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Cytogenetic effects of inhaled ethylene oxide in somatic and germ cells of mice

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Abstract. The induction of cytogenetic effects by inhalation of ethylene oxide was tested in bone marrow cells and primary spermatocytes at diakinesis-metaphase I cells from mouse after a single treatment (6 h/1 day) at 0, 200, 400 and 600 ppm, and multiple treatment (6 h/5 days/2 weeks) at 0, 200 and 400 ppm. Ethylene oxide induced chromosomal aberrations in both somatic as well as in germ cells of mice.

In the single treatment the response observed for germ cells was not equivalent to that observed for somatic cells. In the latter there was a greater sensibility for bone marrow cells. With multiple treatment the effects on the chromosomes were equivalent in somatic and in germ cells.

Key words: Inhaled ethylene oxide – Cytogenetic effects – Somatic cells – germ cells

Introduction

Ethylene oxide is produced on an industrial scale and has many uses involving wide-spread human exposure. It is used as a fumigant for foodstuffs and textiles, as an agricultural fungicide and in the sterilization of surgical instruments. It is also used in organic synthesis, particularly in the production of ethylene glycol and as starting material for the manufacture of acrylonitrile and non-ionic surfactants (Fishbein 1969).

Ethylene oxide is a mono-epoxide derived from ethylene, an environmental pollutant. According to Ehrenberg and Hussain (1981), the epoxides are relatively efficient inducers of chromosomal aberrations when compared to other alkylating agents. A number of studies have demonstrated ethylene oxide induction of gene mutations and chromosomal aberrations in several non-mammalian systems, and of mutations, sister-chromatid exchanges, unscheduled DNA synthesis, and micronuclei in mammalian in vitro assays and somatic tissues (see reviews by Wolman 1979; Ehrenberg and Hussain 1981). The studies in mammals demonstrated that ethylene oxide induces chromosome aberrations in bone marrow cells of rats (Strekalova 1971; Embree and Hine 1975) and micronucleated cells in bone marrow of rats and mice (Embree and Hine 1975; Appelgren et al. 1978). In the mammalian germline, ethylene oxide has been shown to induce chromosomal breakage and rearrangement as measured by the dominant-lethal test in mice (Appelgren et al. 1977; Cumming and Michaud 1979; Generoso et al. 1980, 1983) and rats (Strekalova et al. 1975; Embree et al. 1977), and by the heritable translocation test in mice (Generoso et al. 1980). Ethylene oxide also induces unscheduled DNA synthesis (UDS), and thus presumably primary DNA damage, in the testis (Cumming and Michaud 1979), and causes direct testicular damage, such as cell killing, and abnormalities in sperm motility and morphology (NIOSH 1982). Ethylene oxide-induced alkylations in the mammalian testis (Ehrenberg et al. 1974; Sega et al. 1981; Cumming et al. 1981a; Sega 1982) and specific locus mutation (Russel et al. 1984) have been measured.

In this study, we evaluated the effects of ethylene oxide in the induction of chromosome aberrations in bone marrow cells and in mouse germ cells (primary spermatocytes at diakinesis-metaphase I) after single or multiple exposure to inhalation. The effects of ethylene oxide in bone marrow and germ cell chromosomes were compared and analysed.

Material and methods

Three-week-old Swiss Webster male mice were obtained from the Instituto Butantan and kept in stainless steel cages until exposure (at the age of 8–10 weeks). Food and water were freely available. Room temperature (22–24 °C) and lighting (12 h on and 12 h off) were controlled. At the beginning of treatment the animals were transferred to special inhalation chambers with no water and food, during the exposure. Ethylene oxide (CAS no. 75-21-8) was obtained from Oxiteno S.A. Indústria e Comércio, Mauá, São Paulo, Brazil, and was specified by the manufacturer to be 99.9% pure. Our gas chromatography analyses confirmed this level of purity. The synthetic gaseous mixtures (800 ppm in nitrogen) were prepared in stainless steel cylinders at the Oxiteno Laboratory and were used by us in order to obtain the desired concentrations.

Animals were exposed in dynamically operated inhalation exposure chambers constructed of thermoplastic material with 0.4 m^3 capacity. Ethylene oxide-air concentrations were established and maintained in the exposure chambers by passing the gas at a controlled rate through a flowmeter in a mixing tube, where it was diluted with filtered room air, and thence into the gassing chamber. A

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100 l/min chamber air flow was maintained throughout the study, with five to eight air changes/h. Temperature and humidity were monitored each hour during the exposures and were maintained at 24±3 °C and 50±10%, respectively.

The ethylene oxide concentrations in the chambers were monitored during operation with a CG 3537 – D gas chromatograph equipped with a flame ionization detector. The readings were taken hourly from each chamber during the exposures and a time weighted average was calculated.

