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Cytotoxicity of ethyl methanesulfonate in mice spermatogonia

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Summary. Enumeration of different types of spermatogonia, following a single i.p. administration of different doses of ethyl methanesulfonate in mice, showed that survival of A_1–A_4 and in spermatogonia is markedly reduced due to cell killing while the remaining types of spermatogonia were affected marginally. The cell killing effect was dose-dependent, and replenishment of these cells was observed by the end of one cycle of the seminiferous epithelium comprising of 8.5 days.

According to the latest concept of Oakberg[1,2] and Huckins[3], the stem cell spermatogonia duplicate in succession to produce different types of spermatogonia in the seminiferous epithelium of mice testes. Radiation, as well as a number of mutagenic and/or carcinogenic chemicals, have been reported to be cytoxic to these cells[4–7]. In the present investigation, effect of ethyl methanesulfonate, an alkylating agent, mutagenic in the germ cells of mouse[8–10] and rat[11] was evaluated in relation to the specific spermatogonial cell killing.

Material and methods. Adult Swiss mice were given a single i.p. injection of different doses (100, 200, 300 and 400 mg/kg b.wt) of ethyl methanesulfonate (EMS) in normal saline and the control group received 0.5 ml of the vehicle. The mice were killed at intervals of 1, 3, 5 and 8.5 days post treatment. Deparaffinized sections of testes, fixed in Zenker formal, were stained with periodic acid-Schiff reagent and counterstained with hematoxylin. Differentiating spermatogonia [A_1–A_4, intermediate (In) and B] including stem cell (As) spermatogonia and preleptotene spermatocytes (Pl) were enumerated from comparable samples of tubules of seminiferous epithelium in control and treated mice. Results have been expressed as experimental/control ratio[1,2].

Effect of ethyl methanesulfonate on various types of mice spermatogonia

Days after EMS injection	Types of spermatogonia* A_S	A_1	A_2–A_4	In	B	Pl
Control	51	41	162	102	234	395
Dose 100 mg/kg b.wt						
1	39 (0.77)	24 (0.59)	142 (0.88)	93 (0.91)	197 (0.84)	317 (0.80)
3	47 (0.92)	30 (0.73)	118 (0.73)	73 (0.72)	179 (0.77)	370 (0.94)
5	47 (0.92)	21 (0.51)	91 (0.56)	76 (0.75)	178 (0.76)	308 (0.78)
8.5	49 (0.96)	25 (0.61)	122 (0.76)	75 (0.74)	174 (0.74)	389 (0.99)
Dose 200 mg/kg b.wt						
1	38 (0.80)	35 (0.86)	158 (0.98)	101 (0.99)	196 (0.84)	390 (0.99)
3	38 (0.80)	27 (0.66)	133 (0.82)	68 (0.67)	191 (0.82)	380 (0.96)
5	39 (0.76)	30 (0.73)	139 (0.86)	76 (0.75)	213 (0.91)	386 (0.98)
8.5	54 (1.06)	27 (0.66)	93 (0.57)	64 (0.63)	126 (0.54)	384 (0.97)
Dose 300 mg/kg b.wt						
1	35 (0.69)	19 (0.46)	46 (0.28)	38 (0.37)	80 (0.34)	352 (0.89)
3	25 (0.49)	10 (0.24)	21 (0.13)	12 (0.12)	12 (0.05)	28 (0.07)
5	25 (0.49)	19 (0.46)	20 (0.12)	11 (0.11)	30 (0.13)	96 (0.24)
8.5	41 (0.80)	22 (0.53)	95 (0.59)	86 (0.84)	187 (0.80)	384 (0.97)
Dose 400 mg/kg b.wt						
1	27 (0.53)	15 (0.37)	38 (0.24)	37 (0.36)	84 (0.36)	352 (0.89)
3	31 (0.61)	11 (0.27)	17 (0.11)	1 (0.01)	2 (0.01)	4 (0.01)
5	32 (0.63)	21 (0.51)	23 (0.14)	6 (0.06)	15 (0.06)	2 (0.01)
8.5	52 (1.02)	18 (0.44)	107 (0.66)	86 (0.84)	173 (0.74)	352 (0.89)

*Spermatogonia type A_S includes A-pair and A-align, counted from seminiferous tubular stages 1 through 12; A_1 spermatogonia from stage 1 to 9; A_2 to A_4 type spermatogonia from stage 10 through 2; In (intermediate) in stage 3 and 4; B type spermatogonia in stage 5 and 6 and Pl (preleptotene spermatocytes) in stage 7 only. From each mouse various types of spermatogonia were counted from 99 tubular cross sections. Each set of results is the average number of cells counted from at least 2 mice and also expressed as mean experimental/control ratios (values shown within parenthesis).

