Anti-Tuberculosis Drugs Co-Administration Aggravates the Testicular Toxicity of Ethanol in Rats

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Abstract

Alcohol is well documented to affect male fertility. The association between alcohol use disorders and tuberculosis has long been known and well described. Taking into account that both alcohol and anti-tuberculosis drugs (ATD) could have negative impacts on male gonads, an increase in adverse effects is probable. The study aimed to determine combine effects of long-term alcohol consumption and ATD administration on male rat testes CYP2E1 mRNA and protein expression, DNA fragmentation, spermatogenesis parameters, as well as on male reproductive capacity and antenatal development of its posterity. Wistar albino male rats were divided into three groups: I – control (intact animals), II – chronic alcoholism (15% ethanol self-administration during 150 days); III- chronic alcoholism + ATD (ethambutol, isoniazid, rifampin and pyrazinamide) administration. Combined effects of chronic alcoholism and ATD led to changes in testicular CYP2E1 mRNA and protein expression, as well as in DNA fragmentation processes. These abnormalities were more expressed in alcoholic with ATD co-administration group as compared with alcoholic group. The revealed disorders at levels of testicular genome and proteome caused negative implications on spermatogenic epithelium quantitative and qualitative parameters, sperm count, male fertility, and postimplantational surviving of its offspring. Thus the ATD administration modulated the impact of long-term alcohol consumption on spermatogenesis and aggravated paternal-mediated negative effects on posterity.

ABBREVIATIONS

TB: Tuberculosis; ATD: Anti-tuberculosis Drugs; EMB: Ethambutol; INH: Isoniazid; RMP: Rifampin; PZA: Pyrazinamide; RT-PCR: Reversed Transcriptase Polymerase Chain Reaction; GAPDH: Glyceraldehyde-3-phosphate Dehydrogenase

INTRODUCTION

Alcohol is well documented to affect every organ system in the organism [1]. Particularly the effects on male fertility have been shown, but the level of consumption associated with sperm quality impairment and concomitant risk factors remain unclear [2,3]. Possibility of ethanol and pharmaceuticals combined consumption couldn’t be excluded for the majority of adults [4]. The significant pharmacodynamic interaction and harmful effects on organism occur due to ingestion of medications while alcohol is present in the body or vice versa [5]. In many cases pharmacotherapy is carried out if pathological changes in organism are present and additional load by ethanol could have great negative consequences. In this regard it is of special interest the association between alcohol use disorders and tuberculosis (TB). This phenomenon has long been well known and described [6,7]. As it has been reviewed, alcohol use disorders are the risk factors for an impairment of immune system, and this in turn increases a person’s susceptibility to active TB infection as well as to the reactivation of latent disease [6]. In such situation, the probability of simultaneous intake of alcohol and anti-tuberculosis drugs (ATD) is very high and the consequences could be very serious.

We have previously shown the anti-fertility effect of co-administered ATD in male rats [8,9]. It has been reported that the co-administration of ethambutol (EMB), isoniazid (INH), rifampin (RMP) and pyrazinamide (PZA) (in therapeutic doses) to male rats during the period of spermatogenesis caused a modulation
of pro-/antioxidant system, significant changes in DNA fragmentation, significant decrease of male fertilizing capacity and fertility, and an increase in pre- and post-implantation embryo-lethality [7]. Among this increase in cytochrome P-450 isoenzymes (CYP2E1, CYP3A2 and CYP2C23) mRNA has been demonstrated in rat’s testes after above mentioned drugs co-administration [10,11], as well as morphological and morphometric changes of the spermatogenic epithelium [8]. Also marked induction of testicular CYP2E1 mRNA expression and catalytic activity has been observed after INH administration in rats [12,13].

On the other hand, we have previously reported that chronic ethanol consumption can cause over-expression of CYP2E1 mRNA and protein in rats’ testes accompanied by spermatogenic impairment [14,15]. Established correlative links between testicular CYP2E1 mRNA expression levels in alcohol-dependent rodents allow us to suggest the involvement of this isoenzyme in testicular pathology development [16,17].

