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PYRAZINAMIDE POTENTIAL EFFECTS ON MALE RATS DNA FRAGMENTATION, BONE TYPE I COLLAGEN AMINO ACID COMPOSITION, REPRODUCTIVE CAPABILITY AND POSTERITY ANTENATAL AND POSTNATAL DEVELOPMENT

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Abstract: Current therapeutic regimens with first-line antitubercular agents are associated with a high rate of adverse effects which can lead to therapeutic failure. Understanding the nature and the severity of these effects is important for treatment optimization. The aim of present study was to investigate pyrazinamide potential effects on male rats DNA fragmentation, amino acid composition of bone type I collagen, reproductive capability and their posterity antenatal and postnatal development. Wistar albino male rats (160–200 g b.w.) were divided into three groups: I – received pyrazinamide *per os* at a dose of 1000 mg/kg b.w./day, II – at a dose of 2000 mg/kg b.w./day, in both groups it was given for 60 days; III – control. After 60 days of the experiment, rats of the experimental (groups I and II) and control groups were mated with intact virgin females. The amino acids contents of male rat bone type I collagens were determined using amino acid analyzer; epididymis and testis DNA fragmentation – electrophoretically; posterity antenatal development indices and postnatal development – by standard procedures. The study of pyrazinamide effects (administered in different doses) on males bone type I collagen amino acid contents and testis DNA fragmentation demonstrated the presence of dose-dependent pyrazinamide-mediated quantitative and qualitative changes in male rat reproductive organs DNA and extracellular matrix proteins in comparison with control. Changes in nucleic acids and proteins structure were accompanied by alterations in processes of fertilization (with intact females), embryogenesis and by lowering of posterity survival.

Keywords: pyrazinamide, bone type I collagen, DNA fragmentation, paternal effects

The ongoing progresses in the knowledge of the pathogenic mechanisms of diseases and the availability of innovative technological approaches in pharmaceutic industry have lead to the development of great number of new drugs which add to conventional treatments. Many of these chemicals can interfere with the action of native compounds in organisms and are generally referred to as "metabolism disruptors". Among these compounds, effects of some antitubercular drugs (pyrazinamide) have been correlated with alterations in nucleic acids, amino acids and proteins metabolisms (1, 2).

Thus, providing complex analysis of the chemicals potential effects on genome and proteome are urgently required for developing improved treatment strategies and formulating shorter, more effective, safe regimens for chronic diseases treatment and prevention of drugs adverse effects.

This is especially important for improving first-line antitubercular therapy as current therapeutic regimens are associated with a high rate of adverse effects that can lead to therapeutic failure (3, 4). Understanding the nature and the severity of these adverse effects is very important. At present, it is known that simultaneous and long-term usage of antitubercular drugs often causes various negative effects on amino acids and proteins metabolisms and rates of proteins biosynthesis (5). This allows to suspect potential negative effects on genome and proteome functioning. Previously, we have demonstrated putative changes in rat skin collagen amino acid contents, testis cells morphologic and morphometric parameters and spermatogenesis with using different doses of pyrazinamide (6).

The aim of the present study was to investigate potential effect of pyrazinamide on male rats testis and epididymis DNA fragmentation, bone type I collagen amino acid composition, reproductive capability and their posterity antenatal and early postnatal development.

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EXPERIMENTAL

Wistar albino male and female rats, body weight (b.w.) of 160–200 g, were used in the study. Animals were kept in standard conditions of nutrition, water and light regimes.

The study was carried out according to national and international guidelines and the law on animal protection was observed. All animal studies were performed in accordance with the recommendations of the European Convention for the Protection of Vertebrate Animals Used for Experimental and other Scientific Purposes and approved by the Institutional Animal Care and Use Committee.

