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Chronic Heavy Ethanol Consumption Is Associated with Decreased Platelet Aggregation in Rats

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—— Although moderate alcohol consumption seems to be protective against atherosclerosis, coronary artery disease rate increases with its higher doses. Platelet aggregation is an important process which contributes to the atherosclerosis. The aim of this study was to determine whether heavy ethanol consumption stimulates or inhibits platelet aggregation. Fourteen adult male Wistar rats were used. Ethanol (7.2%, v/v) in a modified liquid diet was given to eight rats for 21 days, which mimicked characteristics similar to human chronic alcoholism. Six rats constituted the control group. Adenosine diphophate (ADP) and collagen-induced platelet aggregation was measured in whole blood. We found reduced ADP-induced mean maximal aggregation in the alcoholic rat group compared to the control group at dose of 5 μM ($p < 0.005$). We also found decreased platelet aggregation responses to collagen in the alcoholic group ($p < 0.006$ for 2 μg/ml collagen, and $p < 0.05$ for 5 μg/ml collagen). In conclusion, chronic heavy ethanol consumption results in the decreased platelet aggregation in a rat model of alcoholism. Therefore, increased mortality from coronary artery disease in chronic alcoholism may be explained by other factors such as dietary imbalances and coexisting conditions, which include hypertension and depression. ———— alcohol consumption; platelet aggregation; coronary artery disease; dietary imbalances; cardiovascular protection

Platelet aggregation is a key step in the coagulation process where it has an important action for homeostasis. On the other hand, platelet aggregation has deleterious effects in terms of occlusive thrombus formation leading to stroke and myocardial infarction. It is evident that platelets contribute to the development of atherosclerosis and hence coronary heart disease.

There is a considerable body of evidence that mild to moderate drinking at one to two drinks

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daily is associated with decreased risk of death due to cardiovascular diseases, including a reduction in the incidence of thrombotic stroke and myocardial infarction (Marmot 1984; Klatzky et al. 1992; Fuchs et al. 1995). Moderate alcohol consumption seems to be protective with a reduced incidence of, and mortality from, coronary heart disease (Kannel and Ellison 1996). However, with higher doses, coronary artery disease and all cause mortality rates increase (Thun et al. 1997).

Several mechanisms have been proposed for the beneficial effects of alcohol. Among them are increased high-density lipoprotein (Dai et al. 1985), decreased low-density lipoprotein (Gaziano et al. 1993), and low fibrinogen (Mennen et al. 1999). Inhibition of platelet aggregation is one of the important mechanisms, which is supposed to be responsible for the risk reduction of coronary heart disease (Rand et al. 1988, 1989).

Alcoholic beverages are not consumed as pure ethanol. The antiplatelet activity of wine is explained by ethanol but also by the polyphenolic components, which are enriched in red wine. In addition to beverage type (e.g., red wine, beer, spirit), its constituents (e.g., flavanoids, polyphenols) may affect platelet aggregation. In subjects with moderate wine drinking, it was shown that wine polyphenols inhibited ADP-induced platelet aggregation whereas alcohol had no inhibitory effect (De Lange et al. 2003).

The objective of this study is to evaluate the effect of chronic ethanol exposure on platelet aggregation in rats. We also assess whether the effect of chronic alcoholism is due to ethanol or other factors.

Materials and Methods

Animals and laboratory

All procedures in this study are in accordance with the guide for the Care and Use of Laboratory Animals as adopted by the National Institutes of Health and Helsinki Declaration. The study was approved by the Ethics Committee of Gulhane Military Medical Academy. Fourteen adult male Wistar rats, 260-300 g in weight at the beginning of the experiments were subjects. They were housed in a quiet and temperature-and humidity-controlled room (21 ± 3°C and 60 ± 5%, respectively) in which a 12 hours light-dark cycle was maintained (07 : 00 - 19 : 00 light).