Taking into account that both alcohol and ATD can induce testicular CYP2E1 expression, and moreover all these compounds could have other negative impacts on male gonads, increase in adverse effects due to their co-administration is probable. Understanding of the nature and the severity of these events could be useful for preventing of undesirable outcomes on male reproduction and particularly on paternal-mediated effects on posterity.

This study aimed to determine combine effects of long-term alcohol consumption and ATD administration on rats’ testes CYP2E1 mRNA and protein expression, DNA fragmentation, spermatogenesis parameters, as well as on male reproductive capacity and antenatal development of their posterity.

**MATERIALS AND METHODS**

Wistar albino male and female rats, body weight (b.w.) of 150 g to 170 g, were used in the study. They were kept under a controlled temperature (from 22 °C to 24 °C), relative humidity of 40 % to 70 %, lighting (12 h light-dark cycle), and on a standard pellet feed diet (“Phoenix” Ltd., Ukraine). The study was 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.

For the experimental (chronic alcoholism) model reproducing male rats were selected according to the method for measuring voluntary alcohol self-administration in rats, which provides continuous choice between an alcohol solution and water (two bottle preference test) [18].

The fourteen selected rats were used for chronic alcoholism models by replacing water with a 15 % ethanol solution during 90 days. The consumption of 15 % ethanol was measured as ml and was calculated as g x kg⁻¹ x day⁻¹ of pure ethanol. On this regimen daily ethanol consumption was in average 10 g x kg⁻¹ x day⁻¹.

After 90 days of ethanol consumption alcohol-treated rats were divided in two groups (each group included 7 experimental males): 1 – continuation of alcohol self-administration (hereinafter referred to as alcoholic group); 2 – continuation of alcohol self-administration with simultaneous ATD co-administration (hereinafter referred to as alcoholic with ATD co-administration group); seven intact male rats (of the same age and weight) were used as controls. From the beginning of the experiment, they were kept in the same conditions as experimental animals but were given only water ad libitum.

ATD: EMB, INH, RMP and PZA were supplied by the SIC “Borzhagovsky Chemical Pharmaceutical Plant” CJSC, Ukraine. ATD were suspended in 1% starch gel and given by gavage in DOTS (directly observed treatment, short-course) regimen at maximal doses used in clinic [19]: ethambutol – 155 mg/kg b.w./day, rifampicin – 74.4 mg/kg b.w./day, isoniazid – 62 mg/kg b.w./day, pyrazinamide – 217 mg/kg b.w./day for 60 days (duration of spermatogenesis process and time of germ cell maturation in epipidymis). The coefficient for conversion of human doses to animal equivalent doses based on body surface area was taken into account (Food and Drug Administration (webpage in the internet)).

After 46 days of repeated administrations the males from all groups were mated with intact females at the ratio 1 male: 2 females during 14 days (3 oestrous cycles). During this period the co-administration of ATD to male rats was continued. Males were mated with females only at night hours, and to avoid the consumption of alcohol by females the bottles with ethanol solution were taken out at this period. According to generally accepted guidelines for the fertility study in laboratory rats [20] the first day of pregnancy was established by vaginal cytology (the first day of sperm detection in vagina). Most males were mated within the first 5 days of cohabitation (i.e., at the females first available oestrous), but part of them demonstrated infertility. This fact was taken into account for evaluation of effect of alcohol and ATD on male fertilizing capacity, which was determined by the index:

\[
\text{number of pregnant females} \times 100 \quad \text{number of females mated with males}
\]

After 150 days from the experiment beginning, both the experimental and control rats were sacrificed by decapitation. The testes and epididymis were used for investigation.

The expression of CYP2E1 mRNA in the testes was determined by a reversed transcriptase polymerase chain reaction (RT-PCR). Testes samples (50 mg) were collected, quickly frozen in liquid nitrogen, and stored at -80 °C before RNA extraction. The isolation of total mRNA was carried out with a TRI-Reagent (Sigma, USA). The integrity and concentration of RNA was analysed in a 2 % agarose gel. First-strand complementary DNA was synthesized using a First-Strand cDNA Synthesis Kit (Fermentas, Germany). The reaction mixture contents for PCR, amplification protocol, and specific primers for the CYP2E1 gene were chosen according to S.M. Lankford et al. [22]. The primer sequences were: sense, 5'-GGTGCCCACTGCCATCTGGTTGG-3' and antisense, 5'-CCCATATCTCAGAGTTGTGC-3'. RT-PCR with primers of β - actin (sense, 5'-GCTCGTCGTCGACAACGGCTC-3' and antisense 5'-CAAACATGATCTGGGTCATCTTCT-3') was carried out for internal control. All of the primers were synthesized by...
RESULTS AND DISCUSSION

