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EFFECT OF ALCOHOL ON LIPID LEVELS IN THE BRAINS OF RATS

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ABSTRACT

Purpose: To investigate the effects of alcohol consumption on rat brain cholesterol, triglyceride, and phospholipids levels.

Materials and methods: The study was performed on 24 Sprague-Dawley rats of which 12 were selected as the control group and 12 as the alcohol group. Both groups were fed regular laboratory chow. In addition, the alcohol group rats were fed 15% (v/v) of ethanol in their drinking water for 2 months. Then, the rats were decapitated under ketamine anesthesia and the brain tissues were removed and homogenized in ice-cold 0.1 M phosphate buffer containing 1% digitonin. The tissues were centrifuged and brain cholesterol, triglyceride, and phospholipid levels of supernatants were measured using commercially available enzymatic kits.

Results: Brain cholesterol, triglyceride, and phospholipid levels of the alcohol group were 29.36 ± 1.68, 39.81 ± 8.5, and 2.91 ± 0.82, respectively, whereas the same parameters of the control group were 22.58 ± 2.29, 32.57 ± 3.7, and 2.47 ± 0.64mg/ g protein, respectively. Brain cholesterol ($p<0.001$) and triglyceride ($p<0.05$) levels differed significantly between the groups whereas phospholipid levels did not.

Conclusion: The effects of alcohol on brain cholesterol and triglyceride levels may result in significant disturbances in brain functions. The underlying mechanism of this finding is not known and needs to be investigated further.

Key Words: Ethanol, Brain, Cholesterol, Triglyceride, Phospholipids, Rat 2.

ALKOLÜN SIÇANLARDA BEYİN LİPİD SEVİYELERİNE ETKİSİ

ÖZ

Amaç: Biz çalısmamızda alkol tüketiminin sıçan beyninde kolesterol, trigliserid ve fosfolipid seviyelerine etkisini arastırdık.

Gereç ve yöntem: Bu amaçla 24 Sprague-Dawley disi sıçan, alkol (n=12) ve kontrol (n=12) grubu olmak üzere iki gruba ayrıldı. Her iki grup çalısma süresince normal laboratuvar yemi ile beslendi. Kontrol grubuna normal musluk suyu verilirken alkol grubunun içme sularına %15 (v/v) oranında etanol ilave edildi. İki aylık beslenme süresinden sonra sıçanlar ketamin anestezisi altında dekapite edildi ve beyin dokuları çıkarılarak %0.1 digitonin içeren, 0.1 M soğuk fosfat tamponu içinde homojenize edildi. Homojenatlar santrifüj edildikten sonra süpernatanları alındı ve rutin metodlarla kolesterol, trigliserid ve fosfolipid düzeyleri çalısıldı.

Bulgular: Alkol grubunda beyin kolesterol, trigliserid ve fosfolipid düzeyleri sırasıyla 29,36 ± 1,68, 39.81 ± 8,5 ve 2,91 ± 0,82 kontrol grubunda ise 22,58 ± 2,29, 32,57 ± 3,7 ve 2,47 ± 0,64 mgr/gr protein olarak bulundu. Her iki gruba ait beyin kolesterol ($p<0.001$) ve trigliserid ($p<0,05$) düzeyleri arasındaki fark istatistiki açıdan önemli, fosfolipid düzeyleri arasındaki fark ise önemsizdi.

Sonuç: Alkolün beyin kolesterol ve trigliserid düzeyini artırıcı yöndeki bu etkisi beyin fonksiyonlarında önemli bozukluklara neden olabilir. Alkolün beyin lipidleri üzerine olan bu etkisinin mekanizması bilinmemekte ve daha ileri çalısmarla aydınaltılması gerekmektedir.

Anahtar Kelimeler: Etanol, Beyin, Kolesterol, Trigliserid, Fosfolipid, Sıçan 3.