Procedure

For chronic ethanol exposure, 8 rats (alcoholic group) were housed individually and ethanol was given in the modified liquid diet as previously described (Uzbay and Kayaalp 1995). At the beginning of the study, rats were given the modified liquid diet without ethanol for 7 days. Then liquid diet with 2.4% ethanol was administered for 3 days. The ethanol concentration was increased to 4.8% for the following 4 days and finally to 7.2% for 21 days. Liquid diet was freshly prepared daily and presented at the same time of the day (10 : 00 a.m.). The weight of the rats was recorded every day and daily ethanol intake was measured and expressed as grams per kilogram. The liquid diet supplies 1,000 kcal liter^{-1}. Six rats receiving no ethanol constituted the control group and were pair fed on isocaloric liquid diet containing sucrose as acaloric substitute for ethanol (96 g sucrose and 75 ml cow milk was replaced with 60.75 g or 75 ml ethanol). The animals did not have access to water in addition to liquid diet with or without ethanol.

Collecting the blood and preparing the platelets

Following intraperitoneal ketamine anesthesia, 5 ml blood was withdrawn by cardiac puncture in 5 ml - silicone coated vacutainer tubes containing 0.5 ml of 0.129 M buffered sodium citrate (3.8% w/v, 9 : 1 v/v).

Blood samples were kept at room temperature and tested within 1 hour. Platelet counts were done by standard laboratory technique using an Abbott Cell-Dyne 4,000 cell counter device (Abbott Park, IL, USA).

Platelet aggregation in whole blood was measured with the use of a Whole Blood Lumi-Ionized Calcium Aggregometer (Chrono-log Corporation, Model 560-Ca Havertown, PA, USA) according to the protocol of Chrono-log Corporation.

Platelet aggregation was induced by adenosine diphophate (ADP) (1, 2 and 5 μM final concentration) and collagen (1, 2 and 5 μg/ml final concentration). Chrono-log Corporation aggregating reagents were used (Chrono-log Corporation). Platelet aggregation was recorded on a chart recorder as change in impedance (delta Ohms). Maximal aggregation (delta Ohm) was obtained from the aggregation curve.

Aggregation data presented as median (minimum-maximum) for the alcoholic and control groups. Mann

Whitney's U-test was used for the analysis of the maximal aggregation. Student's *t*-test was used for the comparison of the weights and hematological parameters. The level of significance was set at 0.05.

Results

Daily ethanol consumption of the rats ranged from 11.52 ± 2.78 to 13.89 ± 4.50 g kg^{-1} for 21 days. Mean weights of the rats at the beginning and the end of the study were 262.58 ± 7.81 g and 273.65 ± 10.20 g, respectively ($p > 0.05$). Median values for ADP-induced maximal aggregation responses were shown in Fig. 1. Median decrease of ADP-induced maximal aggregation responses in the alcoholic group reached statistical significance only at 5 μM ADP dose compared to the control group, (12.0, range: 10.0 - 13.0 in the alcoholic group vs 15.0, range: 13.0 - 17.0, in the control group, $p < 0.005$). Median values for collagen-induced platelet aggregation responses were shown in Fig. 2. Statistical significance for median collagen-induced platelet aggregation responses was reached at 2 and 5 μg/ml collagen doses (9.5, range: 8.0 - 11.0 in the alcoholic group vs. 12.5, range: 10.0 - 16.0, at 2 μg/ml collagen dose ($p < 0.006$) and 12.0, range: 10.0 - 15.0 in the alcoholic group vs. 15.5, range: 12.0 - 18.0, in the collagen group, at 5 μg/ml collagen dose ($p < 0.05$).

There was a significant inhibition of platelet aggregation induced by 5 μM ADP. Moreover, platelet aggregation induced by 5 μg/ml and 2 μg/ml of collagen was inhibited by chronic ethanol consumption.

Chronic ethanol administration did not significantly affect hematological parameters in the groups (Table 1).

Discussion

In the present study, we demonstrated that chronic pure ethanol administration inhibited platelet aggregation in rats.