RT-PCR was performed to examine the combined effect of chronic alcoholism and ATD co-administration on CYP2E1 transcriptional activation in testes. We have demonstrated that the mRNA expression of CYP2E1 in alcohol-treated rats' testes was increased 3.6 folds in comparison with control group (Figure 1). At the same time long-term ethanol and ATD co-administration caused more profound modulation in testicular CYP2E1 mRNA content, it was elevated 5.9 folds as compared to control (Figure 1). At the same time long-term ethanol and ATD co-administration caused more profound modulation in testicular CYP2E1 transcript levels and protein expression.

As it can be noted from Figure 2, the amount of the CYP2E1 protein in the testes of alcohol-treated rats increased 1.4 times (Figure 2). On the contrary to results on CYP2E1 mRNA expression we did not find more prominent testicular CYP2E1 protein elevation following alcohol and ATD co-treatment (Figure 2). In general the level of testicular CYP2E1 protein in this group was almost equal to that of alcoholic group (1.2 folds higher than control).

Investigation of DNA fragmentation in testes of alcohol-treated rats demonstrated its essential intensification in comparison with control group (Figure 3). In control group only two fractions of DNA fragments with weights 400 and 80 b.p. were present, while under alcohol administration their number increased to four (with weights over 1000, 400, 300 and 20 b.p.). In control group single fraction of low-weighted DNA fragments was 80 b.p. Main fraction of DNA fragments in this group was 400 b.p. In testes of alcoholic rats single fraction of low-weighted DNA fragments was 20 b.p. Main fraction of DNA fragments was over 1000 b.p.

"Metabion" (Germany). The MyCycler termocycler (Bio-Rad, USA) was used for amplification. PCR products (CYP2E1-744 bp and β-actin-353 bp) were separated in a 2% agarose gel, stained with ethidium bromide, and visualized under a UV transilluminator (BIORAD, USA). Data analysis was carried out with Quantity One Software (USA) and presented in relative units as the ratio of CYP2E1 mRNA contents and β-actin mRNA contents.

CYP2E1 protein concentration was determined by Western Blot analysis, using specific polyclonal antibodies for mouse CYP2E1 protein, produced in the Institute of Molecular Biology & Genetics of the National Academy of Sciences of Ukraine. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as an internal loading control with polyclonal antibodies (Sigma, USA). The protein concentration in the cell lysate was quantified by the Bradford method [23]. Equal amounts of protein per sample (50 μg) were used. The electrophoretic fractionation of proteins was carried out in 12% PAAG (with 0.1% SDS) according to the Laemmli method [24]. The proteins were transferred to the nitrocellulose membrane. After blocking in a TBS buffer containing 5% defatted dry milk, the membrane was incubated with CYP2E1 antibodies (1/400, v/w), and then with anti-rabbit IgG secondary antibodies (Sigma, 1/500, v/w). The CYP2E1 protein was visualized by chemiluminescence. Blots were photographed and densitometric analysis performed using the ImageJ software. CYP2E1 protein data were presented in relative units as the ratio of CYP2E1 protein contents and control GAPDH protein contents in the same gel band.

DNA from rat testes was isolated as described previously [25,26]. Briefly, tissue was homogenized and digested in digestion buffer (100mM NaCl; 10mM Tris–HCl; 25mM 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. DNA 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-isomyl alcohol (25:24:1) and centrifuged for 10 min at 1700 x g. The DNA was precipitated by adding 0.5 vol 7.5M ammonium acetate and 2 vol 100% ethanol to the aqueous layer; samples were separated by centrifugation at 1700g for 5 min, rinsed with 70% ethanol, and air-dried. The pellets were dissolved in TBE buffer (10mm Tris–HCl and 1mmEDTA, 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).

