CLINICAL RESEARCH

Effect of ethanol on vascular prostacyclin (prostaglandin I_2) synthesis, platelet aggregation, and platelet thromboxane release

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Abstract

A series of experiments with platelets from healthy volunteers showed a concentration related inhibitory effect of ethanol on platelet aggregation and release of thromboxane A_2. This effect was observed at blood alcohol concentrations ranging between 66 and 132 mg/dl (14·3 and 28·6 mmol/l), which are commonly found in alcoholics. Investigations carried out by incubating ethanol with platelet rich plasma in vitro also showed an inverse linear correlation between ethanol concentration and platelet thromboxane synthesis. In contrast, the incubation of a wide range of concentrations of ethanol with human endothelial cells and rat aortic rings did not alter the ability of these systems to synthesise prostacyclin (prostaglandin I_2).

This finding of a selective inhibition of thromboxane A_2 synthesis and platelet aggregation without an alteration of prostaglandin I_2 synthesis may provide an explanation for the reported ethanol mediated protection against vascular disease. This effect of ethanol may also be relevant to the induction of acute gastrointestinal haemorrhage that occurs after bouts of excessive alcohol consumption.

Introduction

Moderate consumption of alcohol apparently protects against cardiovascular disease.[1-6] Although the mechanisms are yet to be defined,[1] we do know that relatively high concentrations of ethanol, both in vivo and in vitro, inhibit platelet function.[7] This effect may be relevant, since platelets probably contribute to the pathogenesis of atherosclerosis.[8-10] In addition, activated platelets release thromboxane A_2, a potent vasoconstrictor and inducer of platelet aggregation, which has also been implicated in the pathogenesis of ischaemic heart disease.[11]

Ethanol may exert its inhibitory effect on platelet aggregation by two mechanisms—inhibition of thromboxane A_2 synthesis by platelets and stimulation of prostacyclin (prostaglandin I_2) synthesis by the vascular endothelium. An increase in the ratio of prostaglandin I_2 to thromboxane A_2 would tend to inhibit platelet aggregation and maintain vascular patency, thus offering protection against vascular disease.[12]

The present study examines whether ethanol at concentrations often achieved after alcohol consumption (*a*) alters platelet aggregation and thromboxane A_2 synthesis and (*b*) alters endothelial prostaglandin I_2 synthesis.

Methods

EFFECT OF ETHANOL ON HUMAN PLATELET AGGREGATION AND THROMBOXANE A_2 RELEASE

In vitro studies—Nine healthy, non-smoking volunteers (five men, four women) were studied. None had taken any drugs for two weeks before sampling. Venous blood was collected in 3·8% trisodium citrate, and platelet rich plasma and platelet poor plasma prepared by centrifugation, as described.[13] Various amounts of ethanol (as ethanol-saline solutions) were added to the platelet rich plasma at 37°C in the aggregometer cuvette, so as to achieve final concentrations of 50, 100, 200, 400, and 800 mg/dl (10·9, 21·7, 43·4, 86·8, and 174·0 mmol/l) (see table I). After 10 minutes of incubation platelet aggregation was induced by adding adrenaline 5 μmol/l (91·6 μg/100 ml), adenosine diphosphate to 10 μmol/l (427 μg/100 ml), and collagen 1 mg/l (see table I). Control experiments consisted of appropriate volumes of saline added to platelet rich plasma. Platelet aggregation was expressed as the percentage fall in optical density three minutes after adding the aggregating agent.[13] At the end of the three minutes the platelet rich plasma (450 μl) was added to 1 ml ethanol (to stop the reaction) and thoroughly mixed. Samples were then stored at −70°C until the time of measurement of thromboxane B_2 (the stable metabolite of throm-

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TABLE I—*Effect of ethanol on in vitro human platelet aggregation and thromboxane B_2 release. Results expressed as medians (range)*