In the experiments, pyrazinamide (pyrazine-2-carboxamide) was used in tablets (500 mg of active compound in each) produced by the Borzhagovsky Chemical and Pharmaceutical Enterprise Ukraine (series D.10.00/02271). Male rats were kept in quarantine for 10 days, then they were randomized into experimental and control groups. Experimental groups included 8 males and control group – 6 males. Pyrazinamide suspended in 1% starch gel was given by gavage at a dose of 1000 mg/kg b.w./day (group I) and 2000 mg/kg b.w./day (group II) for 60 days (duration of spermatogenesis process and time of germ cells maturation in epididymis). The control group received only starch gel in corresponding volumes. Choice of doses was based on the previous data available in NIEHS Technical Report on the Reproductive, Developmental, and General Toxicity Studies of Pyrazinamide (http://ntp.niehs.nih.gov/files/AIDSTox011. pdf). Body weight gain, activity, behavior and appearance of the rats were observed throughout the period of pyrazinamide administration (7).

After 60 days of the experiment, males of the experimental (groups I and II) and control groups were mated with intact virgin females with estimated estrus cycle. The mating ratio was 1:2. First day of pregnancy was established by vaginal cytology (first day of spermatozoids detection in vagina).

After successful induction of pregnancy males were sacrificed *via* cervical dislocation under mild diethyl ether narcosis. Their bones, testis and epididymis were used for biochemical assays.

Bone type I collagens were extracted and purified according to Trelstad et al. (8). All procedures were carried out in cold regime (4°C). From bones (20 g) firstly, mineral component was extracted by 100 mL of 3% EDTA (5 days, 4°C). Then, grinded tissue was washed by distilled water (3 times). Protein was extracted by 100 mL of 15% acetic acid and dialyzed against 0.01 M $NaHPO_4$ at 4°C. Proteins fibrils dredges were centrifuged at 6000 × g for 20 min at 4°C. The obtained pellets were dissolved (100 mg of pellets in 10 mL) in 0,1 M acetic acid (pH 2.5). Pepsin (20 mg/g of pellet) was added into this solution and mixtures were left for 3 days in refrigerator at 4°C. After that, for pepsin inactivation, pH in each mixture was neutralized by addition of powdered crystalline Tris (to pH 7.4). Solutions were centrifuged at 35000 × g for 40 min at 4°C. Pellets were discarded and supernatants were used for further collagen types fractionation. Fractionation of pure type I collagen was carried out with NaCl according to (8). Firstly, at 1.5 M NaCl concentration was formed fraction, which contained type III collagen. It was separated by centrifugation – 40000 × g, 40 min, 4°C. Protein fraction from pellets, which was formed at 2.2 M NaCl concentration, contained type I collagen. Fractions were separated by centrifugation (65000 × g, 60 min, at 4°C). The obtained pellets were recrystallized (3 times) by dialysis (against 15% KCl in 0.02 M $NaHPO_4$ at 4°C) and centrifugation (65000 × g, 60 min, at 4°C) (9). Collagen preparations purity was controlled electrophoretically (10).

Collagen fractions were hydrolyzed – 24 h, 6 M HCl, 105°C (11). Their amino acid compositions were analyzed by ion exchange chromatography on the amino acid analyzer AAA-881 (Czech Republic).

DNA from epididymis and testis was isolated as previously described (12). Tissues were homogenized and digested in digestion buffer (100 mM NaCl; 10 mM Tris-HCl; 25 mM EDTA, pH 8; and 0.5% SDS) and freshly added 0.1 mg/mL proteinase K (Sigma-Aldrich, Inc., USA)) (1:1.2 mg/mL) with shaking at 50°C for 15 h. RNA was degraded by incubation of the samples with 1–100 mg/mL thermostable RNase H for 1.5 h at 37°C. DNA was extracted with an equal volume of phenol-chloroform-isoamyl alcohol (25:24:1) and centrifuged for 10 min at 1700 × g. DNA was precipitated by adding 0.5 vol. of 7.5 M ammonium acetate and 2 vol. of 100% ethanol to the aqueous layer; samples were separated by centrifugation at 1700 × g for 5 min, rinsed with 70% ethanol, and air-dried. The pellet was dissolved in TBE buffer (10 mM Tris-HCl and 1 mM EDTA, pH 8); and then fractionated through 2% agarose gels (50–60 V; 3.5 h). After electrophoresis, gels were stained with ethidium bromide and visualized under a UV transilluminator (BIORAD, USA). Electrophoresis data analysis was carried out with Quantity One Software (USA).