In all cases the right testis was used for the evaluation of morphologic and morphometric parameters and spermatogenesis indices. The tissue was sectioned along the longitudinal axis, then fixed in 10% formalin and embedded in paraffin blocks. Histologic sections (6mm) were stained by haematoxylin and eosin. Histological examination was performed under a light microscope (100 x and 400 x). The determination of the spermatogenic index in testicles was carried out according to four points system. It was based on the estimation of number of cell layers, types of cells, and the presence of late spermatids in the seminiferous tubules. The criteria were as follows: (1) only spermatogonia present; (2) spermatogonia and spermatocytes present; (3) spermatogonia, spermatocytes and round (early) spermatids present with <5 late spermatids per tubule; (4) spermatogonia, spermatocytes, and round spermatids present with up to 25 late spermatids per tubule [14]. Spermatogenic index was calculated as a ratio of stages of spermatogenesis total to number of examined tubules. Two hundred seminiferous tubules per testis of each animal were observed by microscopy. The obtained numerical data were recorded and processed with statistical methods, as described below. Simultaneously occurrences of cells exfoliation (shedding of epithelial elements), desquamation epithelium (detachment) from tubule basal membrane, and presence of cell-free regions (“windows”) were taken into account.

The sperm count in epididymal suspensions was estimated as described Chitra et al. [2003] using Goryaev’s counting chamber and light microscope (200 x) [27].

The females were sacrificed by cervical dislocation on day 20 of pregnancy for determination of embryonic and fetal antenatal development indices. The number of corpora lutea in ovaries, of implantation sites and of live and dead fetuses in each uterine horn was counted after laparotomy of pregnant females. Indices of embryonic death at pre- and post implantation periods of development were calculated according to standard procedures [28-30].

The obtained were calculated by one-way analysis of variance (ANOVA) and expressed as the mean±standard error of the mean (M±S.E.M.). Data were compared using Tukey’s test. Differences were considered to be statistically significant at p<0.05.
Six fractions of DNA fragments with weights over 1000, 500, 300, 200 and 150 b.p.) were present in testes of alcoholic ATD-treated rats. The main two fractions of high-weighted DNA fragments were over 1000 b.p.

High level of testis cells damage in alcohol-dependent rats with ATD co-administration was confirmed by testis histomorphology data (Figure 4). There were destructive changes in spermatogenic epithelium as summarized in Table 1. According to obtained data, number of spermatogonies and the spermatogenic indexes in experimental groups decreased significantly in comparison with control. At the same time number of cells at XII stage of the seminiferous epithelium cycle (characterized by the meiotic division of primary spermatocytes) demonstrated clear tendency toward reduction.

The pronounced qualitative changes in the spermatogenic epithelium were also present. Frequency of epithelial cell desquamation into the lumen of tubules and the presence of cell-free regions ("windows") rose in both experimental groups, but in rats with ethanol and ATD co-administration such alterations were more apparent (Figure 4, Table 1).

The impairment of spermatogenesis in alcoholic and alcoholic ATD-treated rats was accompanied by the reduction of epididymal sperm counts as it has been shown in Table 2. While these changes did not reach the significance level in the alcoholic group, in alcoholic with ATD co-administration group the sperm number was lower than in control alcoholics with the statistical significant level <0.05 (2.4 and 1.6 folds correspondingly). Simultaneously, about 15% decreases in epididymis absolute and in relative weights in alcoholic ATD-treated rats took place in
Figure 3 Levels of DNA fragmentation in rat testis (Mr – marker; 1 – control; 2 – alcoholic; 3- alcoholic with ATD co-administration). Analysis was carried out using the Quantity One Software.

Figure 4 Desquamation of epithelial cells into the lumen of tubules: A - alcoholic, B – alcoholic with ATD co-administration. Hematoxin and eosine, x100.

Table 1: Spermatogenic epithelium morphometric indices of control and diabetic rats.

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<tr>
<td></td>
<td>control</td>
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<tr>
<td>Spermatogenic index (stages of spermatogenesis total / number of examined tubules)</td>
<td>3.623±0.012</td>
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<tr>
<td>Number of spermatogonia (per tubular cross section)</td>
<td>67.605±0.890</td>
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<tr>
<td>XII stage of spermatogenesis, %</td>
<td>2.667±0.715</td>
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<tr>
<td>Desquamated epithelium, %</td>
<td>1.167±0.447</td>
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<tr>
<td>&quot;Windows&quot;,%</td>
<td>0.500±0.342</td>
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*p<0.05 in comparison with control
comparison with control, as well as in comparison with alcoholic group (Table 2).