For the single treatment, groups of 20 animals were exposed to 0, 200, 400 and 600 ppm ethylene oxide for 6 h. The animals were killed by cervical dislocation (ten for each concentration) 24 h after the beginning of the exposure and the bone marrow cells were collected for chromosomal preparations. For the cytogenetic analysis in diakinesis-metaphase I, the animals were killed 12 days after the beginning of the exposure which, according to the time sequence established by Oakberg (1956, 1957), represents treatments at the DNA duplication stage. The animals were injected intraperitonially with colchicine (100 mg/kg/body wt.) 2 h before sacrifice. Cytological preparations from bone marrow and testis were obtained, according to the techniques described respectively, by Hsu and Patton (1969) modified by Zambrano et al. (1982) and Evans et al. (1964). One hundred cells were analysed from either the bone marrow or testis of each animal. The metaphases of the bone marrow cells were evaluated for chromatid and chromosome type aberrations, while in the diakinesis-metaphase I breaks, fragments, interchanges and univalents were take into account in the analysis.

In the multiple treatment, groups of ten animals were exposed to 0, 200 and 400 ppm ethylene oxide during 6 h/5 days/2 weeks; in total, 12 days from the beginning to the end of the treatment. The animals were killed 24 h after the end of treatment and using the same technique, bone marrow and germ cell chromosomes were obtained from each animal. The frequency of chromosome aberrations in bone marrow and germ cells was evaluated in both control and treated groups. The results were tested with the Proportional test, based on approximation of the binomial to the Poisson distribution, due to the small number of cells with aberrations in relation to the total number of cells (Chakravarti et al. 1967).

Results

Table 1 shows the frequency of bone marrow cells with chromosome aberrations both in control and treated groups. After a single treatment with an atmosphere of 200 ppm ethylene oxide, there were no significant differences between treated and control groups. However, the differences were significant when animals were exposed to 400 and 600 ppm, not only for total number of cells with aberrations but also for the separated frequencies of cells with chromatid gaps, chromatid breaks and fragments.

Table 2 shows the frequency of chromosome aberrations in diakinesis-metaphase I both in treated and control groups after a single 6 h treatment with ethylene oxide. As for the somatic cells, in meiosis there was no significant difference when the 200 ppm treated group and the control were compared. At 400 and 600 ppm concentrations, there was a significant increase only for autosomal and X-Y univalents.

Table 3 shows the frequency of chromosome aberrations in bone marrow in both treated and control groups after multiple treatment with ethylene oxide. Comparative analysis of treated and control groups shows a significant increase in chromatid gaps, chromatid breaks and chromatid fragments when the animals were exposed to 200 and 400 ppm. Table 4 shows an increase in the frequncy of chromosome aberration and univalents in germ cells after multiple treatment with 200 and 400 ppm.

The aberrations observed in metaphases of bone marrow were gaps, breaks and chromatid fragments. Metaphases with interchanges resulting from translocations were not found. Each cell showed only one aberration, which the exception of one cell that showed two gaps computed with the cells with only one gap. In diakinesis-metaphase I analysis, the observed aberrations were: chromatid and isochromatid breaks, chromatid and isochromatid fragments and X-Y univalents. Each meiotic cell considered showed only one aberration and interchanges resulting from translocations were not observed.

Discussion

Mutations in germ cells are very important and relevant, since they are the only way in which damage induced in the DNA can be transmitted to the next generations. The analysis of meiotic cells in diakinesis-metaphase I showed that ethylene oxide induced chromosome aberrations in mouse germ cells exposed to the inhalation gas mixtures. However, their frequencies for somatic and germ cells were not equivalent.

For the single treatment, the induction of structural chromosome aberration in germ cells is not equivalent to that observed for somatic cells, since the sensitivity was

Table 1. Frequency of cells with aberrations in bone marrow of mice after single[a] treatment with ethylene oxide (E.O.)

Concentration E.O.	No. of mice used	No. of cells scored	Number of cells with			
			Gap (%)	Break (%)	Fragment (%)	Total (%)
0 ppm	10	1000	5 (0.5)	1 (0.1)	3 (0.3)	9 (0.9)
200 ppm	10	1000	4 (0.4)	6 (0.6)	4 (0.4)	14 (1.4)
400 ppm	10	1000	14 (1.4)*	9 (0.9)*	17 (1.7)**	40 (4.0)**
600 ppm	10	1000	17 (1.7)**	15 (1.5)**	19 (1.9)**	51 (5.1)**

[a] The mice received a single treatment for 6 h

* Significant at 5% level

** Significant at 1% level

Table 2. Frequency of cells with aberrations in diakinesis-metaphase I of mice after single[a] treatment with ethylene oxide (E.O.)

Concentration E.O.	No. of mice used	No. of cells scored	Number of cells with				
			Break (%)	Fragment (%)	Autosome univalent (%)	X–Y univalent (%)	Total (%)
0 ppm	10	1000	1 (0.1)	2 (0.2)	6 (0.6)	30 (3.0)	39 (3.9)
200 ppm	10	1000	1 (0.1)	–	9 (0.9)	31 (3.1)	41 (4.1)
400 ppm	10	1000	5 (0.5)	4 (0.4)	20 (2.0)**	78 (7.8)**	107 (10.7)**
600 ppm	10	1000	4 (0.4)	3 (0.3)	17 (1.7)*	71 (7.1)**	95 (9.5)**

[a] The mice received a single treatment for 6 h
* Significant at 5% level
** Significant at 1% level

Table 3. Frequency of cells with aberrations in bone marrow of mice after multiple[a] treatment with ethylene oxide (E.O.)

