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Operative and postoperative oxidative status after experimental model of ethanol intake

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Oxidative stress appears to be an integral part of the physiologic stress response that accompanies surgical interventions. Excessive or chronic ethanol consumption preoperatively has deleterious effects to surgical outcomes and leads to considerably increased postoperative general morbidity. Alcohol may play a harmful role on the oxidative status of the organism both through its direct interaction with cellular functions as well as through the products of its metabolism. The aim of the current study was to investigate the effect of binge-like ethanol intake on the serum oxidative stress levels during and after ovariectomy. Our results showed that the alcohol intake resulted in aggravation of the oxidative stress associated with the surgical procedure. We assume that the impaired oxidative status of surgical patients, which is particularly augmented in alcohol-abusing individuals, might be considered a crucial component of the surgical stress and closely linked to the various peri- and postoperative complications. The discussion of appropriate therapeutic approaches to prevent the oxidative stress in alcoholics who are undergoing operations is of great clinical importance and an intriguing field for further research.

Key words: surgical stress, oxidative stress, ovariectomy, alcohol intake, perioperative complications

INTRODUCTION

It is well known that during and after surgical procedures there is a physiologic stress response in the organism that involves sympathetic activation, endocrine reaction, immunologic and hematologic changes. The acute period that follows surgical trauma and reperfusion injury is associated with systemic inflammatory response, increased production of different cytokines and neutrophil activation which play a central role in the generation of reactive oxygen species (ROS) [1]. Oxidative stress (OS) which is described as a result of an imbalance between the accumulation of ROS and their elimination by the antioxidant defense systems, appears to be an integral part of the surgical stress response [2, 3]. The importance of OS in all forms of major surgery and the following recovery has been recognized recently [4]. OS is likely to cause cellular and molecular pathophysiological changes associated with complications that may be related to increased intra- and postoperative morbidity and mortality [5, 6].

Increased alcohol intake has been demonstrated to be coupled to variable problems during surgery such as increased bleeding time and cardiopulmonary insufficiency [7, 8]. Furthermore, alcoholism may also be responsible for severe conditions in the recovery period including withdrawal symptoms, increased risk of infection, delayed wound healing, general infections and increased mortality [9, 10].

Ethanol has multifactorial and multisystemic mechanisms by which single abuse or chronic exposure to alcohol may lead to adverse effects on cellular and molecular functions. Alcohol may play a deleterious role on the systemic oxidative and inflammatory state in the organism both through its direct interaction with cellular components and functions as well as through the products of its metabolism [11]. Experimental data show that ethanol metabolism is associated with excessive formation of reactive oxygen species which have deleterious effects on major biomolecules like lipids, proteins and DNA and this is causing disruption of multiple, if not all, molecular processes in the body [12]. In addition, alcohol affects the levels of antioxidant compounds as well as the activity of endogenic enzymes involved in oxidative balance, and both chronic and binge models of alcohol administration have been associated with increased levels of oxidative stress [13].

Given the aforementioned data, it is clear that the mechanisms underlying ethanol-induced complications during surgical procedures, including disruption of oxidative balance, are complex and need more detailed investigation. The aim of the present study was to evaluate the effect of ethanol intake on the serum oxidative stress levels during the su rgical intervention and in the postoperative period of experimental ovariectomy.

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EXPERIMENTAL

The experiments were carried out in accordance with the Bulgarian regulations on animal welfare, in conformance with the European Communities Council Directive of 24 November 1986 (86/609/EEC) and with the approval of Medical University-Sofia ethics committee, Protocol #1156/25.06.2008.

Animals

Female Wistar rats (0.200±0.02 kg) body weight, (BW) were housed in standard polypropylene cages and maintained in a temperature (20±0.5°C) and humidity (65±1%) controlled room. 2-3 days before the experiment the animals were handled and then randomly assigned to two groups (n=5). The alcohol model was developed for 7 days prior the surgical procedure. In that period the control group (C) was gavaged with physiological solution, while the group named "Alcohol" (A) was gavaged with ethanol as described below. On the 8th day OVX was performed.

Alcohol administration

Rats were subjected to a modified binge-like model of alcohol exposure [14]. Briefly, the rats received 2.34 g/kg dose of ethanol (30% water solution of ethanol) every 24 hours for 7 days. Food was removed during the period of alcohol administration and water was ad libitum. Tail blood samples were taken during the surgical procedure, on the first postoperative day and three days later (11th day). On the 12th day all animals were exterminated, their blood was collected and blood serum was extracted and stored at −20°C until further analysis.