Ethanol concentration		Aggregating agent					
		Adrenaline (5 μmol/l; 91·6 μg/100 ml)		Collagen (1 mg/l)		Adenosine diphosphate (10 μmol/l; 427 μg/100 ml)	
mg/dl	mmol/l	% platelet aggregation	Thromboxane B_2 (μg/l platelet rich plasma)	% Platelet aggregation	Thromboxane B_2 (μg/l platelet rich plasma)	% Platelet aggregation	Thromboxane B_2 (μg/l platelet rich plasma)
0	0	65 (54-84)	28 (17-44)	67 (62-84)	34 (20-55)	72 (61-84)	12 (5-16)
50	10·9	68 (13-83)	22* (15-31)	66 (56-78)	32 (21-36)	75 (55-85)	10 (4-15)
100	21·7	61 (5-80)	19† (9-27)	67 (44-80)	28* (14-34)	74 (54-84)	9 (3-14)
200	43·4	28† (6-75)	12† (5-21)	60* (27-76)	26* (14-31)	73 (52-85)	7* (4-13)
400	86·8	28† (6-54)	9† (3-11)	48† (5-66)	19* (13-30)	61† (42-78)	7† (3-11)
800	174·0	10† (2-23)	5† (1-11)	3† (0-39)	9† (4-8)	60† (39-76)	6† (2-10)

* $p<0·05$; † $p<0·01$.

TABLE II—*Effect of ethanol ingestion (1 ml/kg body weight) on platelet aggregation and thromboxane B_2 release. Results expressed as medians (range)*

Sampling (min)	Blood ethanol concentration		Aggregating agent					
			Adrenaline (5 μmol/l; 91·6 μg/100 ml)		Collagen (1 mg/l)		Adenososine diphosphate (10 μmol/l; 427 μg/100 ml)	
	mg/dl	mmol/l	% Platelet aggregation	Thromboxane B_2 (ng/10^8 platelets)	% Platelet aggregation	Thromboxane B_2 (ng/10^8 platelets)	% Platelet aggregation	Thromboxane B_2 (ng/10^8 platelets)
0 (before ethanol)	0	0	70 (37-82)	10·2 (2·9-22·0)	79 (77-84)	16·7 (9·8-27·3)	76 (62-90)	1·8 (0·7-4·3)
30 (after ethanol ingestion)	72† (66-132)	15·6† (14·3-28·6)	41* (16-69)	7·2† (0-14·8)	75 (67-80)	9·8† (6·3-20·9)	74 (63-80)	1·4 (0·7-2·5)
60 (after ethanol ingestion)	86† (76-110)	18·7† (16·5-23·9)	69 (13-74)	8·8 (1·0-19·2)	77 (68-79)	12·3* (9·9-19·4)	71 (65-80)	2·4 (0·8-3·6)

* $p<0·05$; † $p<0·01$.

boxane A_2) concentrations by a specific radioimmunoassay (New England Nuclear, Boston, USA). Platelet counts in platelet rich plasma were not adjusted to a specific value since plasma from the same sample was used in control experiments.

Ex vivo studies—Seven healthy, non-smoking volunteers (four men, three women) were given 1 ml ethanol/kg body weight (roughly 0·79 g/kg) diluted in 200 ml Lilt (pineapple and grapefruit crush; Coca Cola Company, London), water being added (up to 200 ml) according to the volunteers' preference. This solution was drunk over 15 minutes. Blood samples were collected before the ingestion of ethanol (zero time). Two further blood samples were collected 30 and 60 minutes after the end of ethanol ingestion (see table II). Blood samples were processed and evaluated as in the in vitro experiments. Platelet counts in platelet rich plasma, however, were adjusted so that they approximated to the count in the basal sample $\pm 10\%$. Counts were performed using a Coulter counter, model D. Concentrations of thromboxane B_2 were expressed as ng/10^8 platelets. Blood samples were also collected for blood ethanol measurement. This was carried out by a routine gas-liquid chromatography technique. Control experiments consisted of ingestion of Lilt and water only (four subjects).

