Acute Ethanol Ingestion Increases Platelet Reactivity: Is There a Relationship to Stroke?

Matti Hillbom, M.D., Mauno Kangasaho, M.Sc.,* Markku Kaste, M.D., Heikki Numminen, M.D., and Heikki Vapaatalo, M.D.†

SUMMARY The effects of ethanol ingestion on ADP-induced platelet aggregation and associated thromboxane formation were studied in the platelet-rich plasma of 10 healthy male volunteers, each serving as his own control. Ethanol caused a transient decrease in threshold concentration of ADP to produce irreversible aggregation. Over a wide range of ADP total platelet aggregation was increased. In the presence of irreversible aggregation, formation of thromboxane B2 rose from 303 ± 56 to 950 ± 212 fmol per 10^7 platelets (p < 0.01). The effects lasted as long as ethanol was present in blood, did not significantly correlate to blood ethanol levels and exhibited great individual variation. It remains to be proved, whether these observations could contribute to the increased risk of ischemic brain infarction associated with acute ethanol ingestion.

Is There A Relationship to Stroke?

Frank H. L. Hillbom, M.D., Mauno Kangasaho, M. Sc.,* Markku Kaste, M.D., Heikki Numminen, M.D., and Heikki Vapaatalo, M.D. †

ETHANOL INGESTION seems to carry an increased risk of both ischemic and hemorrhagic strokes,1,2 but it is not known how both ethanol precipitates acute cerebrovascular disorders. Platelets play an essential role in thrombus formation and changes in platelet function may well contribute to the onset of strokes. The association of acute ethanol intoxication with the onset of symptoms of ischemic brain infarction in adolescents and young adults3 prompted us to study the effects of acute ethanol ingestion on platelet aggregation and associated thromboxane formation. Thromboxane B2 (TXB2), a metabolite of the potent platelet-aggregating gating agent since it may be important for hemostasis and vasoconstrictor agent thromboxane A2 (TXA2), was measured from platelet rich plasma (PRP) after ADP-induced aggregation. ADP was used as an aggregating agent since it may be important for hemostasis in vivo.4 The results demonstrate significant increases in ADP-induced platelet aggregation and associated thromboxane formation after acute ethanol ingestion.

Subjects and Methods

Ten healthy male students aged 22–30 years volunteered for the trial. After full explanation of the purpose, nature and risks of the study, a written informed consent was obtained from each. The study protocol was approved by the Ethical Committee of the Department of Neurology, University of Helsinki.

The volunteers were nonsmokers and occasional or infrequent drinkers. They had abstained from alcoholic beverage for two weeks before the trial. None revealed arterial hypertension, heart disease, diabetes, hematological disorders, migraine or other known risk factors of ischemic brain infarction in their past history, nor were any taking regular medication.

During the first experimental trial randomly selected subjects drank either 1.5 g ethanol per kg body weight as a 4 M solution in fruit juice or an equal volume of the same fruit juice without ethanol. For the second session, a fortnight thereafter, the roles were changed so that each subject served as his own control. The volunteers had a light breakfast at 8 a.m. of the trial day but had nothing to eat for 26 hours thereafter. They received the first drink at 6 p.m., i.e., after fasting for 10 hours. Five drinks were given over 2.5 hours in order to avoid vomiting and to reach a constant rate of ethanol ingestion. The volunteers could drink water freely until the beginning of the trial, but not thereafter. They were also offered the opportunity to sleep between blood samplings but they only slept about six hours during the night.

The first blood sample was obtained immediately prior to the first drink and the second, four hours later, when the peak of blood ethanol concentration was assumed to occur. The two additional samples were taken at 12 and 16 hours after the first drink, i.e., shortly before and during the period when blood ethanol concentration reached zero and the unpleasant after-effects of ethanol intoxication (hangover symptoms) should appear.

Blood was dripped via siliconized needles to EDTA-tubes and to 1:10 volume of citrate containing plastic tubes. At first we measured the packed cell volume by a microhematocrit method to observe changes caused by drinking of ethanol or fruit juice. The results suggested no need to adjust the amount of anticoagulant with the packed cell volume more precisely. Then, citrated PRP was prepared by centrifuging whole blood at 160 g for 20 min at room temperature, and platelet count was adjusted to 350 × 10^9 μL^-1 by autologous platelet poor plasma.