Ovariectomy (OVX)

OVX is an operative method for removal of large ovarian cysts, cancerous ovaries and other pathological conditions. Since OVX is generally performed by two surgical approaches - laparotomy and laparoscopy, it is commonly used in experimental studies to evaluate the benefits and risks of each of them, including the oxidative status, related to the surgical procedure [15].

Ovariectomy was performed under Ketaminol (0,08ml/100g, i.p.) anaesthesia after the 7th day of the alcoholic model. The surgical procedure involved bilateral removal of the rat ovaries. Complete ovariectomy was evaluated by visual inspection of the ovaries after their extraction.

Chemicals

All chemicals used in this investigation were of highest grade, purchased from SIGMA-ALDRICH. K, Na-phosphate buffers (PBS) of pH 7.45, 7.80 and 7.00 were prepared using bi-distilled water. For the xanthine oxidase (XO) assay was prepared 0.3 mM solution of xanthine (X). TRIS-HCl buffer of pH 8.2 and 3 mM pyrogallol solution were prepared for SOD activity determination. Fe(II)/EDTA solution along with 3 mM water solution of H_2O_2 were used to determine the MDA accumulation, the H_2O_2 being used for analysis of CAT activity.

The biochemical analysis was performed using Shimadzu 1601 spectrophotometer equipped with software package and quartz cuvettes.

Markers of oxidative stress and antioxidant activity

In this investigation we used two markers of oxidative stress: the activity of Xanthine oxidase, which *in vivo* leads to the formation of superoxide and hydrogen peroxide, and malondialdehyde (MDA), which is a product of profound lipid peroxidation. The markers for antioxidant activity were Cu,Zn-SOD, which transfers the superoxide into hydrogen peroxide, and CAT - the enzyme which eliminates the hydrogen peroxide.

Xanthine oxidase assay: The activity of XO was evaluated using the characteristic signal for uric acid (UA) at 293 nm. 1 ml of the sample solution contained blood serum with 1 mg proteins, 0.01 ml X solution and PBS (pH 7.45). The intensity of the band at 293 nm was recorded for 10 minutes. The activity of XO was calculated in units (U), 1U corresponding to the amount of XO which converted 1 μl of xanthine for 1 minute in 1 ml solution at 298K. The activity of XO was measured 5 times for each animal. After elimination of the gross errors, the average and standard deviations of the group activity were determined. The XO activity for each group was presented as percentage of the day 1st ones.

MDA assay: The relative increase of the characteristic band of MDA at 145 nm was monitored using quartz cuvette. One ml of the solution contained 0.02 ml water solution of Fe(II)/EDTA, 0.005 ml H_2O_2 solution, blood serum with 1 mg proteins and PBS (pH 7.45). The background was measured in the presence of blood serum with 1 mg proteins and PBS. In the control measurement the blood serum was omitted. The background, control and sample measurements were used in the calculations. Each measurement took 10 minutes and was repeated 5 times. The amount of MDA formed for 1 minute in the presence of 1 mg proteins was calculated. The average and standard deviations of MDA formed in each group were determined and presented as percentages of the day 1st ones.

***Cu,Zn-SOD (SOD) assay*:** The activity of SOD was determined using pyrogallol auto-oxidation. The relative change of the band at 420 nm was monitored for 10 minutes at 298 K. One ml of the sample cuvette contained blood serum with 1 mg proteins, and pyrogallol. In the control sample the blood serum was omitted, while the blank measurement was performed in the presence of blood serum in TRIS-HCl buffer. The activity of SOD was determined by subtracting the blank from the sample data, dividing the result to the control measurement and finally multiplying it by 100. The average and standard deviations for the groups were calculated and presented as percentage of the day 1st ones.

CAT assay: The CAT activity was measured by monitoring the characteristic band for hydrogen peroxide at 240 nm, for 3 minutes at pH 7 of the medium. One ml of the sample cuvette contained blood serum with 1 mg proteins, 0.02 ml 3 mM H_2O_2 and PBS (pH 7.00). The background was recorded in the presence of blood serum alone in PBS, and in the control sample the serum was omitted. The activity of CAT was calculated in units. One unit of CAT converted 1 μM hydrogen peroxide for 1 minute at 298K. Each measurement was repeated 5 times. The average and standard deviations of CAT activity of each group were calculated and presented as percentage of the day 1st ones.