EFFECT OF ETHANOL ON PRODUCTION OF VASCULAR PROSTAGLANDIN I_2

Rat aortic ring: spontaneous prostaglandin I_2 release model—Rat aortic rings were incubated in Krebs-Ringer bicarbonate buffer (pregassed to pH 7·4 with carbon dioxide-oxygen (95:5)) for 15, 30, and 60 minutes at 37°C. Various volumes of ethanol were added to produce final ethanol concentrations of up to 800 mg/dl (174 mmol/l) (see table III). Seven incubates were studied at each ethanol concentration. Concentrations of 6-oxo-prostaglandin $F_{1\alpha}$ (the spontaneous stable metabolite of prostaglandin I_2) were assayed in aliquots from the incubates using a specific radioimmunoassay kit (New England Nuclear, Boston).

TABLE III—*Effect of ethanol on in vitro spontaneous release of 6-oxo-prostaglandins $F_{1\alpha}$ (6-oxo-$PGF_{1\alpha}$) by rat aortic rings*

Ethanol concentration		6-oxo-$PGF_{1\alpha}$ production (pg/mg/min)		% Change from basal 6-oxo-$PGF_{1\alpha}$ production	p
mg/dl	mmol/l	Median	Range		
0	0	400	378-419	Basal	
250	54·3	470	451-489	+17·5	NS
500	109·0	450	433-477	+12·5	NS
800	174·0	420	394-450	+5·0	NS

NS = Not statistically significant.

Rat aortic ring: arachidonic acid incorporation model—This method has been described.[14] Briefly, rat aortic rings were incubated with ^{14}C-arachidonic acid (specific activity 58·4 mCi/mmol; 192 mCi/g) at 37°C in trometamol-(TRIS)-HCl buffer (containing 0·9% NaCl and 1 mmol (292 mg) edetic acid/l, pH 8·0) in the presence and absence of ethanol. Seven incubates were set up for every ethanol concentration studied (see table IV). The percentage conversion of ^{14}C-arachidonic acid to 6-oxo-prostaglandin $F_{1\alpha}$ was then calculated, using thin layer chromatography for separation and liquid scintillation counting of the appropriate areas for quantification.

TABLE IV—*Effect of ethanol on in vitro conversion of ^{14}C-arachidonic acid (^{14}C-AA) to 6-oxo-prostaglandin $F_{1\alpha}$ (6-oxo-$PGF_{1\alpha}$) by rat aortic rings*

Ethanol concentration		% Conversion of ^{14}C-AA to 6-oxo-$PGF_{1\alpha}$		% Stimulation	p
mg/dl	mmol/l	Median	Range		
0	0	21·2	19·2-22·8	Basal value	
100	21·7	21·8	19·8-23·3	+3	NS
250	54·3	22·2	20·6-24·1	+5	NS
500	109·0	25·2	23·5-26·6	+15	< 0·05
800	174·0	26·7	24·6-28·7	+20·5	< 0·01

NS = Not statistically significant.

Human umbilical endothelial cell: arachidonic acid incorporation model—Umbilical cords were collected after spontaneous labour from women with no obstetric complications. The umbilical vein was filled with 20 ml collagenase (type V, Sigma Chemicals, Poole, UK) solution in Krebs-Ringer bicarbonate buffer (pH 7·4) and incubated for 15 minutes at 37°C. The endothelial cells were then harvested and washed three times before suspension in trometamol-HCl buffer (pH 8·0 containing 0·9% NaCl and 1 mmol (292 mg) edetic acid/l). Aliquots of cell suspensions were incubated with 20 nCi ^{14}C-arachidonic acid and the percentage conversion ascertained, as for the rat aortic rings. Cell concentrations were adjusted to give conversions equivalent to 15 mg rat aortic rings. Seven incubates were set up at each ethanol concentration studied (see table V).

