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Comparative investigation of methionine and novel formulation Metovitan protective effects in Wistar rats with testicular and epididymal toxicity induced by anti-tuberculosis drugs co-administration

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ABSTRACT

Despite clear beneficial effects, studies on the efficacy of different formulations of micronutrients and vitamins in the treatment of male infertility are still very limited. The aim of the study was to compare the efficacy of methionine and novel formulation Metovitan based on the combination of methionine, thiamine, nicotinamide, α-tocopherol acetate and zinc salt as remedies for prevention of anti-tuberculosis drugs (ATD) anti-fertility effects in male rats. ATD co-administration resulted in testicular CYP2E1, CYP2C23 and CYP3A2 transcriptional activation. Methionine and Metovitan regulated the level of CYP2E1 and CYP3A2 mRNA expression. Methionine unaffected CYP2C23 mRNA level, while Metovitan effectively normalized this parameter. The use of methionine did not allow normalizing of DNA fragmentation processes in testes, while Metovitan provided decrease in the number of DNA fragments to the control level. Both substances significantly decreased p-nitrophenol hydroxylase activity and prevented pro/antioxidant parameters imbalance in testes of ATD-treated rats. The end results of methionine and Metovitan application were partial or complete restoration of altered spermatogenesis parameters with subsequent increase in sperm count and fertility. Ameliorating effects may be attributed to antioxidant properties and modulating effects on testicular CYPs. More pronounced beneficial Metovitan effects might be due to synergic effects of its components.

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1. Introduction

A steady increase in the proportion of male factor in infertile couples is observed in recent years (Gannon and Walsh, 2015). As regards to the etiology of the male infertility there is a growing apprehensions about the association between this pathology and environmental and occupational pollutants, changes in lifestyles and dietary habits, and exposure to toxic agents (Deng et al., 2016; Wijesekara et al., 2015; Muhammad et al., 2015). Only recently more attention has been given to the underlying causes of male factor infertility including the association between semen parameters and certain pharmaceuticals (Brezina et al., 2012). We have previously shown strong anti-fertility effect of co-administered ethambutol (EMB), isoniazid (INH), rifampin (RMP) and pyrazinamide (PZA) in male rats (Shayakhmetova et al., 2012). In this case the testicular damage could be a result of direct or mediated action of one or more of used anti-tuberculosis drugs (ATD) on the balance between ROS generation and scavenging activities. As reviewed by Walczak–Jedrzejowska et al. (2012) *in vitro* and *in vivo* studies have demonstrated the beneficial effect of many antioxidant factors on sperm, pregnancy rate and live birth rates. The controversial issue is, however, the effectiveness of antioxidants on sperm, which has not yet been confirmed by other studies. Generally the use of micronutrients and vitamins as antioxidants in infertile men treatment was very popular in recent years. These compounds have properties to scavenge free radicals preventing oxidative stress development and DNA fragmentation in spermatozoa, improve sperm quality, stimulate spermatogenesis, and androgen synthesis and secretion by various mechanisms (Showell et al., 2011; Yu et al., 2014; Rashida et al., 2015). Several studies have

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provided the evidences of positive effects of biologically active compounds (vitamins C, B_{12} and E, zinc, selenium, magnesium, arginine folate, carnitine and carotenoids) on sperm quality in infertile men and pregnancy rate as the main outcome (Zini et al., 2009; Lanzafame et al., 2009; Ross et al., 2010; Showell et al., 2011; Schmid et al., 2012; Sundaram et al., 2013). Recent study has shown ameliorative effect of methionine against adverse effects of cholesterol (Abu Elnaga, 2012). Also it has been shown, that methionine has protective effects on sperm variables in freeze-thawed Merino ram semen (Omur and Coyan, 2016).

Despite clear beneficial effects, studies on the efficacy of different formulations of micronutrients and vitamins in the treatment of male infertility are still very limited. Thus, investigations of biologically active low-molecular compounds' combined protective effects against testicular and epididymal toxicity of xenobiotics are of certain interest.

The aim of the current study was to compare the efficacy of methionine and novel formulation Metovitan based on the combination of methionine, thiamine, nicotinamide, α-tocopherol acetate, and zinc salt as remedies for prevention of ATD anti-fertility effects.

2. Materials and methods

2.1. Animals and treatment

The study was carried out on Wistar albino male rats with initial body weight (b.w.) 150–170 g (8–9 weeks old) and female rats with 150–170 g b.w. (9–10 weeks old) purchased from Biomodel Service (Kyiv, Ukraine). Animals were kept under a controlled temperature (from 22 °C to 24 °C), relative humidity of 40%–70%, lighting (12 h light-dark cycle), and on a standard pellet feed diet («Phoenix» Ltd., Ukraine).