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INTRODUCTION

Excessive consumption of alcohol by a large proportion of the population is still a prominent medical and social problems in many countries. Ethanol permeates to all tissues of the body and affects most vital functions of the organs. It is known that chronic alcoholism leads to degenerative and inflammatory changes in many organs, including the liver, brain, kidney, heart, skeletal muscle, stomach, and pancreas. In addition, many neurological lesions and even cerebral atrophia may develop in alcoholics.[1-3] Lipids are an important constituent of the brain, not only because of myelin, but also because of the large surface-to-volume ratio of neurons, which contain a higher proportion of lipid than other cells.[4] The dry weight of an adult brain is 50-60% lipid, of which approximately 35% is in the form of polyunsaturated fatty acids (PUFAs) [5]. The central nervous system (CNS) accounts for only 2.1% of the whole body weight but it contains approximately 23% of the total amount of the cholesterol present in humans. [6] The brain is a site of high lipid turnover. Although there is selective uptake of essential PUFAs into the brain, cholesterol and nonessential fatty acids do not enter the brain parenchyma. [7] The input of cholesterol into the CNS comes almost entirely from in situ synthesis, and there is currently little evidence for the net transfer of sterol from the plasma into the brain of the fetus, newborn or adult.[8] Thus, there is a highly efficient recycling of cholesterol in the brain, with minimal losses to the circulation.[7] It has been reported that administration of ethanol to rats causes changes in the metabolism of serum and tissue lipids. The lipid abnormalities seen after alcohol consumption include alterations in the level of cholesterol, fatty acid esters, cholesterol esters, and particularly the fatty acyl composition of membrane phospholipids (PLs).[4]

Indeed, ethanol was reported to be a powerful inducer of hyperlipidemia in both animals and humans.[9,10] Furthermore, ethanol treatment in rats was shown to cause the translocation of fat from the peripheral adipose tissues to the liver, kidney and brain for accumulation.[10] Since ethanol is lipophilic, it readily crosses the blood brain barrier and enters the CNS.[11] Alcohol affects brain composition and functions in various ways. One of the various cellular mechanisms of ethanol toxicity is the alteration in membrane structure and function. Alterations in lipid components by ethanol can cause changes in membrane function by altering its fluidity [1] and animals can compensate for the increased fluidity of their membranes by altering the incorporation of PUFAs as a mechanism of adaptation to alcohol.[12] In the present study, we investigated the effect of alcohol consumption on rat brain cholesterol, triglyceride (TG) and PL levels.

MATERIALS AND METHODS

This study was performed on 24 inbred female Sprague-Dawley rats at the age of 4 months. The rats were supplied by the Center for Experimental and Applied Medical Research, University of Selçuk. They were separated into two groups (control group and alcohol-fed group) and fed regular laboratory chow consisting of 24% protein, 3.62% fat, 7% cellulose, 10% ash and 12% water. Environmental conditions (humidity, heat, ventilation etc.) were kept constant for 24 hours daily during the period of the study. The animals were housed in conventional wire-mesh cages at room temperature regulated at 21±1 °C, humidity 45-50% and with 12 h light/dark cycles. The study group animals were fed 15% (v/v) ethanol in their drinking water for 2 months.[13,14] After that period, the rats were decapitated between 9 and 10 am under ketamine anesthesia and their brains were quickly removed and washed in cooled 0.15 M NaCl. The brain tissues were homogenized in 2 ml of ice-cold homogenizing buffer (100 mM KH2PO4-K2HPO4, pH: 7.4, 0.1% digitonin) by an ultrasonic homogenizer (Musonix Microson Ultrasonic Cell Disruptor CML).[15] The homogenates were then centrifuged at 5000 rpm for 15 min at 4 °C. The supernatants were removed and cholesterol, TG and PL levels were measured. All procedures were performed following the Guide for the Care and Use of Laboratory Animals. The study was approved by the Ethics Committee of Meram Faculty of Medicine, University of Selçuk. Cholesterol, TG and PL levels of supernatants were measured by commercially available enzymatic kits: Randox, CH 202; Randox, TR 213; and Roche, MPR 2 691844, respectively. Tissue protein levels were determined by the Biuret method.[16] The results were calculated as mg/g protein. Statistical evaluation.