TABLE V—*Effect of ethanol on in vitro conversion of ^{14}C-arachidonic acid (^{14}C-AA) to 6-oxo-prostaglandin $F_{1\alpha}$ (6-oxo-PGF$_{1\alpha}$) by human umbilical endothelial cells*

Ethanol concentration in incubates		% Conversion of ^{14}C-AA to 6-oxo-PGF$_{1\alpha}$		% Stimulation over basal conversion	p
mg/dl	mmol/l	Median	Range		
0	0	32·2	30·1-33·3	Basal value	NS
80	17·4	32·6	29·7-36·5	1	NS
160	34·7	33·2	30·8-34·9	3	NS
320	69·4	33·5	32·5-36·8	4	NS
640	139·0	32·2	30·2-33·9	0	NS

NS = Not statistically significant.

STATISTICAL ANALYSIS

All measurements were compared with those in control samples using a paired Wilcoxon rank sum test. Results are expressed as medians and ranges.

Results

EFFECT OF ETHANOL ON HUMAN PLATELET AGGREGATION AND THROMBOXANE A_2 RELEASE

In vitro studies—Platelet aggregation (table I) was significantly inhibited at ethanol concentrations of 200 mg/dl (43·4 mmol/l) and above. Adrenaline induced platelet aggregation and collagen induced aggregation were more sensitive than adenosine diphosphate induced aggregation to the inhibitory effect of ethanol. Percentage platelet aggregation and the concentration of ethanol added to the platelet rich plasma showed a significant inverse correlation (fig 1). In contrast to platelet aggregation, production of thromboxane

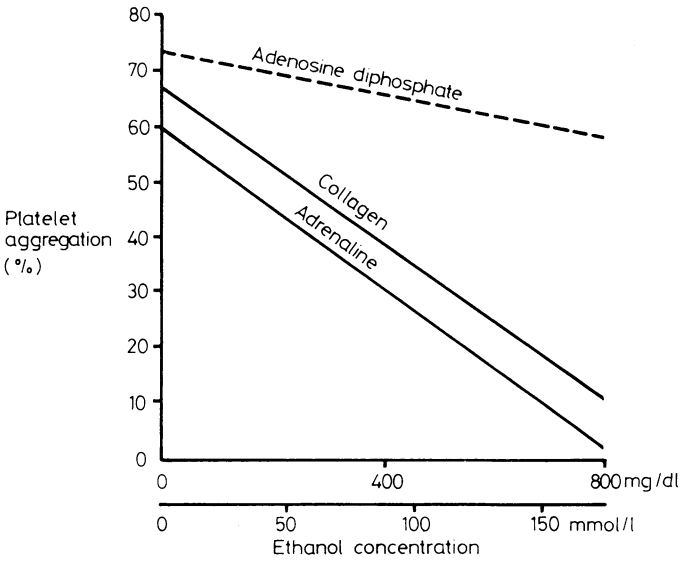

FIG 1—Correlation between ethanol concentrations in platelet rich plasma and in vitro platelet aggregation induced by adenosine diphosphate 10 μmol/l (427 μg/100 ml), collagen 1 mg/l, and adrenaline 5 μmol/l (91·6 μg/100 ml).
Statistical analysis of correlation coefficients: adenosine diphosphate r = −0·38, p = 0·006; collagen r = −0·77, p < 0·0001; adrenaline r = −0·62, p < 0·0001.

B_2 (table I) was significantly inhibited at lower ethanol concentrations (50-100 mg/dl; 10·9-21·7 mmol/l). Furthermore, adenosine diphosphate induced release of thromboxane B_2 was inhibited at ethanol concentrations that did not influence adenosine diphosphate induced platelet aggregation. Release of thromboxane B_2 induced by adrenaline or collagen, but not by adenosine diphosphate, showed a significant inverse correlation with the concentration of ethanol in platelet rich plasma (fig 2). Platelet counts in platelet rich plasma from the volunteers varied from 270 × 10^9 to 480 × 10^9/l. There was no correlation between the platelet count in the platelet rich plasma and the observed response to ethanol.