Half of females from each group were sacrificed at 20-th day of pregnancy for determination

Table 1. Male rats bone type I collagen amino acid contents in control and with pyrazinamide administration at doses 1000 mg/kg and 2000 mg/kg of body weight (M ± m, n = 5, residues/ 1000 residues).

Amino acid	**Control (norm)**	Pyrazinamide 1000 mg/kg	Pyrazinamide 2000 mg/kg
Hydroxylysine	6.70 ± 0.10	5.30 ± 0.90	4.60 ± 0.70*
Lysine	39.1 ± 0.40	48.80 ± 3.10*	49.3 ± 0.90*
Histidine	4.70 ± 0.20	7.50 ± 0.60*	7.80 ± 0.90*
Arginine	48.30 ± 0.60	59.20 ± 3.60*	47.50 ± 6.30
Hydroxyproline	99.00 ± 1.40	90.30 ± 4.30	93.10 ± 3.40
Aspartic acid	38.80 ± 0.80	54.60 ± 4.10*	56.50 ± 6.20*
Threonine	24.00 ± 0.60	29.40 ± 2,50	31.10 ± 0.70*
Serine	38.20 ± 1.40	40.00 ± 0.30	46.0 ± 0.70*#
Glutamic acid	97.50 ± 1.30	125.80 ± 1.90*	107.30 ± 8.60
Proline	101.90 ± 1.80	91.30 ± 1.20*	91.30 ± 10.80
Glycine	305.00 ± 2.50	272.40 ± 12.70*	290.30 ± 6.80
Alanine	109.00 ± 1.10	85.00 ± 7.40*	82.20 ± 5.40*
Valine	23.00 ± 0.50	21.10 ± 1.90	21.80 ± 2.70
Methionine	6.20 ± 0.10	5.80 ± 0.90	6.00 ± 0.30
Isoleucine	13.70 ± 0.30	17.50 ± 0.30*	10.80 ± 2.20#
Leucine	27.90 ± 0.20	36.00 ± 3.50	29.40 ± 1.20
Tyrosine	4.80 ± 0.20	7.30 ± 0.30*	10.00 ± 1.10*#
Phenylalanine	12.00 ± 0.20	10.70 ± 0.40	14.40 ± 0.70*#

M ± m – mean ± mean standard error. * $p < 0.05$ statistically significant in comparison with control; # $p < 0.05$ statistically significant pyrazinamide, 1000 mg/kg group *vs.* pyrazinamide, 2000 mg/kg group.

Table 2. Embryologic indices at 20-th day of pregnancy of female rats coupled with male rats with pyrazinamide administration during all period of spermatogenesis.

Male group Indices	Control (norm)	Pyrazinamide 1000 mg/kg	Pyrazinamide 2000 mg/kg
Number of pregnant females	8	8	7
Total number of corpora lutea	92	94	98
Number of corpora lutea per one female, M ± m	11.5 ± 0.4	11.75 ± 0.58	13.43 ± 1.81
Total number of implantation sites	91	76	31
Number of implantation sites per one female, M ± m	11.38 ± 0.3	11.13 ± 0.41	5.86 ± 2.01 *
Preimplantational loss, abs/ %	1 / 1.09	18 / 19.1	6 / 19.3
Preimplantational loss per one female, M ± m	0.13 ± 0.12	0.63 ± 0.25	8.86 ± 3.18 *
Postimplantational loss, abs/ %	1 / 1.10	4 / 5.26	6 / 19.3
Postimplantational loss per one female, M ± m	0.13 ± 0.12	0.50 ± 0.18	1.29 ± 0.69
Total number of live conceptuses	90	72	25
Number of live conceptuses per one female, M ± m	11.25 ± 0.34	10.63 ± 0.39	4.71 ± 1.90 *

M ± m – mean ± mean standard error. * $p < 0.05$ statistically significant in comparison with control.

fetus antenatal development indices. Other pregnant females were rested to natural delivery for investigation posterity postnatal development.

