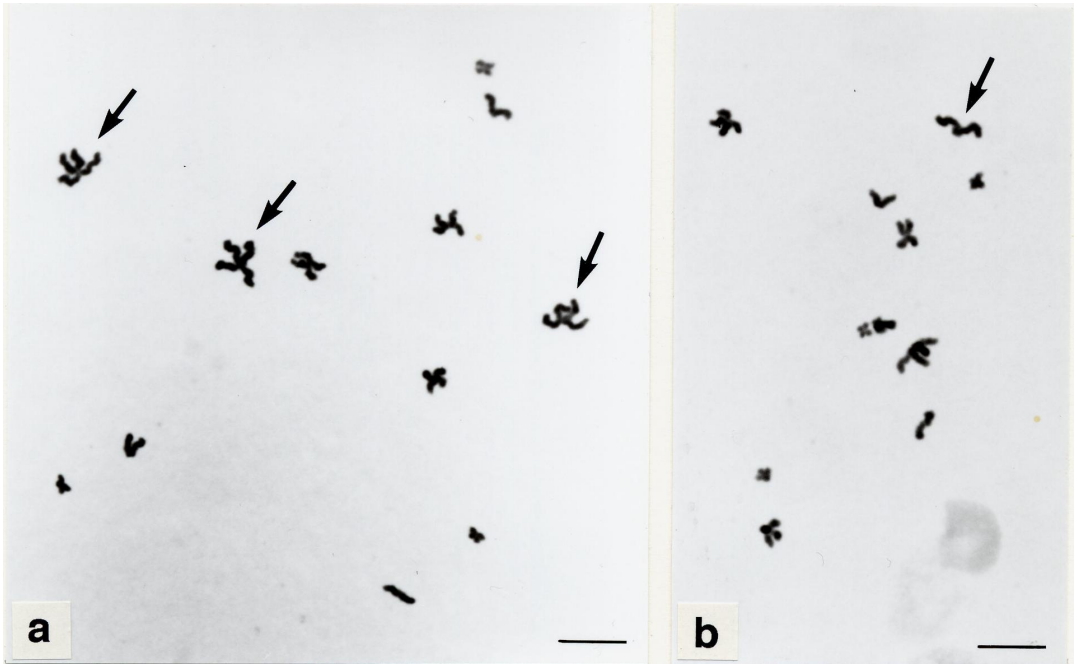

Figure 3

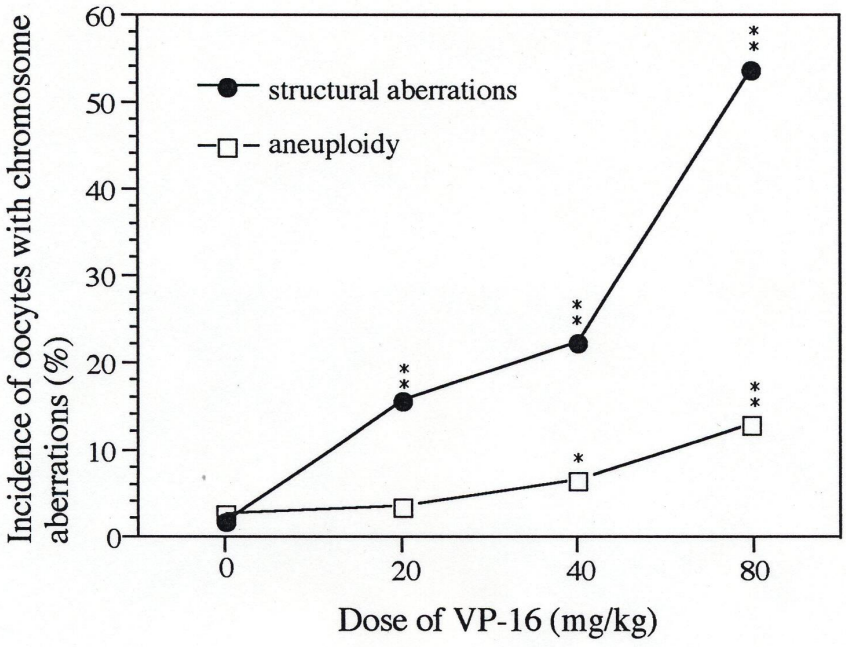
Incidence of oocytes with chromosome aberrations (%)
60
50
40
30
20
10
0
structural aberrations
aneuploidy
**
**
**
**
*
**
0
20
40
80
Dose of VP-16 (mg/kg)

Figure 4

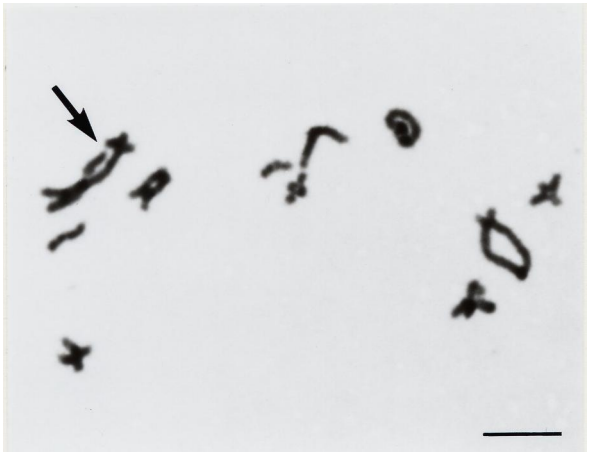

Figure 5