Markedly smaller number of pregnant females in ethanol with ATD-treated group is an evidence of fatal decline of male fertility (Table 3). Only 4 from 14 mating females became pregnant, while the index of fertility in control and alcoholic group was 93% and 71% respectively.

The comparison of embryogenesis indices in alcoholic and control groups demonstrated constitutive negative impact of long-term paternal alcohol consumption on preimplantational lethality rate (Table 4). It was 32%, while in control group – 9.6%. The level of postimplantational embryonal and fetal death had no significant differences between these groups. As to females mated with alcohol and ATD co-administered males, in this group percent of preimplantational loss was equal to alcoholic group, but postimplantational death reached 100%

The total embryonal/fetal death in alcoholic group increased 22% in comparison with control, as well as in alcoholic with ATD co-administration group it was 100% (Table 4).

The results of our current experiments on the increase of CYP2E1 gene expression in rats with chronic alcohol consumption are in good accordance with other researchers’ data, as well as with our previous findings [10,11,31]. But of interest was the combined effect of ethanol and ATD co-administration. While we noted progressive increase of CYP2E1 mRNA content in the testes of rats that consumed alcohol simultaneously with ATD, the amount of CYP2E1 protein remained at the same level as in alcoholic group (Figure 1). Such phenomenon seems best explained by existence of the very complex molecular regulation of CYP2E1 induction, which is realized through transcriptional, post-transcriptional, and post-translational mechanisms depending on the dose, duration, manner of administration, and specific structure of the inducing agents [32,33].

The increase in testicular CYP2E1 mRNA content following ethanol alone or ethanol and ATD administrations suggests a

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<tr>
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<tr>
<td>Sperm number, 10^6 x ml^-1</td>
<td>62.8±3.69</td>
</tr>
<tr>
<td>Epididymis (right) absolute weight , g</td>
<td>0.53±0.020 0.140±0.007*</td>
</tr>
<tr>
<td>Epididymis (right) relative weight , g</td>
<td>0.53±0.040 0.125±0.011</td>
</tr>
<tr>
<td>*p&lt;0.05 in comparison with control; **P&lt;0.05 in comparison with alcoholic group.</td>
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<thead>
<tr>
<th>Groups</th>
<th>Number of mated females</th>
<th>Number of pregnant females</th>
<th>Fertility index, %</th>
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<tr>
<td>Control</td>
<td>14</td>
<td>13</td>
<td>93</td>
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<tr>
<td>Alcoholic</td>
<td>14</td>
<td>10</td>
<td>71</td>
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<tr>
<td>Alcoholic with ATD co-administration</td>
<td>14</td>
<td>4</td>
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<td>control alcoholic alcoholic with ATD co-administration</td>
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<tr>
<td>Number of pregnant females</td>
<td>13 10 4</td>
</tr>
<tr>
<td>Total number of corpora lutea</td>
<td>146 128 75</td>
</tr>
<tr>
<td>Number of corpora lutea per one female, mean±S.E.M</td>
<td>11.23±0.73 12.80±0.90 18.75±1.0* **</td>
</tr>
<tr>
<td>Total number of implantational sites</td>
<td>132   97  46</td>
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<tr>
<td>Number of implantational sites per one female, mean±S.E.M</td>
<td>10.15±0.55 8.70±0.87 11.5±2.15</td>
</tr>
<tr>
<td>Preimplantational loss, abs / %</td>
<td>17 / 9.58 41 / 32.03 28 / 38.67</td>
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<tr>
<td>Preimplantational loss per one female, mean±S.E.M</td>
<td>1.31±0.41 4.10±0.88* 2.57±0.24</td>
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<tr>
<td>Postimplantational loss, abs / %</td>
<td>10 / 7.58 8 / 9.19 46 / 100</td>
</tr>
<tr>
<td>Postimplantational loss per one female, mean±S.E.M</td>
<td>0.76±0.32 0.80±0.36 11.5±2.15* **</td>
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<tr>
<td>Total number of living foetuses, abs / %</td>
<td>125 / 94.69 81 / 93.10 0 / 0</td>
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<tr>
<td>Number of living foetuses per one female, mean±S.E.M</td>
<td>9.62±0.40 8.10±0.82 0</td>
</tr>
<tr>
<td>Total embryonal/fetal death, %</td>
<td>14.38 36.72 100</td>
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<tr>
<td>*p&lt;0.05 in comparison with control</td>
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transcriptional regulation of this isoenzyme expression [34]. It can be explained by the effect of different substances in the mixture on the similar molecules, or synergistic pathways of these substances effects, or additive effects as well [35]. Previously we have shown increase in CYP2E1 mRNA content in rats testes following EMB, INH, RMP and PZA co-administration [8], as well as increase in CYP2E1 mRNA content and enzymatic activity in liver [36]. Moreover, it has been reported that INH induces CYP2E1 in the liver and testes [9,27-29]. Thus, we speculate that a more pronounced increase in the expression of CYP2E1 gene with alcohol and ATD co-administration could be due to synergistic or additive effects of different factors at the level of transcription.

