Acamprosate Modulates Alcohol-Induced Oxidative Stress In Alcoholic Patients
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ABSTRACT
Some authors have suggested possible loss of antioxidant levels in alcoholic subjects. Acamprosate may have modulator effect on antioxidant levels in the alcoholic subject due to its effects on Ca2+ regulation. We investigated effects of acamprosate on serum lipid peroxidation and antioxidant vitamin concentrations in alcoholic patients.

Twelve male health control and twelve alcoholic patients were used in the study. After taken blood samples the patients received orally acamprosate for 3 weeks and blood samples were taken the patients again.

Lipid peroxidation (LP) levels were higher in the alcohol group than in control although vitamin A, vitamin E and β-carotene values were lower in the alcohol group than in control. LP levels were decreased in treatment group compared with the alcohol group. Vitamin A and β-carotene in the treatment group were increased compared with the alcohol group. Serum vitamin C concentrations were not found to be statistically different in any of the groups.

Oxidative stress has been proposed to explain the biological side effects of alcohol intake. Acamprosate modulated alcohol-induced oxidative stress and antioxidant vitamin levels in the alcoholic patients.

Key words: Oxidative stress, alcohol addiction, antioxidant vitamin, blood, acamprosate.

INTRODUCTION
Oxidative stress is defined as an imbalance between higher cellular levels and reactive oxygen species (ROS) e.g. superoxide and hydroxyl radicals (Nazıroğlu, 2007a) and cellular antioxidant defense (Reinke, 2002). Generation of ROS is ubiquitous since ROS are generated during aerobic metabolism i.e., mitochondrial oxidations and phagocytosis. In order to scavenge ROS, various defense systems exist in blood. Vitamin E (α-tocopherol) is the most important antioxidant in the lipid phase of cells (Zingg and Azzi, 2004; Nazıroğlu, 2007a). Vitamin C (ascorbic acid), as well as being a free radical scavenger, also transforms vitamin E to its active form (Frei et al., 1989). Vitamin A (retinol) serves as a prohormone for retinoids and is involved with signal transduction at cytoplasmic and membrane sites (Halliwell, 2006).

Alcohol indirectly induces alterations of membrane fluidity, channel and pumps, and ionic transients. Recently, several studies have examined the role of oxidative stress in development of alcohol mediated blood and tissue toxicity, possibly via the formation of ROS (Shirpor et al., 2008 Çalışkan et al., 2010). Alcohol initiates the oxidation of mitochondrial and cytoplasmic membrane, lipids, damaging membranes and leading to the formation of ROS (Siler-Marsiglio et al., 2004). Some authors have been suggested that alcohol have been shown to induce lipid peroxidation and to decrease antioxidant such as glutathione and vitamin C level due to the generation of ROS in animal and human alcoholics (Fernández-Solá et al., 1998; Shirpor et al., 2009; Çalışkan et al., 2010).

Acamprosate has been introduced for treating alcohol craving and reducing relapses in weaned alcoholics (Bachteler et al., 2005). It is well known that increase of cytosolic Ca2+ causes increased production of ROS via depolarization of mitochondria and activation mitochondrial enzymes (Nazıroğlu, 2007b). Acamprosate may exert antioxidant role via its action through the mitochondrial oxidative stress system and the glutamatergic (via NMDA receptors) or GABAergic systems (Chen et al., 1997; Berton et al., 1998; Allgaier et al., 2000) and the subject should be clarified in an alcoholic human model.

ABBREVIATIONS
LP : Lipid peroxidation
MDA : Malondialdehyde
PUFAs : Polyunsaturated fatty acids
ROS : Reactive oxygen species
SOD : Superoxide dismutase

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To our knowledge there is no research on oxidant/antioxidant effects of acamprosate in alcoholic patients. We aimed to evaluate whether there would be effects of acamprosate on oxidative stress levels and antioxidant vitamin concentrations in serum of alcoholic patients.

### Subjects and methods

#### Chemicals

All chemicals (KOH, NaOH, thiobarbituric acid, 1,1,3,3 tetraethoxy propane) were obtained from Sigma-Aldrich Chemical Inc. (St. Louis, MO, USA) and all organic solvents (n-hexane, ethyl alcohol) from Merck Chemical Inc. (Darmstadt, Germany). All reagents were analytical grade. All reagents were prepared daily and stored at +4 °C. The reagents were equilibrated at room temperature for half an hour before an analysis was initiated or reagent containers were refilled.