Statististical Evoluation

The data were analyzed using SPSS for Windows, version 10.0. Statistical differences between the groups were evaluated using an independent t-test. P values <0.05 were considered statistically significant.

RESULTS

The results are given in Table 1 as mean ±standard deviation (SD). As seen from the table and figures, both cholesterol ($p<0.001$) (Fig. 1) and TG ($p<0.05$) (Fig. 2) levels in the alcohol group were significantly increased, whereas brain PL (Fig. 3) levels were slightly but not significantly increased compared to those in the control group.

Table 1: Brain lipid levels in the control and alcohol groups (mean ± SD).

Groups	Cholesterol (mg/g protein)	Triglyceride (mg/g protein)	Phospholipid (mg/g protein)
Control group	22.58 ± 2.29	32.57 ± 3.7	2.47 ± 0.64
Alcohol-fed group	29.36 ± 1.68*	39.81 ± 8.5**	2.91 ± 0.82

* $p<0.001$ higher than control group
** $p<0.05$ higher than control group

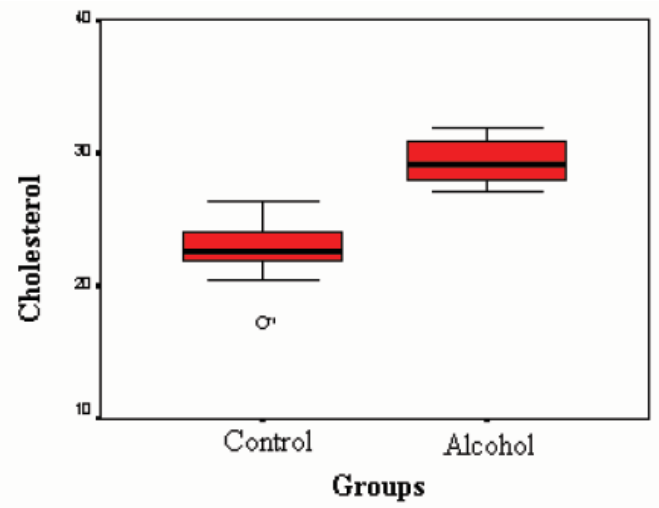

Figure 1: Brain cholesterol levels of the groups.

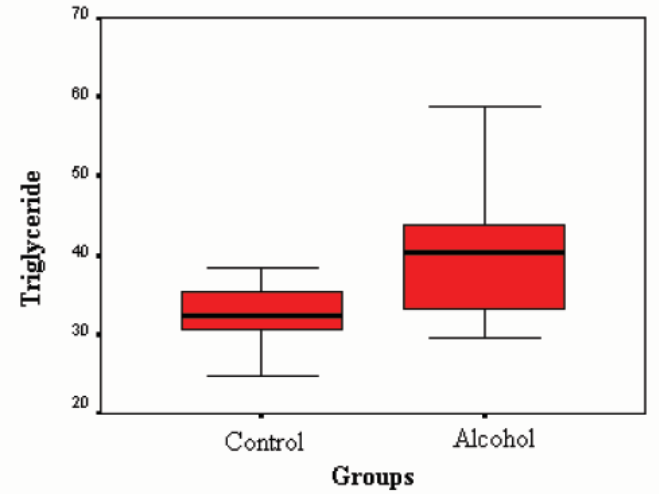

Figure 2: Brain triglyceride levels of the groups.

