EXPERIMENTAL GENETICS

EFFECT OF ANTENATAL ALCOHOLIC INTOXICATION ON MALE GERM CELL DEVELOPMENT IN RATS

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Clinical observations and experimental studies have shown that alcohol disturbs reproductive processes in man and animals In male chronic alcoholics, besides a hormonal imbalance, aplasia of the germ cells, atrophy of the seminiferous tubules with peritubular fibrosis and concentration of the Leydig's cells around the seminiferous tubules [11] and also destructive changes in the spermatogenic epithelium [8] are observed. Pathological changes in the testes have been shown to depend on the dose of alcohol and the time of exposure to it [12]. The present writers regard alcohol abuse as one of the main etiologic factors in the development of azoospermia, and suggest that the latter be qualified as "alcoholic azoospermia." Meanwhile there is virtually no information on the effect of alcohol ("a poison of everyday life") during intrauterine development on spermatogenesis in the fetus and sexually mature offspring.

The aim of this investigation was to determine the degree of disturbance of male germ cell development in the offspring of animals exposed to alcohol in the antenatal period of development, by adopting a system of quantitative criteria to evaluate the gametotoxic effect.

EXPERIMENTAL METHOD

To create a model of a state of alcoholic intoxication in pregnant noninbred rats, unaccompanied by the development of a fetal alcohol syndrome (and thus ensuring that the fetus was exposed to a sufficiently mild degree of alcoholic poisoning, throughout pregnancy (1st-20th days) the mother rats received a 20% solution of ethanol per os in a dose of 0.4 mg/kg body weight. Control animals received distilled water during the same period. The experimental investigation was carried out on testes of 21-day-old fetuses and the sexually mature offspring aged 2 months. The gonads were fixed in Bouin's solution and subjected to the usual histologic treatment. Serial sections 5 μ thick were stained with Ehrlich's hematoxylin and eosin. Altogether 250-400 germ cells were counted in the testes of the 21-day-old rat fetuses every 5-10 sections, and the following parameters were calculated: 1) the fraction of germ cells of the total number of generative (prospermatogonia) and somatic (Sertoli cells) cells in the seminiferous tubules, in percent; 2) the fraction of degenerative germ cells relative to the total number of germ cells analyzed, in percent; 3) the mean diameter (in μ) and volume (in μ^3) of the nucleus of the germ cells [7]. The gametotoxic effect of ethanol in the nature offspring was investigated by quantitative karyologic analysis of generations of germ cells at stage VII of the cycle of the spermatogenic epithelium (CSE) [5, 9] and by quantitative analysis of cells in maturation divisions (MD), suggested by the present writers, using the classification of pathology of mitosis in [7, 8]. The following parameters were chosen as the most informative criteria of disturbance of the normal course of meiosis: 1) the number of pathological MD as a fraction of the total number of cells analyzed in MD, in percent; 2) the number of metaphases with scattering and deletion of the chromosomes in metakinesis as a fraction of the total number of cells analyzed in pathological MD, in percent; 3) the number of anaphases and telophases with deletion of chromosomes

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TABLE 1. Cytophysiological Characteristics of State of Germ Cells in Testes of 21-Day Rat Fetuses after Chronic Exposure to Ethanol during 1st-20th Days of Pregnancy

Group of animals	Total number prospermatogonia counted	Number of fetuses	Number of prospermatogonia as per cent of total number of cells counted in seminiferous tubules	Number of degenerative prospermatogonia, as per cent of total number of prospermatogonia	Mean diameter of nuclei of prospermatogonia, μm	Volme of nuclei of propspermatogonia, μm^3
Control	2001	5	15,3±0,5	0,7±0,2	8,1±0,1	271,3±12,0
Experiment	2034	5	16,9±1,2	0,8±0,1	8,9±0,1 $p<0,001$	362,9±12,2 $p<0,001$

TABLE 2. Dynamics of Spermatogenesis in Sexually Mature Rats after Antenatal Exposure to Ethanol during 1st-20th Days of Pregnancy

Type of cells	Control	Experiment
Type A spermatogonia	7,75±0,95	5,69±0,65
Spermatocytes at preleptotene stage	204,91±3,64	202,18±4,36
Spermatids at pachytene stage	241,90±5,19	243,54±4,90
Spermatids at 7th stage of development	694,48±12,27	621,21±15,1 $p<0,001$

TABLE 3. Pathology of Maturation Divisions of Male Germ Cells of Rats after Antenatal Exposure to Ethanol in a Dose of 0.4 mg/kg during 1st-20th Days of Pregnancy

Group of animals	Number of testes	Total number of cells counted in MD	Number of pathological MD as per cent of total number of cells in MD	Fraction of number of cells in pathological MD, %			
				metaphase I and II with deletion of chromosomes	metaphase I and II with scattering of chromosomes	anaphase and telophase I and II with deletion of chromosomes	anaphase and telophase I and II with bridge formation
Control	4	1681	53,7±1,4	88,7±1,9	2,0±0,4	5,7±1,2	3,6±1,1
Experiment	5	2155	63,2±2,9 $p<0,05$	89,9±1,0	2,2±0,3	5,8±0,7	2,2±0,5

and with bridge formation as a fraction of the total number of cells analyzed in pathological MD, in percent. All the quantitative data were subjected to statistical analysis by the Student–Fisher test, with a level of significance of 0.05.

EXPERIMENTAL RESULTS

After chronic exposure to alcohol throughout pregnancy, the population of male germ cells remained unchanged in number until the end of the antenatal period of development (Table 1). In our previous studies of the effect of antenatal alcohol consumption on oogenesis [9, 10] a very small decrease in the number of oocytes also was found in the rat fetuses toward the end of the period of intrauterine life, possible evidence that male and female gametes are equally sensitive to the action of alcohol at this stage of their development. The gametotoxic effect of alcohol during prespermatogenesis was manifested only as a significant increase in volume of the nucleus of the T1-prospermatogonia compared with the control ($p < 0.001$), possibly due to its action on the cell membranes [11, 12].

Quantitative karyologic analysis of passage of the germ cells of the sexually mature offspring through the stages of meiosis and differentiation after antenatal exposure to ethanol revealed a significant decrease in the number of stage 7 spermatids ($p < 0.001$, Table 2). Whereas the dynamics of spermatogenesis was preserved in the earlier

stages, the observed effect of a decrease in the number of spermatids may evidently be the result of elimination of degenerating spermatocytes during the period of two maturation divisions and of spermatids in stages 1-7 of development.

Quantitative analysis of cells in maturation divisions revealed a significant increase in the index of pathological maturation divisions after antenatal exposure to ethanol despite a sufficiently high level of pathology in metaphase I and II and in anaphase and telophase I and II in the control group (Table 3). The greatest contribution to the structure of pathology was provided by spermatocytes with deletion of chromosomes in metakinesis. In male rats of the control group the fraction of metaphases with delay and scattering of chromosomes was 55.1% of the total number of spermatocytes in metaphase. In the experimental group of animals, this fraction was significantly larger (65.1%, $p < 0.05$). The higher level of pathology in the 1st and 2nd maturation divisions, reflecting the degree of damage to the chromosomes and/or disorganization of the division spindle may be the cause of the appearance of chromosomal mutations in the germ cells and, consequently, the cause of several chromosome diseases.

The results of these investigations thus demonstrate that the damaging effect of alcohol on male germ cells as a result of antenatal exposure is prolonged in character and is manifested in the final stages of spermatogenesis.

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