For the anticipated deleterious effect of heavy drinking on the cardiovascular system, it

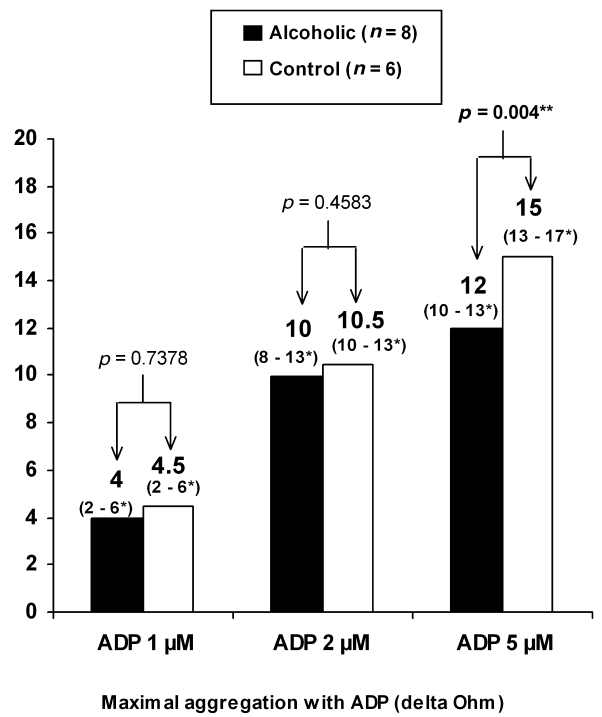

Fig. 1. The comparison of ADP-induced platelet aggregation between the groups.
A, alcoholic group; C, control group.
* range, ** statistically significant.

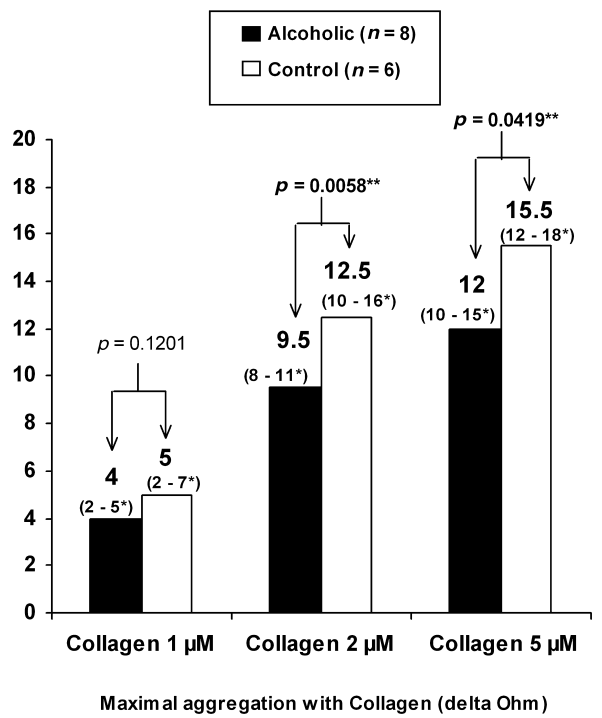

Fig. 2. The comparison of collagen-induced platelet aggregation between the groups.
A, alcoholic group; C, control group.
* range, ** statistically significant.

TABLE 1. *Comparison of groups with regard to hematological parameters*

Group	WBC (/mm^3)	Hb (g)	Htc (%)	RBC (mm^3)	Platelet (/mm^3)
A	5,539 ± 4,082	13.2 ± 1.1	43.4 ± 3.3	8.2 ± 0.7	485 ± 75
C	6,747 ± 4,470	13.7 ± 0.5	44.5 ± 1.4	8.4 ± 0.3	557 ± 43
p value	NS	NS	NS	NS	NS

A, alcoholic; C, control; WBC, white blood cell; RBC, red blood cell.