***Statistical analysis*:** The differences among average data were statistically analyzed using one way ANOVA with Bartlett's test for the standard deviations and Bonferroni post-test.

RESULTS

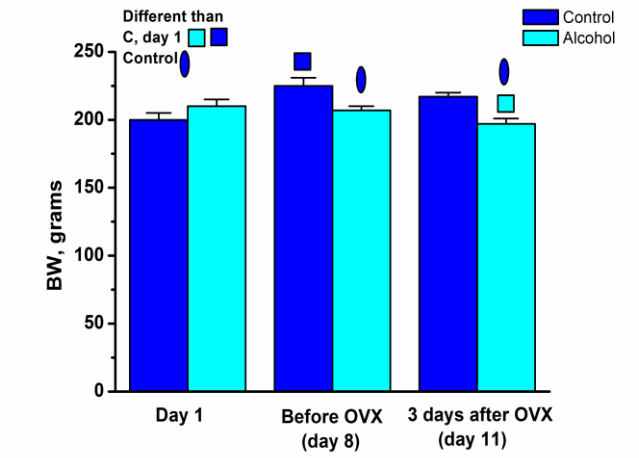

Figure 1. BW of the experimental animals in three time intervals: at the beginning of the experiment, after developing the alcohol model and right before the OVX, and 3 days after the surgical procedure; statistically significant differences at p<0.005.

The control group showed significant increase in BW compared to the first day, while the BW of the alcohol group did not change. The A group's BW at day 8 was significantly lower than this of the controls. Three days after the operation the BW of group C was lower than before the operation, but equal to this at the first day. The BW of group A was lower than this at the first day and lower than this of the C group at the same day.

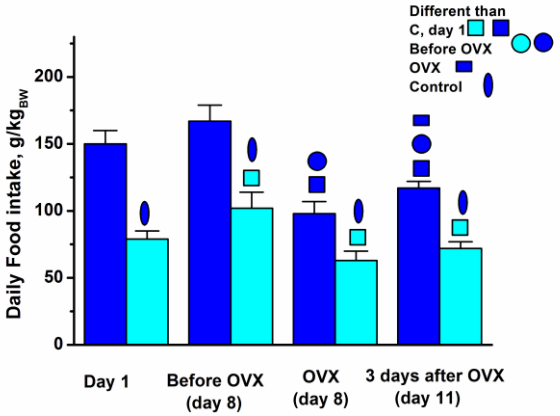

Figure 2. Daily food intake of the experimental animals; statistically significant differences at p<0.005.

Compared to the controls, the animals receiving alcohol showed very poor appetite during the entire experiment. Interestingly, after OVX the appetite of the controls decreased and restored three days after the operation. This trend was not observed in the A group.

The daily water consumption of the alcohol group was significantly higher than this of the control group. The water consumption of the controls at the day of the OVX and in the postoperative period was significantly lower than before the operation.

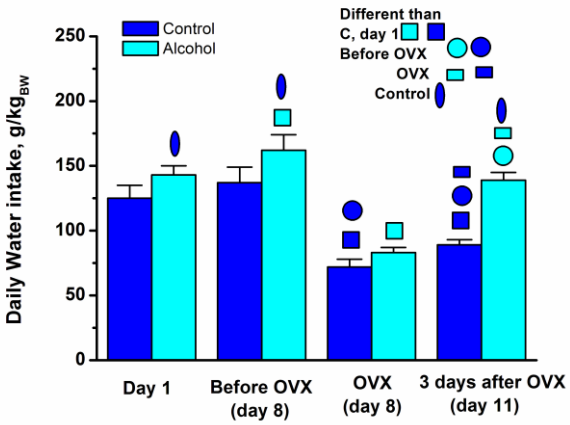

Figure 3. Daily water intake of the experimental animals; statistically significant differences at p<0.005.

The serum XOA was the same in both groups at the first day. Before OVX the A group showed markedly increased XOA compared with the C group. At the day of the OVX XOA of both groups

significantly increased compared to the previous day and the one of group A was significantly higher compared to group C. In the postoperative period the XOA of the controls decreased, but was still higher than the one before the operation. The same trend was observed in group A. XOA of the alcohol group remained much higher than the one of the controls during the postoperative period.