Platelet aggregation was induced two hours after blood sampling in PRP by 4, 8, 12 or 16 μM ADP at +37°C with continuous stirring by magnetic stirrer.
(1200 rpm). Aggregation was allowed to proceed for five minutes and was stopped by adding 25 μl of 1 N HCl per 300 μl of PRP and the tubes were transferred to an icebath. The amount of TXB₂ formed during aggregation was measured directly from plasma⁷ by a double antibody radioimmunoassay technique. Plasma components were found not to disturb the assay and TXB₂ was stable in our acidified samples for more than half a year when immediately frozen and stored at -20°C. Parallel samples treated with ethylenediamine tetra-acetic acid and indomethacin instead of HCl yielded quite similar amounts of TXB₂.

Blood ethanol concentration was measured by a routine gas-liquid chromatography technique.

ADP was from Boehringer-Mannheim GmbH (Mannheim, GFR) and ³H-TXB₂ from Amersham International plc (Amersham, UK). TXB₂ antiserum was from Institut Pasteur (Marnes-la-Coquette, France).

We calculated the average increases across the two treatment periods and the mean differences in the changes from baseline during each of the two treatment periods within individual subjects. The student’s t test for paired observations was also used in statistical comparisons.

Results

Packed cell volumes and platelet counts in PRP are shown in table 1. Slight increases were observed in the platelet yield in PRP preparations during both control and ethanol trials, but for aggregation studies the platelet count in PRP was always adjusted to be the same. Packed cell volumes were significantly increased at the end of the trials probably because the volunteers were not allowed to drink water freely.

High concentrations of ADP (8–16 μM) always induced irreversible (secondary phase) aggregation and the total aggregation was enhanced by ethanol (tables 2–3). The enhancement of platelet reactivity was transient and disappeared when blood ethanol concentration reached zero (fig. 1).

Plasma of 8 subjects was tested with 4 μM ADP. During the control trial this concentration usually failed to induce irreversible aggregation. However, at 16 hours two subjects showed irreversible aggregation which probably was due to some change in plasma components caused by prolonged fasting such as an increase in free fatty acids. In contrast, during the ethanol trial all 8 subjects transiently developed irreversible aggregation. An example of this type of reaction is shown in figure 2. Before starting to drink ethanol all the subjects showed reversible aggregation to 4 μM ADP, but irreversible aggregation could be seen in 7 subjects at 4 hours and in 5 subjects at 12 hours. At 16 hours all again showed reversible aggregation. These findings clearly demonstrate that the lowest concentration of ADP needed to produce irreversible aggregation was less when ethanol was present in blood.

When compared to pre-ethanol values, TXB₂ formation increased significantly but transiently during the ethanol trial. Ingestion of fruit juice alone did not influence platelet aggregation and associated TXB₂ formation. In some subjects the enhancement of TXB₂ formation by ethanol was very large (4–8 fold). We retested one such subject after a year, but the effect was less the second time. Ethanol did not increase TXB₂ formation if the aggregation remained reversible. On the other hand, the ethanol-induced enhancement of TXB₂ formation was seen with all the concentrations of ADP that caused irreversible aggregation, and adding more ADP did not further increase TXB₂ formation.

Blood ethanol levels were not directly related to TXB₂ formation. In some subjects higher TXB₂ levels were found at 12 hours compared with 4 hours even though blood ethanol was higher at 4 hours. After partialing out initial variation in TXB₂ levels between the subjects no significant correlation could be found between blood ethanol and TXB₂ formation in PRP.

Discussion

The effects of ethanol on platelet function reported in the literature are inconsistent. Human platelet aggregation has been reported to be unchanged⁶ or inhibited⁹ by acute ingestion of moderate doses of ethanol. In one recent study where the effects were followed only for one hour after ethanol ingestion neither ADP-in-