Concentration E.O.	No. of mice used	No. of cells scored	Number of cells with			
			Gap (%)	Break (%)	Fragment (%)	Total (%)
0 ppm	10	1000	3 (0.3)	–	1 (0.1)	4 (0.4)
200 ppm	10	1000	19 (1.9)**	14 (1.4)**	23 (2.3)**	56 (5.6)**
400 ppm	10	1000	35 (3.5)**	29 (2.9)**	16 (1.6)**	80 (8.0)**

[a] The mice were treated for 6 h/5 days/2 weeks
** Significant at 1% level

Table 4. Frequency of cells with aberrations in diakinesis-metaphase I of mice after multiple[a] treatment with ethylene oxide (E.O.)

Concentration E.O.	No. of mice used	No. of cells scored	Number of cells with				
			Break (%)	Fragment (%)	Autosome univalent (%)	X–Y univalent (%)	Total (%)
0 ppm	10	1000	–	–	5 (0.5)	29 (2.9)	34 (3.4)
200 ppm	10	1000	5 (0.5)*	7 (0.7)**	30 (3.0)**	59 (5.9)**	101 (10.1)**
400 ppm	10	1000	17[b] (1.7)**	11[c] (1.1)**	48 (4.8)**	85 (8.5)**	161 (16.1)**

[a] The mice were treated for 6 h/5 days/2 weeks
[b] 0.5 (5%) of these cells showed aberrations of chromosome type
[c] 0.3 (3%) of these cells showed aberrations of chromosome type
* Significant at 5% level
** Significant at 1% level

higher for bone marrow cells. But for multiple treatment, in the same animal, there seems to be a good correlation between the effects observed for somatic and germ cells.

The different responses of bone marrow and germ cells after a single treatment with ethylene oxide, at 400 and 600 ppm, may be explained on the basis of the toxokinetics and toxodynamics of this chemical. A single exposure may have led to a very quick detoxification, which, in association with a probable reduction in the passage of the substance through the testis hystological barrier, would result in less active substance reaching the germ cells. As observed by Ehrenberg et al. (1974), inhaled ethylene oxide has a rapid distribution to all organs, and a rapid detoxification and excretion. On the other hand, since ethylene oxide-induced alkylations in the mouse testis have been studied, two studies have demonstrated that after exposure of animal to tritium labelled ethylene oxide, the radioactivity (Ehrenberg et al. 1974) and the alkylation of DNA (Cumming et al. 1981b) was very low in testis compared to other tissues.

The increase in the frequency of cells with autosomal univalents and X-Y univalents suggests that even a short-term exposure (400 or 600 ppm for 6 h) may interfere with the pairing of the chromosomes. This probably occurs because of the interaction between this substance and the proteins that form the synaptonemic complex. The occurrence of univalents induced by ethylene oxide indicate the possibility that this substance induces non-dysjunction.

In bone marrow, the higher frequency observed in our study for total cells with aberrations was 8.0% (Table 3) in mice exposed to 400 ppm of ethylene oxide for 6 h/5 days/2 weeks. However, Embree and Hine (1975) related an increase in total aberrations in bone marrow samples from 7/120 cells in the control group to 101/120 cells of rats exposed to 250 ppm ethylene oxide for 7 h/day for 3 days. The discrepancy between our results and those of Embree and Hine (1975) could be due to differences in experimental condidions such as species animal, exposition mode, ethylene oxide purity and exposition time.

The majority of aberrations observed in diakinesis-metaphase I were of the chromatid type (break or chromatid fragments), although a smaller proportion of cells showed aberrations of the chromosome type (break or fragments) (see Table 4). The observation of chromosomic aberrations argues against the assumption established for somatic cells in which aberrations induced by alkylating agents are S-dependent, and that the resulting aberrations are of the chromatid type. As ethylene oxide is an alkylating agent and induces mainly aberrations of the chromatid type, our suggestion is that it may also induce chromosome aberrations, depending on the stage and the type of cell treated. The mechanism by which the chemically-induced damage in the germ cell chromosomes are converted into aberrations may depend on many factors: the stage of the treated germ cells, repair mechanism of the germ cell and the type of the chemical used. Several investigators (Embree et al. 1977; Cumming and Michaud 1979; Generoso et al. 1980; Generoso et al. 1983) reported that ethylene oxide induced dominant lethal mutations in rats and mice after postmeiotic germ cells treatment. This fact supports the idea that ethylene oxide may also induce chromosome aberrations.

Our results in mammalian germ cells in association with data reported by other authors show the potential risks of the ethylene oxide to human populations and may represent a useful contribution in further studies of occupational exposed men.

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