#### Healthy untreated controls and alcoholic patients

The Ethics Committee of the Medical Faculty of Suleyman Demirel University (SDU) approved the study plan. All subjects volunteered for the trial and they gave written consent. Alcoholic patients were recruited from outpatient clinics within the Medical Center of SDU. The controlled study was performed on 12 male patients and 12 controls. Mean ages of patients and control subjects were 42.3 and 39.2, respectively. Male nurses and workers in the Medical Center of SDU formed the healthy untreated control groups in the study. Routine blood tests, erythrocyte sedimentation rate, liver and kidney function tests and enzymes, thyroid hormone concentrations and sex hormone profiles of the patients and controls were evaluated. Patients taking anti-inflammatory and systemic drugs or antioxidant vitamin supplements within the previous 6 months were excluded, as were patients with liver or thyroid diseases. Both control and patients were smokers. They had no clinical or biochemical evidence of severe liver disease, encephalopathy, ascite. Liver biopsies were not performed due to ethical reasons.

#### Study Groups and Exercise

Acamprosate was dissolved in physiological saline (0.9%, w/v). Forty male rats were randomly divided into four equal groups as follows:

- **Group I** was a control group (n=12) and placebo was orally given to this group for 21 days.
- **Group II** was an alcoholic patient group. Blood samples were taken the patients.
- **Group III** was an acamprosate group. The patients in group II received oral 1998 mg/dL of acamprosate tablet [Acamprosat calcium (campral), Merck Medicine and Chemistry Inc., Istanbul, Turkey] for 21 days. After 12 hours from the administration of acamprosate, fasting blood samples were taken from the patients again.

#### Lipid Peroxidation (LP) Level Determinations

LP levels in the serum samples were measured with the thiobarbituric-acid reaction by the method of Placer et al. (1966). The quantification of thiobarbituric acid reactive substances was determined by comparing the absorption to the standard curve for malondialdehyde (MDA) equivalents generated by acid catalyzed hydrolysis of 1,1,3,3 tetramethoxypropane. The values of LP in the serum were expressed as µmol/L.

Although the method is not specific for LP, measurement of the thiobarbituric-acid reaction is an easy and reliable method, which is used as an indicator of LP and ROS activity in biological samples.

#### Serum Antioxidant Vitamin Analyses

Vitamins A (retinol) and E (α-tocopherol) were determined in the serum samples by a modification of the method described by Desai (1984) and Suzuki and Katoh (1990). Serum samples of about 250 µL were saponified by the addition of 0.3 ml 60 percent (w/v in water) KOH and 2 ml of one percent (w/v in ethanol) ascorbic acid, followed by heating at 70°C for 30 min. After cooling the samples on ice, 2 ml of water and 1 ml of n-hexane were added and
mixed with the samples and then rested for 10 min to allow phase separation. An aliquot of 0.5 mL of n-hexane extract was taken and vitamin A concentrations were measured at 325 nm. Then reactants were added and the absorbance value of hexane was measured in a spectrophotometer at 535 nm. Calibration was performed using standard solutions of all-trans retinol and α-tocopherol in hexane.

The concentrations of β-carotene in serum samples were determined according to the method of Suzuki and Katoh (1990). Two ml of hexane were mixed with 250 µL serum. The concentration of β-carotene in hexane was measured at 453 nm in a spectrophotometer.

Quantification of ascorbic acid in the serum samples was performed using the method of Jagota and Dani (1982). The absorbance of the samples was measured spectrophotometrically at 760 nm.

Statistical analyses

All results are expressed as means ± SD. Presence of statistical significances were firstly analyzed by Kruskall-Wallis test. To determine the effect of treatment, data were analyzed using Mann-Whitney U test. P-values of less than 0.05 were regarded as significant. Data was analyzed using the SPSS statistical program (version 9.05 software, SPSS Inc. Chicago, Illinois, USA).

Results

Lipid peroxidation (LP)

The serum LP levels in three groups are shown in Figure 1. Mean LP values as μmol/L in control, patients and treatment groups were 1.07, 1.45 and 1.05, respectively. The results showed that serum LP levels were significantly (p<0.05) in patient group than in control. However, serum LP levels were significantly (p<0.05) lower in treatment group than in control.

Antioxidant vitamin concentrations

The mean serum antioxidant vitamin concentrations in the three groups are shown in Table 1. The results showed that the serum vitamin A (p<0.05), β-carotene (p<0.01) and vitamin E (p<0.01) concentrations were significantly lower in alcohol group than in the control. Hence, serum vitamin A (p<0.05), β-carotene (p<0.01) and vitamin E concentrations were decreased by the alcohol consumption. Vitamin A (p<0.05) and β-carotene (p<0.01) concentrations were significantly higher in treatment group than in the patient. However, the serum vitamin C concentrations did not change significantly in the three groups as a result of the treatments.

Discussion

We found that, serum LP values increased in alcoholic patients, whereas serum vitamin A, vitamin E and β-carotene concentrations decreased. Hence, the alcoholic human model is characterized by increased LP and decreased antioxidant vitamins concentrations. To our knowledge we are the first to report that administration of acamprosate caused increase of serum vitamin A and β-carotene concentrations in alcoholic patients.