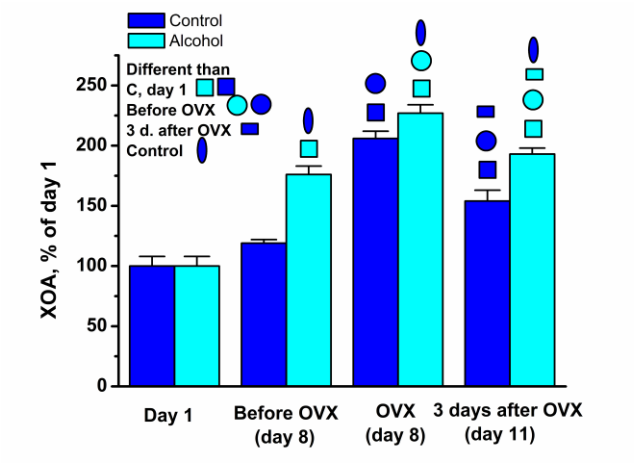

Figure 4. Serum xanthine oxidase activity of the experimental animals expressed as percentage of the 1st day XOA activity of each group; statistically significant differences at $p<0.005$.

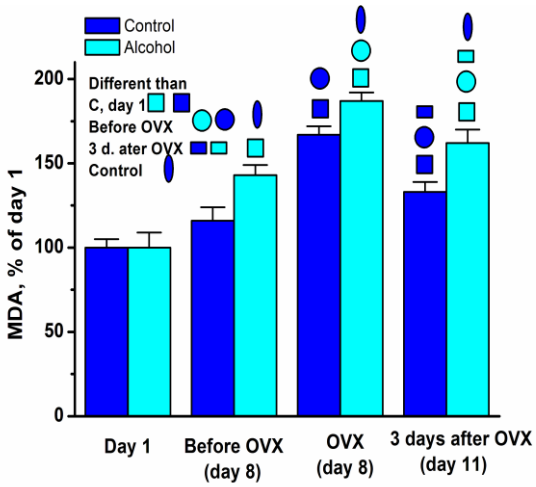

Figure 5. Serum MDA levels of the experimental animals expressed as percentage of the 1st day levels of each group; statistically significant differences at $p<0.005$

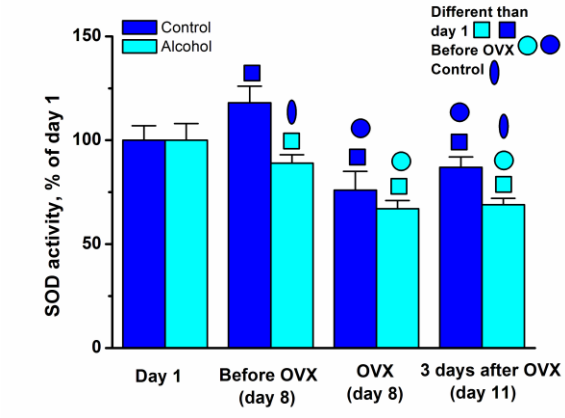

Figure 6. Serum SOD activity of the experimental animals expressed as percentage of the 1st day SOD activity of each group; statistically significant differences at $p<0.005$.

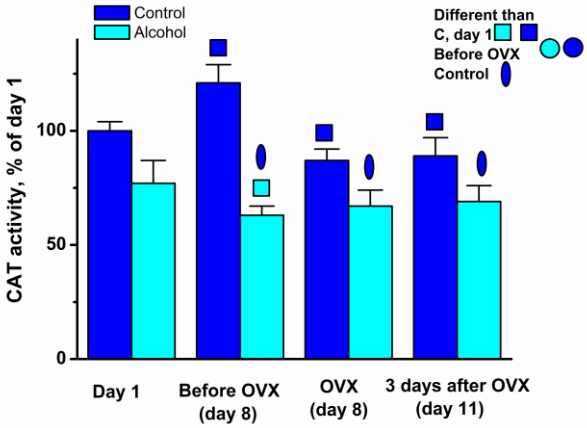

Figure 7. Serum CAT activity of the experimental animals expressed as percentage of the 1st day CAT activity of each group; statistically significant differences at $p<0.005$.

The alcohol consumption led to increased MDA accumulation compared to the control group. On the OVX day both C and A groups demonstrated increased MDA accumulation. The serum MDA of group A during the OVX was significantly higher than this of group C. In the postoperative period the MDA accumulation decreased in both groups, but remained higher for the alcohol consuming animals.