Mating behavior of males was estimated *via* mating index determination (12).

Number of implantation sites in each uterine horn, number of live and dead conceptuses, number of corpora lutea in ovaries were determined after pregnant female laparotomy. Indices of embryonal death were calculated according to standard procedures at pre- and postimplantation periods of development (12).

Live fetuses of each female were examined for any external alterations of development (including

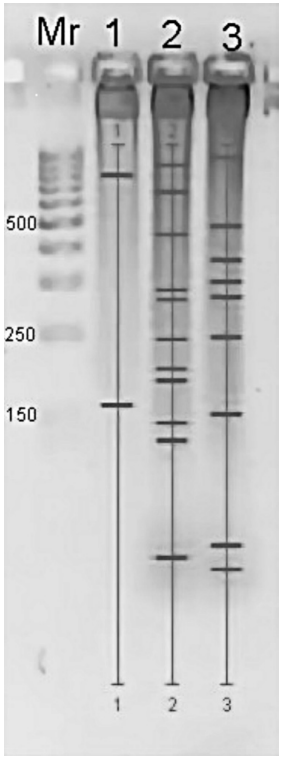

Figure 1. Levels of DNA fragmentation in rat testis with different doses of pyrazinamide (Mr – marker; 1 – control group; 2 – pyrazinamide 1000 mg/kg b.w.; 3 – pyrazinamide 2000 mg/kg b.w.)

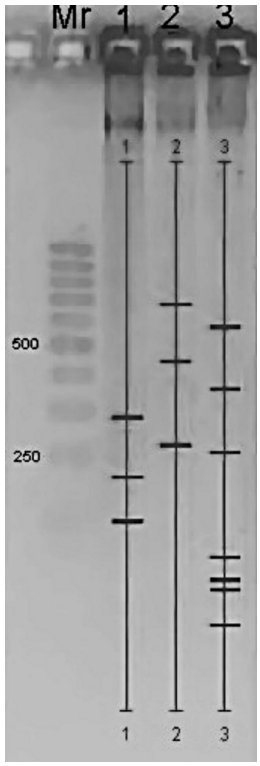

Figure 2. Levels of DNA fragmentation in rat epididymis with different doses of pyrazinamide (Mr – marker; 1 – control group; 2 – pyrazinamide 1000 mg/kg b.w.; 3 – pyrazinamide 2000 mg/kg b.w.)

Table 3. Loss of posterity from male rats with pyrazinamide administration during all period of spermatogenesis.

Male group Indices			Control (norm)	Pyrazinamide 1000 mg/kg	Pyrazinamide 2000 mg/kg
Females number			7	7	7
Offspring number		total	80	69	72
		M ± m	11.43 ± 1.21	9.85 ± 0.34	10.29 ± 0.61
Days of registration	4th	abs / %	0	6 / 4.3	5 / 6.9
		M ± m	0	0.43 ± 0.29	0.71 ± 0.36
	7th	abs / %	0	3 / 4.3	8 / 11.1
		M ± m	0	0.43 ± 0.29	1.14 ± 0.46 *
	10th	abs / %	0	12 / 17.4	4 / 5.5
		M ± m	0	1.71 ± 0.52 *	0.57 ± 0.29
	14th	abs / %	11 / 13.75	11 / 15.9	1 / 1.4
		M ± m	1.57 ± 0.78	1.57 ± 0.71	0.14 ± 0.14
	21th	abs / %	8 / 10	10 / 14.5	3 / 4.16
		M ± m	1.14 ± 1.14	1.42 ± 0.75	0.43 ± 0.29
Total mortality		abs / %	18 / 22.5	39 / 56.5	21 / 29
Mean mortality per litter		M ± m	2.57 ± 1.32	5.57 ± 1.21	3.0 ± 1.23