### Table 1 Changes in Packed Cell Volumes and Platelet Counts of PRP

<table>
<thead>
<tr>
<th>Variable</th>
<th>Trial</th>
<th>0</th>
<th>4</th>
<th>12</th>
<th>16</th>
<th>Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Packed cell volume</td>
<td>control</td>
<td>45.9±0.8</td>
<td>47.2±0.7</td>
<td>47.4±0.8</td>
<td>48.2±0.8</td>
<td>%</td>
</tr>
<tr>
<td></td>
<td>ethanol</td>
<td>46.9±0.6</td>
<td>47.4±0.8</td>
<td>47.9±0.6</td>
<td>48.8±0.7*</td>
<td>%</td>
</tr>
<tr>
<td>average increase‡</td>
<td>1.5±0.7</td>
<td>0.5±0.9</td>
<td>0.3±0.7</td>
<td>0.6±0.7</td>
<td></td>
<td>%</td>
</tr>
<tr>
<td>mean difference</td>
<td></td>
<td>-1.0±0.9</td>
<td>-1.0±1.1</td>
<td>-0.7±0.7</td>
<td></td>
<td>%</td>
</tr>
<tr>
<td>Platelet count in PRP</td>
<td>control</td>
<td>4.5±0.3</td>
<td>4.8±0.3</td>
<td>5.1±0.4*</td>
<td>4.8±0.3</td>
<td>10⁸ ml⁻¹</td>
</tr>
<tr>
<td></td>
<td>ethanol</td>
<td>4.7±0.3</td>
<td>4.9±0.3</td>
<td>5.1±0.3</td>
<td>5.2±0.3*</td>
<td>10⁸ ml⁻¹</td>
</tr>
<tr>
<td>average increase§</td>
<td>0.2±0.2</td>
<td>0.1±0.2</td>
<td>0.0±0.2</td>
<td>0.4±0.2</td>
<td></td>
<td>10⁸ ml⁻¹</td>
</tr>
<tr>
<td>mean difference</td>
<td>-0.1±0.1</td>
<td>-0.2±0.2</td>
<td>0.2±0.2</td>
<td></td>
<td></td>
<td>10⁸ ml⁻¹</td>
</tr>
</tbody>
</table>

The figures represent the mean values ± SEM of 10 volunteers. *p < 0.05, †p < 0.01 for difference from the value at the beginning (0 hours) of the same session.

‡Average increase from the control to the ethanol trial. §p < 0.05.

† Mean paired differences in the changes from baseline (0 hours) during each of the two treatment periods.
were significantly changed. But ethanol caused a rapid
duced aggregation nor associated TXB₂ formation
even been reported. 13-14 Preliminary experiments made
be without effect. 12 Inhibition of TXB₂ formation has
instead of ADP. 9
Incubation of human platelets with ethanol in vitro
has shown that even addition of eth-
and transient inhibition of platelet aggregation and as-
sociated TXB₂ formation, when epinephrine was used
achieved in the present study increases platelet reactiv-
ty to ADP and associated TXB₂ formation (Kanga-
saho, unpublished observation).
Accordingly, previous observations are not consis-
tent with our results. As far as we know, our findings
are the first to suggest enhanced platelet aggregation
may well depend on the aggregating agent
used. 9

TABLE 2 Effects of Ethanol Ingestion on ADP-induced Platelet Aggregation and Associated TXB₂ Formation

<table>
<thead>
<tr>
<th>Variable</th>
<th>Trial</th>
<th>Sampling time (hours)</th>
<th>Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>4</td>
<td>12</td>
</tr>
<tr>
<td>Blood ethanol</td>
<td>control</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>ethanol</td>
<td>34.1 ± 1.7</td>
<td>10.3 ± 1.4</td>
</tr>
<tr>
<td>Platelet aggregation</td>
<td>4 µM ADP</td>
<td>control</td>
<td>45 ± 5</td>
</tr>
<tr>
<td></td>
<td>ethanol</td>
<td>50 ± 4</td>
<td>59 ± 5</td>
</tr>
<tr>
<td></td>
<td>8 µM ADP</td>
<td>control</td>
<td>62 ± 2</td>
</tr>
<tr>
<td></td>
<td>ethanol</td>
<td>69 ± 3</td>
<td>82 ± 2†</td>
</tr>
<tr>
<td></td>
<td>16 µM ADP</td>
<td>control</td>
<td>74 ± 5</td>
</tr>
<tr>
<td></td>
<td>ethanol</td>
<td>77 ± 2</td>
<td>83 ± 2</td>
</tr>
<tr>
<td>TXB₂ formation</td>
<td>4 µM ADP</td>
<td>control</td>
<td>7.1 ± 1.0</td>
</tr>
<tr>
<td></td>
<td>ethanol</td>
<td>7.6 ± 1.6</td>
<td>24.5 ± 8.1</td>
</tr>
<tr>
<td></td>
<td>8 µM ADP</td>
<td>control</td>
<td>10.8 ± 2.3</td>
</tr>
<tr>
<td></td>
<td>ethanol</td>
<td>10.6 ± 1.8</td>
<td>33.2 ± 7.4†</td>
</tr>
<tr>
<td></td>
<td>16 µM ADP</td>
<td>control</td>
<td>11.7 ± 2.3</td>
</tr>
<tr>
<td></td>
<td>ethanol</td>
<td>13.8 ± 3.0</td>
<td>35.0 ± 7.5†</td>
</tr>
</tbody>
</table>