There is clear evidence for ethanol ability to stabilize the CYP2E1 molecule structurally and to suppress proteolysis by inhibition of the proteolytic system[37,38]. Our results on increase of testicular CYP2E1 protein content in experimental rats (Figure 2) reflected the possibility of its stabilization by ethanol and the prolongation of its half-life time [39,40]. After ATD co-administration the level of testicular CYP2E1 was also higher than in control, but we did not observe its subsequent rise as compared with alcohol group (Figure 2).

It could be related to changes in CYP2E1 protein turnover, which like transcription, mRNA stability and translation can be influenced by certain exogenous factors. For example some compounds like carbon-tetrachloride and glucagon have been shown to affect the stability of CYP2E1 [41,42]. Also oxidative stress seems to be important factor for start of CYP2E1 degradation by initiating proteasome-dependent proteolysis [43]. However the methodology of the current study does not allow us to give exact mechanistic explanation of this phenomenon because of multiplicity of four ATD and ethanol combined effects on CYP2E1 posttranslational regulation.

Anyway, increased expression of CYP2E1 in the rats’ testes of both experimental groups could have serious toxicological outcomes due to CYP2E1 ability for massive generation of oxygen reactive species (ROS), such as superoxide radical [23]. Increased oxidative stress at the level of testicles after repeated administration of alcohol or ADT has been confirmed by our previous findings and those of other authors [7,9,35-44]. Oxidative stress can lead to serious disruption of nucleic acids and proteins metabolism, structure and functions and may play an important role in induced DNA disruption in the germ cells [45]. ROS produced during ethanol oxidation are the major source of DNA damage, causing strand breaks, removal of nucleotide, and a variety of modifications of the organic bases of the nucleotides [46]. This supposition has been confirmed by our results on testicular DNA fragmentation level (Figure 3) as marker of apoptotic processes intensity [47,48]. Apoptosis is an integral component of normal testicular function and it has been hypothesized to limit the germ cell population and prevent maturation of aberrant germ cells [49,50]. Ethanol can induce apoptosis by up-regulation of the Fas system and the activation of caspases [51]. Chromatin started to fragment at high levels of oxidative stress, regardless to the factors that caused it (ethanol or ethanol with ATD-co-administration) [52]. Importantly, the losses of fertilizing potential and DNA integrity can be occurred at different rates of oxidative stress, with the latter being the more sensitive [53,54]. It can be supposed that testicular injury in ethanol or ethanol with ATD-co-administration treated rats might be due to intensified germ cell apoptosis caused by oxidative stress stimulation [42].

