Arch Toxicol (1987) 60: 331–333

Archives of
Toxicology

Short communication

Activity of ethylene oxide in the mouse sperm morphology test

Lucia Regina Ribeiro[1], Daisy Maria Fávero Salvadori[2], Carlos Alberto Bragança Pereira[3], and Willy Beçak[2]

[1] Laboratório de Toxicologia e Genética Toxicológica, Departamento de Patologia e Clínicas, Escola de Medicina Veterinária, Universidade Federal da Bahia, Av. Ademar de Barros, 500, Ondina, 40000 – Salvador, Bahia, Brazil
[2] Serviço de Genética, Instituto Butantan, São Paulo, Brazil
[3] Instituto de Matemática e Estatística da Universidade de São Paulo, São Paulo, Brazil

Abstract. Inhaled ethylene oxide induced an increased frequency of abnormal sperm cells in mice when the animals were treated at 200 and 400 ppm (6 h per day; 5 days) in three stages of cell development: spermatozoa, spermatid and preleptotene spermatogonial cells. These results suggest that sperm head morphology changes can be induced by interference of ethylene oxide with spermatogenesis, which, depending on the stage of the treated germ cells, may be correlated with the mutagenic potential of this chemical agent.

Key words: Ethylene oxide – Mouse sperm – Morphology test

Introduction

Recent studies indicate that in mice a certain percentage of sperm cells shows abnormal morphology. The frequency of such abnormalities varies among different strains but exhibits a constant pattern within a particular strain. Wyrobek and Bruce (1975) proposed that studies on sperm abnormalities might be used as an "in vivo" test for mutagenic potential in mammals. The mouse-sperm morphology test has been developed to study the effects of mutagens on male germ cells and it can also be used to compare the sensitivity of the different germ-cell stages (Wyrobek and Bruce 1975, 1978). Despite the great number of papers published in this field (see review by Wyrobek et al. 1983), evaluations of the effects of chemical agents in different stages of spermatogenesis are still insufficient.

This study was developed to evaluate the effects of ethylene oxide inhalation in the incidence of abnormal sperm cells in mice. The animals were killed at 1, 3 and 5 weeks after the beginning of exposure in order to verify which stages of spermatogenesis were the most sensitive to the agent, as well as the persistence of damage induced.

Material and methods

Three-week old Swiss Webster male mice obtained from the Instituto Butantan were maintained until the age of 11–15 weeks old at room temperature (22–24 °C) with food and water freely available and in a 12 h dark: 12 h light photophase. For exposure the animals were transferred to special inhalation chambers and maintained without water and food during the treatment. Ethylene oxide (CAS no. 75-21-8) was obtained from Oxiteno S. A. Indústria e Comércio, Mauá, São Paulo, Brasil, and specified by the manufacturer to be 99.9% pure. Our gas-chromatography analyses confirmed this level of purity. The synthetic gaseous mixtures (500 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 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 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.

Groups of ten animals were exposed during 5 days, in the inhalation chamber to an atmosphere of gaseous mixture containing 0, 200 and 400 ppm ethylene oxide. Groups of five animals, used as positive control, received daily, and during 5 days, an intraperitoneal injection of 100 mg/kg body wt. of cyclophosphamide (Enduxan-Pravaz) dissolved in 0.9% NaCl. The groups of treated animals, negative control and positive control were killed, by cervical dislocation, 1,3 and 5 weeks after the beginning of the exposures, representing treatments of spermatozoa, spermatid and preleptotene spermatogonial cells, respectively, in accordance with the schedule established by Oakberg (1956, 1957) and Goldberg et al. (1977). A modification of the technique of Wyrobek and Bruce (1975) was used in the preparation of the sperm samples. Cauda epididymides were removed and minced in 1 ml isotonic NaCl solution. The preparations were flushing through a Pasteur pipette about 15 times and left 15 min at room temperature, allowing the tissue fragments to settle at the bottom of the tube. Supernatant was transferred to another tube where 0.1 ml 1% Eosin Y aqueous solution was

Offprint requests to: Lúcia Regina Ribeiro

Table 1. Frequency of sperm head abnormalities after treatment[a] with ethylene oxide (E.O.) and cyclophosphamide (CPA) at differents stages of spermatogenesis

Groups	Treatment		Sacrifice, weeks after the treatment	Population of the treated cells	No. of mice	No. of cells scored	Sperm abnormalities[b] %
1	E.O.	0 ppm	1	Spermatozoa	10	10.000	1.76 ± 0.5
		200 ppm	1		10	10.000	3.02 ± 0.5**
		400 ppm	1		10	10.000	3.95 ± 0.6**
	CPA[c]	100 mg	1		5	5.000	3.12 ± 0.7**
2	E.O.	0 ppm	3	Spermatid	10	10.000	1.62 ± 0.4
		200 ppm	3		10	10.000	3.62 ± 0.6**
		400 ppm	3		10	10.000	5.81 ± 1.5**
	CPA	100 mg	3		5	5.000	2.60 ± 0.8**
3	E.O.	0 ppm	5	Spermatogonial	10	10.000	1.32 ± 0.4
		200 ppm	5	cells in	10	10.000	2.32 ± 0.5**
		400 ppm	5	preleptotene	10	10.000	5.54 ± 1.4**
	CPA	100 mg	5		4	4.000	10.40 ± 1.6**