M ± m – mean ± mean standard error. * $p < 0.05$ statistically significant in comparison with control.

the head (shape, pinnae, eyes, eyelids, snout, jaw, nares, lip, palate), skin, tail, back, ventral wall, genitalia, limbs, feet and digits, umbilical herninas, hematomas, newborn anasarcas). Fetal weights and their cranio-caudal parameters were also determined, as soon as placental weights, sizes and condition (hyperemia, edema and fibrinous covering).

For females rested till delivery there were determined sizes of litters, their weights, number of live and dead pups. Number of survived pups were registered at 4-th, 7-th, 14-th and 21-st days of postnatal development. Observations on litters state and physical development were carried out daily till the end of lactation period according to standard procedures, viability index and lactation were calculated (12).

In statistical processing of experimental data, the mean of corresponding parameter (for each animal or each litter from one female) was used as independent variable. The obtained data were calculated by one-way analysis of variance (ANOVA). Data were compared using Tukey test. Differences were considered to be statistically significant at $p < 0.05$.

RESULTS AND DISCUSSION

DNA is an important molecular target for products of tuberculostatics biotransformation (1), which induced endonucleases for its lethal splitting. Such compounds could inhibit processes of DNA repair by nuclear DNA-polymerases. The level and character of DNA fragmentation are markers of apoptotic processes in organism (13). Our results on DNA-fragmentation intensification in rat testis with different doses of pyrazinamide are in good accordance with our previous data (which demonstrated the presence of epigenetic effects with administration to rats different doses of this compound) and with results of other authors, which established pyrazinamide negative effects on chromosomes structure and functioning (14).

Dysregulation of physiological germ cells apoptosis, which could cause male infertility (15), may be a result of external disturbances such as exposure to certain chemotherapeutic agents (16). Investigation of rats testis and epididymides DNA fragmentation (with pyrazinamide administration at different doses) demonstrated its essential intensification in testis in comparison with control group (Fig. 1). At lower dose of pyrazinamide, 11 fractions of DNA fragments with weights 900, 700, 450, 300, 270, 200, 170, 140, 100, 70 and 50 b.p. were present. The main fractions of low-weighted DNA fragments were 50 and 70 b.p. At higher dose of pyrazinamide, 9 fractions of DNA fragments with weights 1000, 500, 350, 300, 270, 200, 100, 60 and 40 b.p. were present. Main fractions of low-weighted DNA fragments were 100 – 40 b.p.

Somewhat another situation was in case of rats epididymides (Fig. 2). At lower dose of pyrazinamide, 3 fractions of DNA fragments with weights 700, 450 and 230 b.p. were present. At higher dose of pyrazinamide, 7 fractions of DNA fragments with weights 600, 350, 220, 80, 65, 60 and 40 b.p. were present. The main fractions of low-weighted DNA fragments were 200 b.p. Here the absence of low-weighted DNA fragments (at both doses of pyrazinamide) may be a result of their further splitting to single nucleotides during necrotic cell death intensification or acceleration of apoptotic cells removal, as it was previously demonstrated in experiments of other authors (17).

Differences in DNA fragmentation processes between testis and epididymis (Figs. 1 and 2) could be caused by tissue specificity of nucleases activities (13). Depending on nucleases set and effectiveness, DNA fragmentation could produce only fragments with high molecular weights, or with low and hight molecular weights, or with only low molecular weights, or even single nucleotides in different cell lines (13).

Thus, adverse effects of pyrazinamide, this widely used antitubercular drug, are much more serious and more profound than it was considered earlier. Pyrazinamide treatment could cause qualitative changes in nucleic acid molecules, changing their length and structure (1).

