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Subacute alcohol and/or disulfiram intake affects bioelements and redox status in rat testes

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ABSTRACT

The aim of the study was to investigate if alcohol and disulfiram (DSF) individually and in combination affect bioelements' and red-ox homeostasis in testes of the exposed rats. The animals were divided into groups according to the duration of treatments (21 and/or 42 days): C_{21}/C_{42} groups (controls); OL_{21} and $OL_{22\text{-}42}$ groups (0.5 mL olive oil intake); $A_{1\text{-}21}$ groups (3 mL 20% ethanol intake); $DSF_{1\text{-}21}$ groups (178.5 mg DSF/kg/day intake); and A_{21}+$DSF_{22\text{-}42}$ groups (the DSF ingestion followed previous 21 days' treatment with alcohol). The measured parameters in testes included metals: zinc (Zn), copper (Cu), iron (Fe), magnesium (Mg) and selenium (Se); as well as oxidative stress (OS) parameters: superoxide anion radical ($O_2^{\bullet -}$), glutathione reduced (GSH) and oxidized (GSSG), malondialdehyde (MDA), hydrogen peroxide (H_2O_2) decomposition and activities of total superoxide dismutase (tSOD), glutathione-S-transferase (GST) and glutathione reductase (GR). Metal status was changed in all experimental groups (Fe rose, Zn fell, while Cu increased in A_{21}+$DSF_{24\text{-}32}$ groups). Development of OS was demonstrated in $A_{1\text{-}21}$ groups, but not in $DSF_{1\text{-}21}$ groups. In A_{21}+$DSF_{22\text{-}42}$ groups, OS was partially reduced compared to A groups ($A_{1\text{-}21}$>MDA>C; $A_{1\text{-}21}$<GSH<C). High metal-binding affinity of DSF/DDTC changes red-ox homeostasis in rat testes.

1. Introduction

Alcoholism is a global social and health problem. Functioning of almost all organ systems is compromised by chronic alcohol intake (Altura and Altura, 1999; Thurman et al., 1999; Kershaw and Guidot, 2008; Djukić et al., 1999a). Recent reports confirmed that prolonged alcohol consummation in man is associated with infertility, decreased testosterone level and increased oxidative stress (OS) (Emanuele and Emanuele, 2001; Uygur et al., 2014).

Metabolism (i.e. elimination) of excessively consumed ethanol follows zero-order kinetics, as capacity of catabolizing enzymes, alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH) in the liver is overloaded (Lieber, 1994). In such circumstances (when blood alcohol concentration exceeds 20 mM), ethanol oxidation proceeds additionally by hepatic microsomal monooxygenases, particularly cytochrome P450 2E1 isoenzyme, which rate of the reaction is ten times higher than those with ADH (Cederbaum, 2010a). Leaking of reducing equivalents (protons, H^+ and electrons, e^-) adheres to such types of redox reaction and contributes to OS development (Rukkumani et al., 2004). Generated reactive oxygen and/or nitrogen species (ROS/RNS) cause peroxidation of unsaturated lipids and oxidation/nitration of proteins and DNA. Disturbance of overall antioxidative defense capacity implies changed antioxidative enzyme activities, depletion of glutathione (GSH) and nicotine amide dinucleotide phosphate (NAD(P)H, accompanied with energy devastation, results in OS and cell death by apoptosis, eventually (Kershaw and Guidot, 2008; Cederbaum, 2010a).

Disulfiram (tetraethylthiuram disulfide, bis[diethylthiocarbam-

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yl] disulfide) (DSF) has been used in aversive therapy of alcoholism for almost 60 years. It inhibits enzyme ALDH (both isoforms, ALDH1A1 and ALDH2) (Moreb et al., 2012). Increased acetaldehyde blood concentration initiates unpleasant symptoms, so-called DSF-ethanol reaction (DER): flushing, palpitation, dyspnea, hypertension, increased pulse rate nausea, vomiting, cyanosis and decreased blood pressure (Djukić et al., 1999a, 1999b; Marcato et al., 2011). Since messenger ribonucleic acid (mRNA) for ALDH1A1, ALDH1A2 and ALDH1A3 is detected in human testes, DSF may induce inhibitory effect on testicular ALDH1 isoenzymes (Nishimtura and Naito, 2006; Zhai et al., 2001).

The DSF metabolism comprises several pathways, including instant reduction into two molecules of diethyldithiocarbamates (DDTCs) that bind copper (Cu); undergoe methylation and conjugation with glucuronic acid; become oxidized into sulphate; and decomposed to carbon disulphide and diethylamine (Deitrich and Erwin, 1971; Lipsky et al., 2001). Dithiocarbamates are potent metal-chelating agents, used in the therapy of nickel and Cu poisonings (Sunderman, 1964). Additionally, many studies have reported on Cu-DDTC anticancer effect (Barth and Malcolm, 2010; Guo et al., 2010).

Essential bioelements, such as zinc (Zn), iron (Fe), Cu, magnesium (Mg) and selenium (Se) play an important role in physiological, metabolic and oxidative cell signaling pathways in mammals (Rotter et al., 2015).

