ACUTE EFFECT OF ETHANOL ADMINISTRATION ON PLATELET FUNCTION

S. SUDHIR, L. C. GUPTA, J. SINGH, AND R. D. BUDHIRAJA

Department of Pharmacology and Blood Bank,
Medical College, Rohtak - 124 001 (Haryana)

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Summary : The acute effect of single dose of ethanol (1.5 *g kg*) and aspirin (10 *mg/kg*) alone and in combination, on platelet aggregation time and platelet adhesiveness were studied in rabbits. There was a significant and comparable increase in aggregation time both by aspirin and ethanol. Similarly platelet adhesiveness was decreased by both the agents.

Key words : ethanol aspirin platelet aggregation platelet adhesiveness

INTRODUCTION

Altered platelet function has been associated with widely varying types of cardiac diseases including arrhythmias, congestive heart failure, coronary disease, thromboembolic disorders (5, 11, 18) and with sudden death. Alcohol ingestion in the past has been clinically associated with exacerbation of these disorders (14, 15), although a recent report exists on protection by ethanol against atherogenesis in man (16). The pharmacological alteration in platelet function may be of therapeutic benefit in certain patients. In some studies decreased platelet aggregation has been observed after ethanol administration (3, 6, 30). Conversely, in a more recent *in vitro* study increased microaggregation has been observed (4) after ethanol.

In view of the conflicting reports regarding the effect of acute ethanol administration on platelet functions, our aim was to study the acute effect of ethanol on platelet function and compare the effect with that of aspirin – known inhibitor of platelet aggregation and PG synthetase.

MATERIAL AND METHOD

Rabbits of either sex weighing (2-2.5 *kg*) were divided into 4 groups of 10each. The first group served as control and received 0.9% saline orally. Rabbits of the second group

received ethanol alone (1.5 *g/kg*, po). Third group received aspirin alone (10 *mg/kg*, po) and fourth group received ethanol along with aspirin.

Platelet aggregation : 1½ hr after drug administration, 6 *ml* blood was withdrawn directly from the heart and collected in 3.8% sodium citrate solution (proportion, 9:1). Platelet rich plasma (PRP) was obtained by centrifuging the blood samples at 150-200 G for 15 min. Platelet aggregation was induced by adding 20 μg of sodium salt of adenosine 5' diphosphate (ADP, Sigma 0.02% solution) to 3 *ml* of vigorously stirred PRP. The resulting formation of platelet clumps produced an alteration in the optical density which was measured with photoelectric colorimeter at 550 $m\mu$ noting readings every 5 sec till value became constant. The time taken to attain maximum change after addition of ADP was expressed as platelet aggregation time.

Platelet adhesiveness : Platelet adhesiveness was determined by a glass bead column technique using a modification of Salzman's method (1). Two *ml* blood was obtained from the heart 1½ hr after drug administration and collected in vials containing 2 *mg* of Na_2EDTA as anticoagulant. A second sample (2 *ml*) was obtained in 10 *ml* dry siliconised syringe containing grease free soda lime silica glass beads (34 beads, 0.5 mm D). Syringe was rotated between palms of both the hands for 40-50 sec so that blood came in contact with all the beads avoiding frothing. The blood was transferred to a vial containing 2 *mg* of Na_2EDTA. Platelet counts were made in both the samples and adhesive index was calculated as $\frac{x-y}{x} \times 100$) where x is the platelet count of the blood not passed over the beads and y is the count of the blood passed over beads.

RESULTS

Ptatelet aggregation : The animals treated with ethanol or aspirin exhibited a significant increase in platelet aggregation time (Table I). The aggragation time was also increased in animals receiving both aspirin and ethanol as compared to ethanol alone treated group. However, there was no significant difference in aggregation time between animals receiving both ethanol and aspirin alone (Table I).

Platelet adhesiveness : Oral administration of ethanol and aspirin significantly decreased the platelet adhesiveness in all drug treated animals. The effect was more marked in animals receiving ethanol alone (Table I). However, there was no significant difference in platelet adhesiveness between animals receiving both ethanol and aspirin and aspirin alone.

DISCUSSION

In the present study ethanol and aspirin produced a significant and comparable increase in ADP indudced platelet aggregation time and decrease in platelet adhesiveness.

TABLE I : Effect of aspirin and ethanol alone and in combination on platelet functions in rabbits. Each result mean (±SEM) from 10 animals.

Group	*PC (Before Beads)*	*PC (After Beads)*	*Adhesive Index %*	*Platelet aggregation time (Sec.)*
Control	273.0±9.58	164.0±5.47	40.14±2.05	21.0±0.41
Aspirin treated	110.0±3.93	85.0±2.28	22.43±2.39*	40.4±1.27*
Ethanol treated	270.5±4.41	206.5±3.14	24.71±2.15*	35.7±1.70*
Aspirin + Ethanol treated	111.5±3.57	90.0±1.30	19.27±0.96*[a]	40.1±1.81*[a]

(*) Values significantly ($P<0.01$) differ from corresponding values in control group (students 't' test) PC = mean platelet count ($\times 10^{-3}$±S.E.)

(*a) Value differs ($P<0.05$) from ethanol treated group

The antiaggregatory effect of ethanol is supported by earlier reports (3, 6, 20) that the decreased aggregation with high ethanol blood levels probably resulted from the direct inhibition of ADP induced aggregation. However, this may not be the only factor involved. It is possible that the alcohol may alter the reactivity of platelet membrane or may change the adenine nucleotide storage pool of the platelets. Some studies have demonstrated that ethanol or its metabolite acetaldehyde interferes with the binding and transport of myocardial calcium and other mitochondrial enzymes (2, 17). Ethanol may produce similar effect in platelets as well and diminished cellular calcium may inhibit ADP induced aggregation. On the other hand role of ethanol on platelet prostaglandin production is also very important and it is possible that alcohol inhibits synthesis of TA_2 - a proaggregatorry prostanoid as in human platelets (8). Further, aspirin is known to impair haemostatic properties of human platelets by inhibiting the synthesis of cyclic endoperoxides (21) and ethanol may act similarly. On the other hand alcohol has been found to cause increased formation of microaggregates *in vitro* with high ethanol levels (450 to 500 *mg*/100 *ml*) in pig and rabbit blood (4). Horak *et al.* (7) have demonstrated in rats that low dose of ethanol (iv) suppresses the platelet aggregation, while aggregation is enhanced with high doses (>300 *mg*/*dl*). The discrepancy found between our results and those of other, may be due to the difference in methodology and routes of ethanol administration employed.

Although the relationship between consumption of alcohol and coronary artery disease is controversial at present, more recent studies have found preventive effect from daily ingestion of ethanol (22) while other reports note no apparent effect or a deleterious effect of alcohol in coronary artery disease (19). Both acute and chronic ingestion of ethanol decrease left ventricular contractility (10) which could have a beneficial effect on angina by decreasing myocardial oxygen consumption. However, Mallov and Gilmour (9) reported inhibition of epinephrine induced myocardial necrosis by ethanol and this effect may be related to a platelet antiaggregatory effect. Role of ethanol in coronary disease may be mediated via platelet prostaglandins, which are modified in biphasic manner by increasing doses of ethanol (7). Platelet prostaglandin production has significant effects on coronary tone and motion and is related to platelet aggregation and deaggregation (12). From the results of the present study it is difficult to propose the exact mechanism responsible for prevention of platelet aggregation and platelet adhesiveness by ethanol. Elucidation of these mechanisms would be of interest.

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