In the pre-operative period the SOD activity (SODA) of the controls increased, while this of the alcohol group decreased, compared to the first day. During OVX SODA decreased in all animals to levels lower than the first day ones. In the post-operative period SODA did not significantly change compared to the OVX day and was higher in group C, compared to group A.

At the first day CAT activity (CATA) was not significantly different among the groups. At the 8-th day the CATA of the controls was improved, while this of the alcohol group decreased. During OVX the CATA decreased in both groups, and its level did not change in the postoperative period.

DISCUSSION

The results of the current study showed that alcohol intake led to delay in the body weight gain of the experimental rats and was associated with a longer recovery in the immediate postoperative period. Naturally, the anesthesia and the surgical

procedure caused a decrease in the appetite of both groups. However, the appetite of the controls increased with time in the postoperative period, while that of the alcohol group did not improve significantly.

In addition, the thirst of the alcohol consuming animals before OVX was much stronger than this of the controls. In the alcohol group, unlike in the control one, the thirst in the postoperative period increased and the amount of drinking water they used was equal to the first day of the experiment. The body weight gain seemed to be related to the appetite of the animals rather than any water retention.

Our data presented here are in agreement with previous reports for postoperative fatigue and decreased appetite, which are particularly pronounced in patients consuming alcohol [16]. Both physiological and psychological factors can be related to decreased or loss of appetite which can exacerbate patient wellness, increase the risk for nutrient-related illnesses and prolong the postoperative recovery period of those suffering from alcohol dependence or withdrawal [17].

The current study showed that administration of ethanol resulted in increased activity of serum XO in the alcoholic experimental animals compared to the non-alcoholic controls (Fig. 4). Most likely, there was an intensive production of superoxide radicals and hydrogen peroxide in the alcohol group which could be associated with a prominent tissue damage. Furthermore, this was related to higher serum levels of MDA in the alcohol consuming rats (Fig. 5), which is a well-known marker for lipid peroxidation and profound tissue damage and acts as an indicator of oxidative stress.

Moreover, the data in Figure 6 and Figure 7 indicate an alteration in the antioxidant activity of the endogeneous antioxidant enzymes in the group with binge-like alcohol consumption. There was detected a decreased SOD activity, accompanied by lower CAT activity in the serum of the alcoholic group, compared with the control group. This suggested accumulation of superoxide and hydrogen peroxide which possessed an essential pro-oxidant potential. In consent with our observations, a considerable amount of experimental reports show that excessive ethanol consumption disrupts the anti-/pro-oxidative balance in the organism which is associated with a series of pathological conditions [18, 19]. In addition, we have recently reported for increased XOA and MDA levels and oxidative stress in brains of rats in a model of chronic alcohol intake [20].

Numerous experimental and clinical studies demonstrate increased oxidative stress during and after major surgical procedures including ovariectomy [21, 22, 23]. Besides, the occurrence of potential side effects and complications peri- and postoperatively is more significant with acute or chronic alcohol use prior to surgery [24]. It is reported that preoperative ethanol consumption is deleterious to surgical outcomes, with a considerable increase in postoperative general morbidity [9]. The adverse effects of perioperative alcohol use include biochemical, immunological and hematological abnormalities [25]. Since oxidative stress is proposed to be a substantial determinant of surgical stress severity [26, 27], it is intriguing to discuss our results on the impact of ethanol consumption on the oxidative status during OVX and postoperatively.

Our data demonstrate that the OVX procedure was associated with increased XOA and MDA levels in both groups (Figures 4 and 5). Also, there was observed impaired physiological antioxidant capacity of the experimental animals - both SOD and CAT activities were decreased (Figures 6 and 7). Figure 4 and 5 show that alcohol intake further increased the MDA levels, throughout the entire experiment and particularly during the OVX. Additionally, the activities of SOD and CAT were lowest during the surgical intervention and immediately after it, and both were significantly lower in the alcohol-consuming group compared with the controls.

According to our results it may be accepted that the binge-like alcohol model resulted in aggravation of the oxidative stress associated with the OVX procedure. We assume that the impaired oxidative status of surgical patients, which is particularly augmented in alcohol abusing individuals, might be considered a crucial component of the surgical stress and closely linked to the various peri- and postoperative complications.

Our conclusion is that in surgical individuals that have been using alcohol in the short or long terms prior to operations, assessment of oxidative stress levels, improving understanding of its role, as well as discussing appropriate therapeutic approaches to prevent it are of great clinical importance and represent an intriguing field for further research.

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