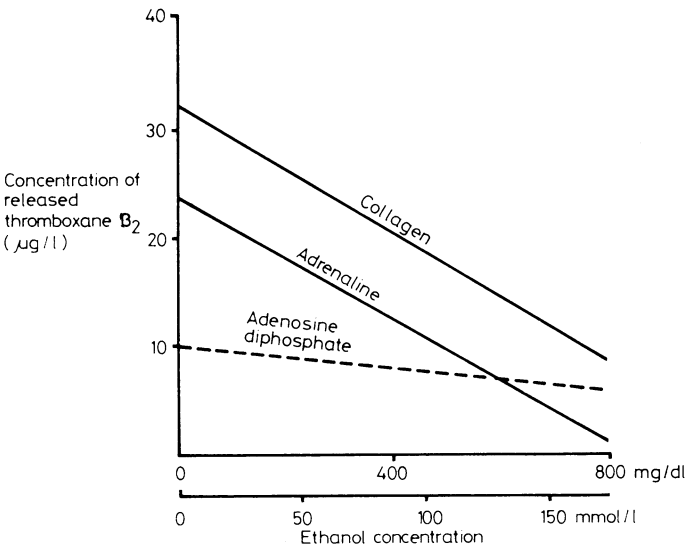

FIG 2—Correlation between ethanol concentration in platelet rich plasma and concentration of thromboxane B_2 released in vitro from human platelets after aggregation induced by collagen 1 mg/l, adenosine diphosphate 10 μmol/l (427 μg/100 ml), and adrenaline 5 μmol/l (91·6 μg/100 ml).
Statistical analysis of correlation coefficients: collagen r = −0·67, p < 0·001; adenosine diphosphate r = −0·26, p = NS; adrenaline r = −0·73, p < 0·001.

Ex vivo studies—Only adrenaline induced platelet aggregation was significantly inhibited (30 minute sample) after ingestion of ethanol (table II). Adrenaline and collagen induced release of thromboxane B_2 was inhibited by ethanol ingestion. Adenosine diphosphate induced release of thromboxane B_2 was also inhibited, but this change did not achieve statistical significance (table II). Neither platelet aggregation nor release of thromboxane B_2 correlated significantly with the blood ethanol concentration. There were no appreciable changes in platelet counts in platelet rich plasma during the experiment, most samples requiring no adjustment of the count. Control experiments showed no inhibition of platelet aggregation or release of thromboxane B_2. Median blood ethanol concentrations showed a progressive rise during the experiment (table II).

EFFECT OF ETHANOL ON VASCULAR PROSTAGLANDIN I_2 PRODUCTION

Rat aortic ring: spontaneous prostaglandin I_2 release model (table III)—Ethanol had no significant effect on release of prostaglandin I_2 (measured as 6-oxo-prostaglandin $F_{1\alpha}$, the spontaneous stable metabolite of prostaglandin I_2,) from rat aortic rings. Production of 6-oxo-prostaglandin $F_{1\alpha}$ after 30 minutes of incubation is reported, since production of prostaglandin I_2 by control aortic rings was linear between zero and 30 minutes of incubation. Beyond that time the rate of production tended to decline.

Rat aortic ring: arachidonic acid incorporation model (table IV)—No inhibition of the rate of conversion of ^{14}C-arachidonic acid to 6-oxo-prostaglandin $F_{1\alpha}$ occurred. At very high ethanol concentrations (600-800 mg/dl; 130-174 mmol/l) significant stimulation of conversion occurred.

Human umbilical endothelial cell: arachidonic acid incorporation model (table V)—There was no significant change in the rate of conversion of ^{14}C-arachidonic acid to 6-oxo-prostaglandin $F_{1\alpha}$, even at high ethanol concentrations.

Discussion

These data are clear evidence that ethanol inhibits platelet thromboxane A_2 synthesis at concentrations commonly observed in alcoholics[15-17] and at concentrations not too distant from those achieved by non-alcoholics after a "moderate" intake of alcohol.[2]

It is also clear that platelet thromboxane A_2 synthesis is inhibited at significantly lower concentrations of ethanol than platelet aggregation itself.