Alterations on the level of genome in our experiments were accompanied with alterations on the level of proteome. Changes in male rat bone type I collagens amino acid contents induced by pyrazinamide were profound as compared to control (Table 1). Statistically significant changes were registered in bone collagen with pyrazinamide administration at a dose of 1000 mg/kg for 10 amino acids and at dose 2000 mg/kg – for 9 amino acids.

Bone type I collagen of male rats with pyrazinamide at dose 1000 mg/kg contains lower contents of proline (–10.0 %), glycine (–11%) and alanine (–22.0%) simultaneously with higher contents of lysine (+25.0%), histidine (+60.0%), arginine (+22.6%), aspartic acid (+40.7%), glutamic acid (+29.0%), isoleucine (+27.7%) and tyrosine (+52.0%). Collagen of rats with pyrazinamide at adose of 2000 mg/kg contains lower contents of hydroxylysine (–31.3%) and alanine (–24.6%) simultaneously with higher contents of lysine

(+26.0%), histidine (+66.0%), aspartic acid (+45.6%), threonine (+29.6%), serine (+20.4%), tyrosine (+108.0%) and phenylalanine (+20.0%). For the majority of amino acids pyrazinamide effects were dose-dependent.

Our experiments demonstrated the presence of qualitative changes in male rats bone type I collagens with pyrazinamide (in comparison with norm) (Table 1). With pyrazinamide administration possibly could be formed bone type I collagen molecules with changed helix structure (changes of proline and glycine residues), surface charge (changes in number of arginine, aspartic acid, threonine, serine and glutamic acid residues), rigidity (changes in quantity of isoleucine, tyrosine and phenylalanine residues), number and types of cross-links (changes in ratio hydroxylysine : lysine : histidine residues) and specific locuses responsible for cell adhesion, interaction with chaperons and procollagen processing to collagen (changes in arginine, glycine and aspartic acid residues) (18–23). Such collagen molecules changes could hence affect the properties of connective tissues, mineralization processes and calcium metabolism.

We can suppose that such changes could be caused by pyrazinamide *via* its influence on nucleic acids (coding information for this protein) as it was mentioned previously. Besides this, on changes in collagen metabolism and structure, pathologic changes in amino acid metabolism could also affected (2). And at last, taking into account collagen genes polymorphism not only with pathologies but also normal (24, 25) such changes could be a result of pyrazinamide-caused disturbances in rates of transcription of different genes from the same collagen type I superfamily as it was previously demonstrated for other pathology (26).

Taking into consideration the role of apoptosis processes for male infertility, established pyrazinamide effects on DNA fragmentation and proteins amino acid compositions deserve detailed investigation of their influence on reproductive capability and paternal effects. Our observations showed that males treatment with pyrazinamide did not negatively change their sex behavior during cohabitation with intact females and did not cause mating index decrease (data not shown). Intact females indices of fertility (mean number of live pups, born by one female) after cohabitation with males of experimental groups, as well as their placentas (weights and sizes) and integral parameters of posterity antenatal development (fetal weights and sizes) also did not change in comparison with control (data not shown).

According to data of embryologic investigation (Table 2), pyrazinamide administration to males during all period of spermatogenesis negatively influenced indices of their posterity embryonic development (dose-dependent fetal death at different stages of ontogenesis).

The greater increase of preimplantational embryonal loss was at pyrazinamide administration to males at a dose of 2000 mg/kg.

Pyrazinamide at a dose of 1000 mg/kg caused only weak postimplantational loss (5.26 %), while dose 2000 mg/kg caused 19.3% loss of embryos at this stage of development (3.6 times greater in comparison with lower dose of pyrazinamide).

In both experimental groups dead newborns were absent. Number of live conceptuses greatly lowered at a dose of 2000 mg/kg. Data on pups surviving during first 3 weeks are presented in Table 3.