[a] The mice were treated daily for 6 h/5 days
[b] Mean ± standard deviation
[c] Cyclophosphamide – the mice received daily an intraperitoneal injection (100 mg/kg body wt.) during 5 consecutive days
** significant at 1% level

added. After 30 min, smears were prepared on clean slides. The preparations were air dried and mounted with Permount. The slides were coded and examined in a blind test. One thousand sperms from each animal were analysed under the light microscope with a green filter. All preparations were examined by the same scorer and using criteria as close as possible to those established by Wyrobek and Bruce (1975). Only the head morphology was examined. Abnormal sperm had forms readily recognizable as slightly distorted, narrow, triangular, without hook, peaked and filamentous. For statistical evaluation we used the Proportional test for comparisons between ratios based upon the approximation of the binomial distribution to the Poisson distribution of those values of treated and control groups (Chakravarti et al. 1967). This test was used due to the small number of abnormal sperms in relation to the total number of cells examined.

Results and discussion

Table 1 shows the frequency of abnormal sperm in mice after treatment with inhaled ethylene oxide. These data clearly show that ethylene oxide produces a statistically significant ($p < 0.01$) increase in the percentage of abnormal sperms in the three different populations of cells: spermatozoa, spermatid and spermatogonia in preleptotene. Similar findings were obtained (Table 1) when we analysed the effects of cyclophosphamide, the positive control, during the three different stages of the spermatogenesis.

The results arising from the sperm head morphology analysis presented in this study indicate that ethylene oxide is a potent inducer of alteration in the morphology of this cell. A highly significant increase in the frequency of abnormal sperms took place for the three stages of germinal cell treatment. The significant results found in the group of mice, exposed 3 weeks before being killed can be explained by ethylene oxide action on the development of spermatogenic cell. In accordance with Oakberg (1956, 1957) and Goldberg et al. (1977), this timing corresponds to the exposure during the period of spermatid differentiation; hence it is possible that the process of differentiation of sperm head morphology was directly affected by ethylene oxide interference in the expression of already existing genic products. Similarly, the significant results found in the group of mice exposed 1 week before being killed, representing treatment in the spermatozoa cell stage, may be explained by the action of ethylene oxide in the development of young sperms that had not completed the process of differentiation. Sperm changes as a result of treatment in the spermatogonia in preleptotene stage (5 weeks before sacrifice) must have been induced by interference of ethylene oxide in this stage of spermatogenesis, and may be correlated with the mutagenic potential of this chemical agent.

The results of this study that the sperm head morphology test in mice can be useful in detecting chemical agents that induce spermatogenic alterations and mutagenic potential agents. Moreover, further investigations with different agents are needed to assess precisely the treatment-related effects using chemical agents in different stages of the germinal cell.

Acknowledgements. This research was supported by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Financiadora de Estudos e Projetos (FINEP) e Oxiteno S. A. Indústria e Comércio – Brazil. The authors thank Mr. H. J. Targa for critical reading of the manuscript.

References

Chakravarti IM, Laha RG, Roy JR (1967) Handbook of methods of applied statistics. Vol. I, John Wiley, New York

Goldberg RB, Geremia R, Bruce WR (1977) Histone synthesis and replacement during spermatogenesis in the mouse. Differentiation 7: 167–180

Oakberg EF (1956) Duration of spermatogenesis in the mouse and timing of stages of the cycle of the seminiferous epithelium. Am J Anat 99: 507–516

Oakberg EF (1957) Duration of spermatogenesis in the mouse. Nature (Lond) 180: 1137–1138

Wyrobek AJ, Bruce WR (1975) Chemical induction of sperm abnormalities in mice. Proc Natl Acad Sci USA 72: 4425–4429

Wyrobek AJ, Bruce WR (1978) The induction of sperm-shape abnormalities in mice. In: Hollaender A, de Serres FJ (eds) Chemical mutagens – principles and methods for their detection, Vol. 5. Plenum, New York, pp 257–285

Wyrobek AJ, Gordon LA, Burkhart JG, Francis MW, Kapp RW Jr, Letz G, Malling HV, Topham JC, Wharton DM (1983) An evaluation of the mouse sperm morphology test and other sperm tests in non-human mammals: A report of the US EPA Gene-Tox-Program, Mutat Res 115: 1–72

Received April 28, 1986/Accepted August 25, 1986