Two other studies [18 19] have attempted to show diminished thromboxane A_2 synthesis after ethanol ingestion. In one[18] thromboxane synthesis was assayed by measuring the conversion of ^{14}C-arachidonic acid into thromboxane B_2 by washed platelets; no direct measurement of thromboxane B_2 synthesised from endogenous arachidonic acid was carried out. In the other study[19] serum thromboxane B_2 was measured by a specific radioimmunoassay similar to ours. Coagulation of blood, however, leads to a massive release of thromboxane A_2, which may mask any alteration in thromboxane release.[20] Such subtle alterations are probably more easily observed in a system like ours, in which release of thromboxane B_2 is observed in platelet rich plasma at the end of a three minute aggregation period. This is probably why we were able to show significant inhibition of platelet thromboxane A_2 release by much lower concentrations of ethanol than those used in previous work. For example, in the study by Kontula *et al*,[19] a blood alcohol concentration of 120 mg/dl (26 mmol/l) caused a mere 22% reduction in thromboxane A_2, whereas we found a 41% inhibition at 72 mg/dl (16 mmol/l).

Interestingly the inhibitory effect of ethanol on platelet aggregation was more pronounced when adrenaline or collagen was used to initiate aggregation rather than adenosine diphosphate. This was probably due to the fact that aggregation induced by adrenaline and collagen is associated with a much greater release of thromboxane A_2 than that induced by adenosine diphosphate[21]; this is also clearly seen in our data. Ethanol may therefore have a more specific effect on platelet thromboxane synthesis, rather than a uniform effect on all processes leading to aggregation. It should, however, be noted that although the degree of inhibition of adenosine diphosphate induced platelet aggregation correlated significantly with the ethanol concentration in the platelet rich plasma, the amount of thromboxane A_2 released did not. This suggests that ethanol may also inhibit platelet aggregation by a mechanism independent of the production and release of thromboxane A_2.

Our observations may also explain the apparent discrepancy in a recent study[22] showing potentiation by ethanol of aspirin induced prolongation of the bleeding time in the absence of any gross effect by ethanol alone. This potentiation by ethanol may have been mediated through a thromboxane A_2 independent mechanism as described above or by further inhibition of thromboxane A_2 release, since total inhibition of thromboxane A_2 release by platelets may not have occurred after aspirin.[20]

It is, however, intriguing that the effect of ethanol ingestion was both rapid and transient. It was pronounced at 30 minutes, but had almost reversed by 60 minutes. We have no explanation at present. Nevertheless, it is possible that ethanol induces its antiaggregatory effect through alterations in the platelet membrane by reducing the availability of arachidonic acid for thromboxane A_2 synthesis. Such an alteration could be compensated for rapidly by the normal platelet. Continued, chronic ingestion of ethanol, on the other hand, might alter the platelet membrane "permanently" from the time of its formation from megakaryocytes. This concept is supported by our observations in chronic alcoholics (unpublished data), where thromboxane A_2 production by platelets is diminished.

Finally, all our attempts—in the different models—to show an alteration in the synthesis of prostaglandin I_2 failed to detect any ethanol induced inhibition. This absence of an inhibitory effect of ethanol on vascular production of prostaglandin I_2 is important, since it will induce a selective inhibition on platelet prostaglandin synthesis and shift the balance of platelet aggregatory-vasoconstrictive versus platelet antiaggregatory-vasodilatory processes towards the latter.

The effect of ethanol ingestion on in vivo production of prostaglandin I_2 was not assessed because in our hands, and in those of others,[23-27] the techniques for measuring plasma 6-oxo-prostaglandin $F_{1\alpha}$ concentrations are unsatisfactory.

We conclude that at concentrations of ethanol commonly found in the blood of alcoholics[15-17] there occurs an inhibition of platelet aggregation and thromboxane release in vivo and in vitro. Similar and higher ethanol concentrations have no effect on the synthesis of prostaglandin I_2 by vascular tissue in vitro. The selective inhibitory effect of ethanol on platelets may contribute to the protection against vascular disease observed in moderate drinkers,[2] and may also contribute to the pathogenesis of acute gastrointestinal bleeds after bouts of alcohol intake.

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