It must be noted that while at a dose of 2000 mg/kg of pyrazinamide pups loss was greater at early terms, the lower dose caused increasing of pups loss at later terms (from 10-th to 21-st day). Total loss (during all period of registration) in control group was 22.5%, at a dose of 1000 mg/kg of pyrazinamide – 56.5%, at a dose of 2000 mg/kg of pyrazinamide – 29%. Viability index (4-day survival index) in control group was 100%, while at doses of 1000 and 2000 mg/kg of pyrazinamide was 96 and 93%, respectively. Lactation index (% of pups alive at 4 days that survive the 21-days lactation period) in control was 77.5%, at doses of 1000 and 2000 mg/kg of pyrazinamide was 43.5 and 71%, respectively.

Pyrazinamide adverse effects on parental organisms nucleic acids and proteins structures and functions could cause profound changes in posterity. There is increasing evidence that epigenetic information can be inherited across generations in mammals (27). Epigenetic modifications could pass on through generations, causing alterations in genes expression and leading to disease phenotypes (28). Such changes in genotype of male parents can influence the phenotype of their offspring (27).

In our experiments, the influence of pyrazinamide or its metabolites on male germ cells (6) greatly altered implantation processes of ovums fertilized by them (Table 2). On the one hand, testis DNA fragmentation might be associated with altered reproductive outcome (29), on the other, considerable dose dependent increase of pre- and postimplantational losses could be the consequence of spermatozoids mutagenic injury induced by this compound. This supposition was confirmed by investigations of other authors, which demonstrated genotoxic action of pyrazinamide (dose-dependent mitotic index decrease, spermatozoids pathologic forms and

number of cells with chromosomes aberrations increase) (14). It should be also noted that embryo lethality increasing almost always is associated with induction of posterity physical defects (30).

While postimplantational loss is caused by genotoxic action of compounds (30), preimplantational loss could be also a result of nonmutagenic factors, which negatively influence on processes of fertilization *via* spermatozoids quantitative and qualitative changes (total number, activity, transport and introduction into ovum) (30). Previously, we demonstrated that different doses of pyrazinamide could cause considerable decrease in rat spermatozoids number (6).

An increase of posterity postnatal loss in our experiments could be a result of inherited changes in rat genome after pyrazinamide administrations (1, 27, 28).

Higher level of postnatal loss in pups from rat males treated with pyrazinamide with a dose of 1000 mg/kg b.w. in comparison with posterity from rat males treated with pyrazinamide with a dose of 2000 mg/kg b.w. could be a consequence of longer life period of concepts with inherited defects at lower dose of the compound. Increasing of pyrazinamide dose caused shortening of life time of concepts (high embryonal loss at early terms of pregnancy) (Table 2).

Differences in effects of pyrazinamide higher and lower doses could be a result of different drug-induced mechanisms of embryonal death realization. Higher dose possibly accelerates apoptotic mechanism, related to activation of the embryonic genome (31). As a result, the induced embryos did not reach even the blastocyst stage and we registered early high embryonal loss (Table 2). This supposition is confirmed also by our previous results (1) and data of other authors, which demonstrated that alterations in male germ cell DNA methylation patterns may be one of the underlying mechanisms of alterations in fertilization and early embryo development (32).

At lower dose of pyrazinamide, additional mechanism of sudden embryonic death may be realized, connected with important role of type I collagen in establishing the mechanical stability of embryonic tissues(33).

Thus, our experiments demonstrated the ability of different doses of pyrazinamide to cause changes in rat male nucleic acids and proteins structure, which were accompanied by alterations in processes of fertilization (with intact females), embryogenesis and lowering of posterity survival. Such effects could be an evidence of pyrazinamide mutagenic and/or spermatotoxic potential. They made obvious further detailed investigations as in this situation danger is associated not only with mutagenic events and genetic damages but also with irreversible alterations of mans reproductive system in case of pyrazinamide use at prepubertal period.

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