Results and discussion. The data showing the effect of EMS on different types of spermatogonia are given in the table. At the dose of 100 mg/kg, only a small fraction of all A types, In and B spermatogonia were reduced by 3 days after EMS administration. At higher doses (300 and 400 mg/kg) all types of spermatogonia were affected within 24 h with maximum killing of A_1–A_4 cells. Highly profound depletion (96%) in Pl cells, 3 days after administration of 300 and 400 mg/kg, reflected killing of a large fraction of A_4 and In spermatogonia. Similarly 97% decrease in the number of B cells, 3 days after treatment with the same doses indicates damage of late A_3 and early A_4 cells, since spermatogenesis is a dynamic process, the cell-types scored are different from the cell types treated. Although EMS was found to affect most of the types of differentiating spermatogonea, all the cells are not uniformly killed.

EMS increases the incidence of dominant lethals in the post meiotic phase of spermatogenesis largely affecting the spermatids and the sperms[8-11]. EMS has also recently been reported to induce in vivo somatic mutation in mice at relatively lower dosage levels of 50 and 100 mg/kg[12]. However, 300 mg/kg of EMS has failed to increase the mutation frequency in the specific locus test in the mouse spermatogonia[13]. The present study shows that EMS is capable of inflicting biological damage in the premeiotic phase and affects the various spermatogonial cells in a differential manner.

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Comparative studies on the covalent binding of the carcinogen benzo(a)pyrene to DNA in various model systems[1]

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Summary. The covalent binding of tritiated benzo(a)pyrene (BP) to DNA has been determined in rat liver in vivo, in rat liver perfused in situ, after incubation of BP with liver single cells, with liver homogenate, with liver microsomes and DNA, with fibroblasts from a rat granuloma pouch, and with 2 cell lines. Liver single cells were found to be a valuable compromise between the most sensitive system (microsomal incubation of BP with DNA) and the biologically most relevant system (in vivo).

It is now widely accepted that the first step in the chemical induction of a tumour involves binding of the chemical or one of its metabolites to a biological macromolecule[3]. Good correlations between that binding and carcinogenicity have been found particularly with DNA as target molecules[4]. Before such a binding can occur most substances have first to be converted by enzymatic action to reactive metabolites[5]. Only a very small fraction reacts with nucleic acids so that this type of binding can normally be measured only by using radiolabelled chemicals. Because of the cost of radioactive compounds and the limited availability of high specific activities, the yield of chemical bound to DNA as a fraction of the total amount of chemical used should be optimized so that a radioactivity on DNA can be measured from a minimal dose administered.

We have measured the binding of a standard carcinogen (tritiated benzo(a)pyrene, BP) to DNA in various model systems and have calculated the specific binding of BP per mg DNA as well as the radioactivity which can be recovered in a scintillation vial from the total DNA isolated. This comparison will help to choose the most appropriate model system for measuring the binding of other suspected carcinogens to DNA.

Materials and methods. Chemicals. Inactive BP was obtained from Fluka AG, Buchs, Switzerland. Generally tritiated BP (purified on silica-gel before use) was from the Radiochemical Centre, Amersham, England, 36 Ci/mmole. NADP, glucose-6-phosphate and glucose-6-phosphate-dehydrogenase were from Boehringer, Mannheim, GFR. The other reagents used were of the purest grade available from Merck, Darmstadt, GFR.

Isolation of DNA. DNA was isolated according to Markov and Ivanov[6] with the modifications described before[7].

Rat liver, in vivo. 0.5 ml of a BP solution in oil was given to male rats (SIV 50, Sprague-Dawley derived, about 300 g) by i.p. injection (4 mg, 10^9 dpm). After 50 h, the liver was excised and DNA was isolated.

Liver perfusion in situ. Female rats (250 g) were given 60 mg/kg pentobarbitone and the liver was perfused in situ for 2 h by cannulation of the portal vein (in) and the thoracic portion of the vena cava (out). Tritiated BP (0.6 µg, $1.83 \cdot 10^8$ dpm) was deposited on the bottom of a 25 ml conical flask by evaporating the hexane from the stock solution under a stream of nitrogen and 20 ml of perfusion buffer (0.8% NaCl, 0.04% KCl, 0.005% Na_2HPO_4, 0.006% KH_2PO_4, pH 7.6 with NaOH) was added. Oxygen was supplied by keeping the perfusion buffer reservoir at 37 °C under oxygen. The blood present in the liver at the beginning of the perfusion was included in the closed circuit in order to solubilize the BP.

Liver single cells. Single cells were prepared after perfusion of a rat liver with a solution of collagenase[8]. BP (0.73 µg, $2.05 \cdot 10^8$ dpm) was added to a 25 ml conical flask as described above and the cells were incubated for 3 h at 37 °C in Hanks' solution.

Liver homogenate. Rat liver was treated in a loose-fitting Potter-Elvehjem homogenizer with a teflon pestle in 3 vol. of buffer (0.25 M sucrose, 0.01 M Tris/HCl, pH 7.4). The