seems controversial to find platelet aggregation inhibition in chronic ethanol administration concerning our study. Nevertheless, several factors such as, hypertension-related cardiovascular events, depression and its cardiovascular consequences might also be attributed to the increased cardiovascular risk in chronic alcoholic subjects (Sher et al. 2003). Moreover, chronic alcoholism often results in dietary imbalances, which may result in a higher risk of coronary diseases. This may explain the apparent contradiction between platelet function impairment and a higher risk for coronary diseases. Accordingly, it was shown that beneficial effects of wine on cardiovascular health are greater if wine is associated with a healthy diet (Caimi et al. 2003). Quercetin, a flavonoid present in the human diet, which is found in high levels in wine, has been shown to inhibit platelet aggregation and signaling (Hubbard et al. 2003). Animal studies demonstrated that resveratrol; a phytoalexin found in red wine modulates vascular cell function, inhibits LDL oxidation, suppresses platelet aggregation and reduces myocardial damage during ischemia-reperfusion (Hung et al. 2002; Orallo et al. 2002; Bradamante et al. 2004). Mediterranean-type diet and moderate consumption of red wine were shown to have complementary beneficial effects on hemostatic cardiovascular risk factors. Wine intake resulted in a significant increase in ex vivo platelet aggregation and secretion after stimulation with collagen (Mezzano 2004).

Although low incidence of coronary artery disease was initially attributed to the red wine and its antioxidant components (Renaud and De Lorgeril 1992), similar findings have been reported with the use of other alcoholic beverages (Yano et al. 1984; Rimm et al. 1991). In a human study, dealcoholized beer inhibited thrombogenic activity in young adults and this action could have a beneficial effect on the development of coronary artery disease (Bassus et al. 2004).

We implemented pure ethanol at relatively high dose (7.2%) to alcoholic rats. This might better reflect heavy drinking. Approximately nine liters wine or twenty liters of beer should be consumed to be in the state of chronic alcoholism similar to the rats in our alcohol feeding method. From a methodological point of view, duration of exposure might be important to assess chronic effect of alcohol on platelet function; hence, we thought that a feeding method could be accepted as better suited for a chronic study. Our feeding method did not significantly alter the weights of the rats also in our previous studies (Bilgi et al. 2003; Uzbay et al. 2004). Rats receiving ethanol with our method developed physical dependence and also withdrawal syndrome (Uzbay et al. 2000). We also observed ethanol-induced motor behavioral damages (Uzbay et al. 1999). This rat model was also used in a previous study to evaluate its effect on myocardial ischemia (Erinc et al. 2003).

We preferred whole blood platelet impedance aggregation method for evaluating alcohol-platelet relationship, because it is quite similar to the physiological condition of the platelets. Although the optical aggregation method in platelet rich plasma (PRP) is used more frequently, it was suggested that whole-blood aggregation was more sensitive indicator of platelet reactivity than the optical aggregation in PRP (Littleton et al. 1982; Mikhailidis et al. 1987).

Chronic alcoholism may also result in hepatic injury (Dakeishi et al. 2004). In human subjects, chronic alcoholism cause liver cirrhosis,

which leads thrombocytopenia. A low platelet number would effect platelet aggregation measured by the impedance method (Muller et al. 1995). In our study, chronic ethanol administration did not significantly affect the platelet, RBC and WBC counts in any group.

Data about platelet-alcohol relationship in chronic heavy drinkers were relatively rare: Inhibited platelet aggregating responses to various inducing agents such as ADP and collagen in chronic alcoholic subjects was reported (Mikhailidis et al. 1986; Neiman et al 1989). These human studies were not performed with pure ethanol.

In terms of limitations, one might speculate the value of the findings based on relatively small number of subjects in our study. However, it was quite difficult to conduct such a study on animals, which were not so much resistant to heavy alcohol consumption. On the other hand, we think that the differences were quite impressive despite relatively small number. The other point is the different metabolism of ethanol in rats and human subjects. Human liver has higher alcohol dehydrogenase activity and higher low Km aldehyde dehydrogenase activity than rat liver. Ethanol metabolism in rats was shown to be much faster than in human subjects. Hepatic ethanol-metabolizing activities and the in vivo ethanol elimination rate are quite different in rats and human subjects (Zorzano et al. 1990).

In conclusion, chronic heavy ethanol consumption by a modified liquid diet seems to have an inhibitory effect on platelet aggregation in rats. Since ingredients in alcoholic beverages were also reported to inhibit platelet aggregation before, increased incidence of coronary heart disease in chronic alcohol consumption might then be attributed to the dietary imbalances and accompanying diseases. Further studies are needed to explore the mechanisms involved.

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