*p < 0.05, †p < 0.01 for difference from the value at the beginning (0 hours) of the same session. The figures represent the mean values ± SEM of 8-10 volunteers.

Incubation of human platelets with ethanol in vitro
has been found to inhibit platelet aggregation 13-14 or to
be without effect. 13 Inhibition of TXB₂ formation has
even been reported. 13-14 Preliminary experiments made
in our laboratory has shown that even addition of eth-
anol to PRP in similar concentrations that were
achieved in the present study increases platelet reactiv-
ty to ADP and associated TXB₂ formation (Kanga-
saho, unpublished observation).
Accordingly, previous observations are not consis-
tent with our results. As far as we know, our findings
are the first to suggest enhanced platelet aggregation
and thromboxane formation under the influence of eth-
anol. However, different techniques and inducers have
been used, and the effect of ethanol on platelet aggre-
gation may well depend on the aggregating agent
used. 13

TABLE 3 Effects of Ethanol Ingestion on ADP-induced Platelet Aggregation and Associated TXB₂ Formation after Removing the Effects of Subject Variation and Time

<table>
<thead>
<tr>
<th>Variable</th>
<th>Sampling time (hours)</th>
<th>Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>4</td>
</tr>
</tbody>
</table>
| Platelet aggrega
| 4 µM ADP         | average increase†  | 6 ± 8  | 16 ± 6* | 11 ± 8 | -5 ± 9 | % |
|                  | mean difference ** | 7 ± 9  | 5 ± 14  | -12 ± 14 | % |
| 8 µM ADP         | average increase   | 7 ± 7  | 17 ± 4† | 20 ± 4‡ | 8 ± 7 | % |
|                  | mean difference    | 9 ± 7  | 13 ± 8  | -2 ± 10  | % |
| 16 µM ADP        | average increase   | 3 ± 5  | 8 ± 3*  | 12 ± 3† | 5 ± 5 | % |
|                  | mean difference    | 6 ± 5  | 9 ± 6  | 0 ± 8  | % |
| TXB₂ formation   | 4 µM ADP          | average increase | 0.6 ± 1.5 | 22.1 ± 8.0* | 12.2 ± 6.0 | -0.2 ± 2.6 | mmol 1⁻¹ |
|                  | mean difference   | 21.5 ± 8.3§ | 11.6 ± 6.7 | -1.0 ± 1.9 | mmol 1⁻¹ |
| 8 µM ADP         | average increase   | -0.1 ± 2.7 | 25.7 ± 6.7† | 20.8 ± 5.1‡ | 9.7 ± 3.5* | mmol 1⁻¹ |
|                  | mean difference   | 25.8 ± 6.6| 20.9 ± 4.8 | 8.3 ± 2.8§ | mmol 1⁻¹ |
| 16 µM ADP        | average increase   | 2.1 ± 3.2 | 26.8 ± 5.9† | 16.2 ± 4.0‡ | 7.7 ± 3.4 | mmol 1⁻¹ |
|                  | mean difference   | 24.7 ± 6.3| 14.1 ± 3.8 | 5.1 ± 3.7 | mmol 1⁻¹ |

†Average increase from the control to the ethanol trial. *p < 0.05, †p < 0.01, ‡p < 0.001.
**Mean paired differences in the changes from baseline (0 hours) during each of the two treatment periods. §p < 0.05, |p < 0.01.
The figures represent the mean values ± SEM of 8-10 volunteers.
For the clinician it is important to know how platelets are activated in human blood circulation. Whether ADP is important as an aggregating agent in vivo is still uncertain, but there is strong evidence in favour of this concept.4 If ADP plays a key role in platelet aggregation in vivo our findings may prove to be important.