Increase of testicular CYP2E1 levels might have negative impact not only on germ cells via oxidative stress and cells death enhancement. It is important to emphasize that in testes CYP2E1 is localized in Leydig cells, where testosterone biosynthesis takes place [55]. In our opinion activation of CYP2E1-dependent metabolizing systems in steroidogenic cells, at least partially, could determine alcohol and ATD negative effects on spermatogenesis. Excessive CYP2E1 inducers administration could be one of the causes of steroidogenesis enzymes inhibition due to Leydig cells and their microenvironment damages by free radicals. This assumption is consistent with the identified by us violations of spermatogenesis and decrease in the number of sperm in experimental groups (Figure 4, Tables 1,2). However, it should be noted that despite lack of more prominent CYP2E1 levels increase, the more profound impairments in sperm count and fertility have been observed in rats that consumed alcohol and ADT as compared with the group of alcoholic. In this context other (independent from CYP2E1) possible mechanisms of ATD testicular toxicity should be considered. Little experimental evidences are available to permit a clear assessment of each ATD toxic effects on male gonads. EMB-induced loss of fertility and evident regressive lesions in the testes of rats, and spermatogenic epithelium disturbances with further blocking of spermatogenesis in rats and cocks has been reported [56,57]. Recently Awodele et al. (2010) have also presented evidence that RMP in dose 9 mg/kg/day significantly decreased the sperm count and motility, as well as increased sperm abnormalities in rats [58]. On the other hand there are findings on stimulation the activity of 17 alpha-hydroxylase in the microsomal fraction of rat testes by rifampicin leading to an increased formation of testosterone and androstenedione [59]. The roughness is the unavailability of information on any mechanistic aspects. In relation to PZA and EMB we can only suspect that involving of the xanthine oxidase ROS-generating system and the chelation of zinc and copper could contribute to an imbalance of oxidative processes and antioxidant defense in the testicular tissues [46,47]. However there is no direct evidence either for or against such supposition.

The DNA damage in male germ cells can be accompanied with poor fertilization rates, defective preimplantation embryonic development, high rates of miscarriage and morbidity in the offspring [48]. In our experiments very high postimplantational lethality level in alcoholic with ATD co-administration group (Table 4) may have been caused by genotoxic action of substances [20]. The data of experiments on mice demonstrating weak genotoxicity of PZA at doses of 125, 250 and 500 mg/kg b.w. confirm this assumption [49]. In vitro experiments have shown mutagenic action of INH metabolite – mono acethylhydrazine increasing the number of Salmonella typhimurium TA100 and TA1535 revertant mutations and the number of micronuclei in polychromatic erythrocytes [50]. The weak mutagenic effect of INH and its ability to cause liver DNA injury has been also demonstrated by Braun et al. (1984) and Yue et al. (2009) [51,52]. Moreover the recent publication has provided evidences that all ATD possess significant cytotoxic and mutagenic potential, especially in combination [53]. It remains difficult to disentangle the results of mutations from epigenetic modifications in perpetuating paternal impacts. However there
are some facts for speculation on the role of epigenetic factors in the effects of paternal alcohol consumption on offspring. For example D.M. Bielawski et al. (2002) have revealed reductions in DNA-methyltransferase RNA in sperm after chronic alcohol treatment of male rats [54]. On the other hand French (2013) has reviewed that induction of CYP2E1 can lead to prominent epigenetic effects [55]. The weak mutagenic effect of INH and its ability to cause liver DNA injury has been also demonstrated by Braun et al. (1984) and Yue et al. (2009) [56,57]. Moreover the present publication has provided evidences that all ATD possess significant cytotoxic and mutagenic potential, especially in combination [58]. It remains difficult to disentangle the results of mutations from epigenetic modifications in perpetuating paternal impacts. However there are some facts for speculation on the role of epigenetic factors in the effects of paternal alcohol consumption on offspring. For example D.M. Bielawski et al. (2002) have revealed reductions in DNA-methyltransferase RNA in sperm after chronic alcohol treatment of male rats [59]. On the other hand French (2013) has reviewed that induction of CYP2E1 can lead to prominent epigenetic effects [60].

CONCLUSION

Thus our results on combined effects of chronic alcoholism and ATD on rats’ testes have demonstrated changes in CYP2E1 mRNA and protein expression, as well as in DNA fragmentation processes. Abnormalities were more expressed in alcoholic with ATD co-administration group as compared with alcoholic group. The revealed disorders at testicular genome and proteome levels have had negative implications on spermatogenic epithelium quantitative and qualitative parameters, sperm count, male fertility, and postimplantational surviving of offspring. Finally, the ATD administration may modulate the impact of long-term alcohol consumption on spermatogenesis and aggravate paternal-mediated negative effects on posterity.

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Cite this article