Many studies have shown that various toxicants can cause OS in the testes with detrimental consequences to male fertility (Turner and Lysiak, 2008). In particular, alcohol metabolism contributes to the production of ROS, while DSF demonstrates both, antioxidant and pro-oxidant effect (Cederbaum, 2010b; Das and Vasudevan, 2005; Cunningham and Bailey, 2001; Wadhwa and Mumper, 2013). Since bioelements status interlopes with red-ox homeostasis, herein, we investigated if and if positive, in which extent alcohol and/or DSF *per se* affect bioelements' and red-ox status in testes of the subacutely exposed rats during individual and/or combined exposures (the later one assumes cessation of further alcohol intake, since DSF is introduced). In this line we measured content of bioelements (Zn, Cu, Fe, Mg and Se) and OS parameters [superoxide anion radical ($O_2^{\bullet-}$), GSH and oxidized glutathione (GSSG), lipid peroxidation (LPO) by measuring malondialdehyde (MDA), hydrogen peroxide (H_2O_2) decomposition and activities of total superoxide dismutase (tSOD), glutathione-S-transferase (GST) and glutathione reductase (GR)] in testes of the exposed rats.

2. Materials and methods

2.1. Animals

Adult male Wistar rats (weights of 220–250 g), kept in cages under standardized housing conditions (ambient temperature: 23 ± 2 °C, relative humidity: 55± 3% and a light/dark cycle: 13/11 h), had free access to standard laboratory pellet food and tap water. The rats were adapted to laboratory conditions for two-weeks. Lately, the applied procedures, were performed between 9 a.m. and 1 p.m. The experimental animals were treated accordingly to Guidelines for Animal Study, No. 12032014/9 (Ministry of Agriculture and Environmental Protection – Veterinary Directorate).

2.2. Experimental design

The animals were randomly divided into five major groups, subdivided into minor groups (n = 6), according to the duration of applied treatment: control (C) groups – not treated rats, C_{21} and C_{42}, rats that had free access only to food and water for 21 and 42 days, respectively; olive oil (OL) groups – OL_{21} and $OL_{22\text{-}42}$, rats that received 0.5 mL of olive oil (solvent for DSF) from 21-42 days; alcohol (A) groups – A_1, A_3, A_{10} and A_{21}; DSF groups – DSF_1, DSF_3, DSF_{10} and DSF_{21}; and A+DSF groups – A_{21}+DSF_{22}, A_{21}+$DSF_{22\text{-}24}$, A_{21}+$DSF_{22\text{-}31}$ and A_{21}+$DSF_{22\text{-}42}$ – rats previously treated with alcohol for 21 days and lately with DSF only, from 22 to 42 days.

The animals received daily 3 mL 20% ethanol/kg and/or 0.5 mL of suspended 178.5 mg DSF/kg in olive oil, by gastric intubation. After the treatment, the animals were anesthetized with a 50 mg of sodium pentobarbital/kg injection, followed by decapitation. The testes were removed immediately and stored at -80 °C, until analyzing. The concentrations of Zn, Fe, Cu, Mg and Se and OS parameters ($O_2^{\bullet-}$, total SOD, decomposition of H_2O_2, MDA, GSH and GSSG and activities of GST and GR) were measured in testes homogenates.

2.3. Reagents

All reagents and chemicals were of analytical grade. We used ethylenediaminetetraacetic acid – EDTA, nitrobluetetrazolium - NBT, nitric acid - HNO_3 (65%) from Fisher Chemical, UK; perchloric acid - $HClO_4$ (65%), hydrochloric acid - HCl, sodium hydroxide-NaOH, metaphosphoric acid - MPA, sodium perchlorate, orthophosphoric acid, diethyl ether, 2-methyl butane, kits for Glutathione-S-transferase and Gluthatione reductase determination from Sigma–Aldrich, St. Louis, USA; sodium phosphate -Na_2HPO_4, potassium dihydrogen phosphate - KH_2PO_4, glycerol, paraformaldehyde, trichloroacetic acid - TCA, thiobarbituric acid - TBA, cadmium chloride ($CdCl_2 \cdot H_2O$), metals standard solutions (Cd, Cu, Zn, Mg, Fe) and malondialdehyde bis(dimethylacetal) - MDA from Merck, Germany; sodium pentobarbital Vetanarcol (0.162 g/mL) from Werfft - Chemie, Vienna, Austria; saline solution (0.9% w/v) and deionized water from Hospital Pharmacy Military Medical Academy, Belgrade, Serbia; and Ransod commercial kits for Superoxide dismutase determination from RANDOX Laboratories, UK.

2.4. Analysis of metals

Testes samples (around 1 g) were mineralized with a mixture of the concentrated acids, HNO_3 and $HClO_4$ (4:1, v/v). Mineralized dried samples were diluted to 10 mL with 0.1 M HNO_3. The concentrations of Cu, Zn, Fe, Mg and Se were analyzed with atomic absorption spectrometer (Analyst 200, PerkinElmer) equipped with air-acetylene flame. Relevant metal standard solutions were prepared according to the PerkinElmer Pure Atomic Spectroscopy Standards guidelines (NIST traceable CRM, AccuStandard). The absorption wavelengths were 216.51 nm, 213.86 nm, 305.91 nm, 285.21 nm and 196.05 nm for Cu, Zn, Fe, Mg and Se measurements, respectively. Metal contents in the testicular tissue are expressed as μg/g wet tissue (Cu, Zn, Fe and Mg) and ng/g wet tissue (Se).

2.5. Tissue homogenates for biochemical analysis of redox status

The whole procedure of tissue preparing was performed on ice. Around 100 mg of the testes tissue was transferred into 1 ml ice-cold buffered sucrose (0.25 mol/L sucrose, 0.1 mmol/L EDTA in sodium– potassium phosphate buffer, pH 7.2) and homogenized twice with a Teflon pestle, at 800 rpm for 15 min, at 4 °C (Tehnica Zelezniki Manufacturing, Slovenia). The homogenates were centrifuged at 2500 rpm, for 30 min, at 4 °C. Supernatants were sonicated by three cycles (30s sonication and 15s pause) and used for analysis of OS markers and protein measurements. The samples were stored at -80 °C until analysis.