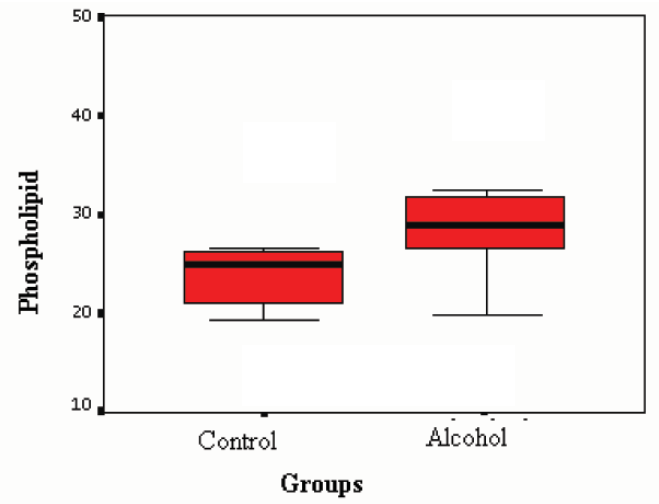

Figure3: Brain phospholipid levels of the groups.

DISCUSSION

The interaction of ethanol with lipid metabolism is complex.[9] It has been reported that long-term ethanol consumption is associated with altered membrane lipid and fatty acid profiles.[12,17-19] The change in fluidity of membranes from ethanol-treated animals has been attributed to changes in the lipid composition of the membranes.[12]

Our findings of increased brain cholesterol and TG levels are in accordance with the findings reported by other investigators.[1,9,10,14,17,18,20] However, the findings of PL levels are controversial. One possible mechanism of increased cholesterol levels of the brain might be the deterioration of the blood-brain barrier, which may permit passage of cholesterol. Moreover, it can be argued that the effect of alcohol on brain cholesterol may be due to the influence of alcohol on de novo synthesis, the mechanism of which is not known. On the other hand, the elevation of cholesterol levels in tissues in alcohol-fed animals has been attributed to the increased hydroxymethylglutaryl-CoA (HMG-CoA) reductase activity in the tissues.[1] The membraneous cholesterol is reported to intercalate among the hydrophobic residues of lipids and modulate the lateral mobility of amphipathic lipids.[14] Therefore, increased cholesterol levels in alcohol-fed rats may drastically alter the fluidity and functions of the cell membranes. It may make the membranes more rigid and may result in nonfunctional lipid-dependent membrane bound enzyme.[10,17]

Furthermore, an altered cholesterol metabolism has been reported to contribute to the development of neurodegenerative disorders, including Alzheimer and Niemann-Pick Type C disease.[21] When ethanol is present, it becomes the preferred fuel for the liver and replaces fat as a source of energy. This blocks fat oxidation and favours fat accumulation, which acts as a stimulus for secretion of lipoproteins into the bloodstream and the development of hyperlipidemia.[1,9,22] Therefore, high TG levels in the brains of the alcohol-fed group may have resulted from increased plasma TG levels. Indeed, it has been reported that reductions in the activities of lipolytic enzymes, namely plasma lipoprotein lipase and hepatic TG lipase, in ethanol-treated rats may lead to decreased removal of TG from circulation and accumulation of TG in tissues.[1]

Cholesterol and PL are used for membrane synthesis and for many other activities by cells throughout the body including the CNS. The PL class and its fatty acid composition and cholesterol content in biomembranes are basic determinants of the physical properties of membranes, and alterations in these lipid species are of special interest, as functional and pathological consequences may be correlated. The importance of PL for structure and integrity of cellular membranes suggests that many functional disturbances in alcoholism may be related to changes in PL content and composition.[12] Some studies showed that chronic alcohol intake resulted in changes in PL content[1,9,10,17] but some studies have reported minor changes in PL levels, as found in our study.[14,23,24] Ethanol was shown to elevate plasma [1] and brain[1,10] free fatty acid levels, which may promote the synthesis of PL in the brain and other tissues. According to some investigators, impaired fatty acid and PL metabolism may play a major role in the etiology of psychiatric disorders.[4] Our results prove that ethanol intake leads to cholesterol and TG increases in the brains of rats. The underlying mechanism of these changes and their effects on brain functions are not known and needs to be investigated further.

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