The current study indicated that alcohol administration produced a significant increase in LP levels of the serum of alcoholic patients. Our results are in accordance with the previous reports of LP increment in brain, hippocampus and cerebellar granule cells (Siler-Marsiglio et al., 2005; Shirpoor et al., 2008 and 2009). On the other hand, the current study is the first report of serum LP in acamprosate administered alcoholic patients. Alcohol may also trigger a variety of biochemical process including the activation of membrane phospholipases, proteases and nucleases (Lieber, 2000). Marked alterations in membrane phospholipid metabolism resulted in the oxidation of mitochondrial and membrane lipids, damaging membranes and leading to the formation of highly reactive oxidative components (Siler-Marsiglio et al., 2005). Free radical generation by the alcohol-induced brain cytochrom p450 plays also a key role in oxidative stress (Shirpoor et al., 2008). Inhibitors of this enzyme have great promise, and are presently being evaluated clinically. The system is particularly interesting because of its innocuity (Lieber, 2000). Hence, involvement of lipid peroxidation as MDA in alcohol administration can be attributed to the activation of membrane phospholipases and cytochrom p450 in brain. In the current study, vitamin A, vitamin E and β-carotene concentrations in the serum of alcoholic patients were decreased by alcohol intake. If the antioxidant vitamins decrease, superoxide radical production may increase and finally lead to oxidative stress and LP (Halliwell, 2006; Naziroğlu,
This reduces the capacity of the brain cell to rid itself of excess LP levels in the cytoplasm and mitochondria because of decreasing antioxidant vitamin concentrations (Reinke, 2002).

In the current study we observed decrease of vitamin A, vitamin E and β-carotene concentrations in serum of alcoholic patients. These results are similar with previous findings, suggesting a link between decrease in tissue antioxidants and pathogenesis alcoholic disease. In this sense, Ward and Peters (1992) reported a reduction in antioxidant vitamins in muscle of short-term ethanol administrated rats, as well as a decrease of plasma values in alcoholic patients with alcoholic cirrhosis or myopathy. Similarly, Cook et al. (1991) reported low concentrations of serum vitamin E in alcoholics. Fernandez-Solà et al. (1998) reported that serum and muscle levels of vitamin A, vitamin C and vitamin E were found to be normal in well-nourished chronic alcoholic patients not submitted to previous vitamin supplementation.

Two main pathways, plasma membrane Ca\(^{2+}\)- permanent channels and intracellular Ca\(^{2+}\) releasing channels for transient changes in free cytosolic Ca\(^{2+}\) amount that are most important in cell signaling (Naziroğlu, 2009). One of the plasma membrane Ca\(^{2+}\)-permanent ion channels in neuronal plasma membrane is NMDA receptors, and this has been the focus of many studies related to the inhibitory effects of ethanol on receptor function (Al Qatari et al., 1998; Allgaier et al., 2000). It is well known that increase of cytosolic Ca\(^{2+}\) causes increased production of ROS via depolarization of mitochondria and activation mitochondrial enzymes (Naziroğlu, 2007b). Acamprosate has been introduced for treating alcohol craving and reducing relapses in weaned alcoholics (Bachteler et al., 2005). In the current study we observed antioxidant effect of acamprosate in serum of alcoholics. Acamprosate may exert its action through the mitochondrial oxidative stress system and the glutamatergic (via NMDA receptors) or GABAergic system (Chen et al., 1997; Berton et al., 1998; Allgaier et al., 2000). The underlying mechanism of acamprosate-induced neuronal repair appears to be due to its action in decreasing the intracellular Ca\(^{2+}\) level. In accordance with the reports, we observed in the current study that the dose of acamprosate induced antioxidant effects due to its regulator effect on NMDA receptors. Contrary to results of the current study we observed oxidant effects of acamprosate alcohol-induced oxidative stress in brain cortex of rat (Çalışkan et al., 2010).

Inactivation of ROS can be carried out by antioxidant vitamins (Naziroğlu, 2007a). Vitamin E, α-tocopherol, is the most important antioxidant in the lipid phase of cells. Vitamin E acts to protect cells against the effects of free radicals, which are potentially damaging byproducts of the body’s metabolism (Zingg and Azzi, 2004). Therefore, low antioxidant levels result in limited antioxidant defense in blood. Vitamin E concentrations in the serum of alcoholic patients were slightly increased in the acamprosate group. The increased concentration of the antioxidant vitamins at the dose of acamprosate tested in our study could be due to its inhibition as a result of the increased production of free radicals. Similarly Siler-Marsiglio et al. (2005) reported that LP levels were decreased after administration of mitochondrially targeted vitamin E (MitoE) and normal vitamin E in the brain homogenates of ethanol consumption rats.

In conclusion, serum concentration of antioxidants, vitamin A, E and β-carotene were found to be influence the presence in alcoholic patients. However, acamprosate modulated alcohol-induced antioxidant vitamin decrease in alcoholic patients. The alcoholic patients should take the antioxidant vitamins because of the oxidant effect of alcohol intake.

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References