Substances of ATD (EMB, RMP, INH, and PZA) were supplied by the SIC «Borzhagovsky Chemical-Pharmaceutical Plant» CJSC, Ukraine. Methionine was supplied by PC «Kyiv Vitamin Factory». Formulation Metovitan based on the combination of methionine, thiamine, nicotinamide, α-tocopherol acetate, and zinc salt was developed in O. V. Palladin Institute of Biochemistry of NAS of Ukraine.

The male rats were divided randomly into 4 groups: 1—control (n = 8); 2—ATD administration (n = 8); 3 - ATD administration + methionine; 4 - ATD administration + Metovitan.

ATD suspended in 1% starch gel were given by gavage in DOTS (directly observed treatment, short-course) regimen at maximal doses used in clinic (Jochi, 2011): EMB—155 mg/kg b.w./day, RMP—74.4 mg/kg b.w./day, INH—62 mg/kg b.w./day, PZA—217 mg/kg b.w./day for 60 days (duration of spermatogenesis process and time of germ cell maturation in epididymis). The coefficient for conversion of human doses to animal equivalent doses based on body surface area was taken into account (Food and Drug Administration, 2005). Methionine and Metovitan suspended in 1% starch gel were given by gavage in dose 50 mg/kg b.w./day every last week of the month, daily, in one hour before ATD administration. The control group received only starch gel in corresponding volumes (5 ml/kg b.w.).

After 46 days of repeated ATD 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 administration of ATD, Methionine, and Metovitan to male rats was continued.

According to generally accepted guidelines for the fertility study in laboratory rats (Male reproductive toxicology (Chapter 16), 2005) 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 oestrus), but part of them demonstrated infertility. This fact was taken into account for evaluation of effects of ATD co-administration and Methionine, and Metovitan on male fertilizing capacity, which was determined by the index:

$$\frac{\textit{number of pregnant females}}{\textit{number of females mated with males}} \times 100\%$$

The pregnancy was confirmed by necropsy. The females were sacrificed via cervical dislocation on day 20 of pregnancy. Males were sacrificed after 60 days of experiment. Euthanasia was performed by the decapitation. The animals were sacrificed under mild ether anaesthesia. Concentration of ether 80 μl per liter of volume of a container was used; time of induction took ~5 min.

Testes were removed. Right testis was used for histological examination and left testis - for biomolecular investigations.

The study was carried out in accordance with the U.K. Animals (Scientific Procedures) Act, 1986 and associated guidelines, the European Communities Council Directive of 24 November 1986 (86/609/EEC) and approved by the Institutional Animal Care and Use Committee (approval number 01/12/09).

2.2. Evaluation of cytochrome P450s (CYPs) CYP2E1, CYP2C23 and CYP3A2 mRNA expression

The expression of CYP2E1 mRNA in testes was determined by a reversed transcriptase polymerase chain reaction (RT-PCR). After collection of the testes samples (25 mg), they were snap 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-Aldrich, Inc., USA). The integrity and concentration of RNA was analysed in a 2% agarose gel. First-strand complementary DNA (cDNA) was synthesized using a First-Strand cDNA Synthesis Kit (Fermentas, Germany) according to the manufacturer's protocol. The reaction mixture contents for PCR, amplification protocol, and specific primers gene were chosen according to Lankford et al. (2000) for the CYP2E1, according to Imaoka et al. (2005) for the CYP2C23 (ortholog of the CYP2C19 and CYP2C9), and according to Jager et al. (1999) for the CYP3A2 (ortholog of the CYP3A4). RT-PCR with primers of β − actin was carried out for internal control. The primer sequences are shown in Table 1.

All of the primers were synthesized by «Metabion» (Germany). The MyCycler termocycler (BioRaD, 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 CYP2E1 mRNA contents/β-actin mRNA ratio.

Table 1
Sequences of primers used to amplify specific mRNAs by RT-PCR.

Target gene	Oligonucleotide	Primer sequence
CYP2E1	sense	5′-CTTCGGGCCAGTGTTCAC-3′
	anti-sense	5′-CCCATATCTCAGAGTTGTGC-3′
CYP2C23	sense	5′-GATGCTGTCTTCCGTCATGC-3′
	anti-sense	5′-GTAATAGGCTTGATGTCAAG-3′
CYP3A2	sense	5′-TACTACAAGGGCTTAGGGAG-3′
	anti-sense	5′- CTTGCCTGTCTCCGCCTCTT-3′
β-actin	sense	5'-GCTCGTCGTCGACAACGGCTC-3′
	anti-sense	5'-CAAACATGAT CTGGGTCATCTTCT-3′

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