Whether the increased platelet reactivity to ADP and associated increased TXB₂ formation are due to ethanol-induced alterations in platelet metabolism, platelet membrane or some effect of ethanol on plasma components is not known. Our results suggest a direct effect of ethanol although this seems to be independent of concentration. Whether acetaldehyde, acetate or some metabolic effect of ethanol plays a role is unclear. Plasma free fatty acids are not significantly influenced by ethanol during the early hours of intoxication,13 and even though endogenous catecholamines are increased they seem neither to initiate platelet activation nor to increase thromboxane formation.16 The two subjects who showed increased platelet activity at the end of the control trial also had very clear rises in TXB₂ formation (3–8 fold) during the ethanol trial. Thus we cannot yet exclude a contributing role of fasting, and more work is needed to clarify the mechanism by which ethanol ingestion increases platelet reactivity. Furthermore, the platelets should also be challenged with other aggregating agents in order to see if the effect is really specific to ADP. It has been reported that ADP-induced biphasic aggregation, and thromboxane formation with ADP, is an in vitro artefact caused by the presence of citrate which reduces the amount of ionized Ca²⁺ in the plasma.17 Fenn and Littleton have already demonstrated that platelet aggregation to exogenous arachidonic acid is potentiated by ethanol.11

We have earlier found that the plasma levels of TXB₂ and 6-keto-prostaglandin F₁α are slightly decreased after ethanol ingestion. TXB₂, being more reduced than the prostacyclin metabolite.18 However, we suggest that plasma and platelet TXB₂, behave differently and independently. The plasma levels do not tell much of the capacity of platelets to form thromboxane when stimulated. Ethanol ingestion does not stimulate platelets, but probably acts via increasing their reactivity which, on the other hand, is not necessarily reflected by increased plasma levels of TXB₂. It is unclear what the plasma levels of TXB₂ reflect.

It is tempting to speculate that ethanol could precipitate ischemic brain infarction by sensitizing platelets to ADP and by increasing platelet thromboxane formation. Of course other contributing factors such as vessel wall disease may be needed.

Our previous epidemiological observations among stroke patients do not unambiguously indicate proneness to thrombosis. Acute ingestion of ethanol was even found to increase the risk of subarachnoid hemorrhage.7 This latter finding cannot be explained by enhanced platelet reactivity but, interestingly, ethanol was recently shown to potentiate aspirin-induced prolongation of the bleeding time19 and to precipitate spasms in cerebral and coronary arteries of experimental animals.20 Therefore, other mechanisms should be taken into account when trying to explain the increased risk of subarachnoid hemorrhage during ethanol intoxication.

In conclusion, the present results indicate that acute...
ethanol ingestion increases platelet reactivity to ADP and associated TXB₂ formation. Whether this suggests that an individual risk factor for ischemic brain infarction might be associated with a change in platelet reactivity remains to be elucidated.

Acknowledgment

We would like to thank Mr. Kari Soini (Orion Pharmaceutical Co.) for excellent technical assistance.

References


Impact Of Digital Subtraction Angiography On Carotid Evaluation

DAVID C. ANDERSON, M.D., GREGORY G. FISCHER, M.D.

SUMMARY Impact of digital subtraction angiography by intravenous injection (DSAV) was examined in a private neurology clinic. In the evaluation of threatened stroke, advent of DSAV was associated with reduced use of both traditional noninvasive tests (from 100% of patients to 36%), and conventional arteriograms (from 29% to 4%). Less compelling indications were often prescreened with noninvasive tests; more compelling symptoms usually had initial DSAV. Conventional arteriograms were done for compelling indications and negative or inadequate DSAV. The average cost of evaluation was increased significantly in patients treated medically and reduced greatly in those having surgery. While cost and convenience might support such utilization, issues of quality of evaluation require consideration.

PATIENTS WITH SUSPECTED CAROTID ATHEROSCLEROSIS were selected for endarterectomy, until very recently, on the basis of conventional contrast arteriography, sometimes after screening with one or more of the traditional noninvasive tests (eg. oculoplethysmography, Doppler flow evaluation, or ultrasound imaging). There has been little disagreement about the preliminary or screening role of the traditional noninvasive tests or about the definitive role of conventional arteriography. The advent of digital subtraction angiography by intravenous contrast injection (DSAV) raises new questions and controversies. DSAV uses computer technology and the subtraction principle to enhance contrast differences, allowing
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