Urinary excretion of 2, 3-dinor-6-keto prostaglandin $F_{1\alpha}$ and platelet thromboxane formation during ethanol withdrawal in alcoholics

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SUMMARY The excretion of 2,3-dinor-6-keto prostaglandin $F_{1\alpha}$, a major urinary metabolite of prostacyclin, and the formation of thromboxane B_2 , a stable metabolite of thromboxane A_2 , by platelets stimulated by adenosine diphosphate, were studied in alcoholics, who had been admitted for detoxification. Once prolonged heavy drinking had stopped, platelet count and thromboxane formation, calculated either per 10^7 platelets or per litre of blood, significantly increased ($p < 0.05$), while the skin bleeding time and urinary excretion of the metabolite of prostacyclin decreased ($p < 0.05$). The balance between prostacyclin and thromboxane therefore seemed to favour the excretion of prostacyclin while it shifted to favour thromboxane formation about a week later.

The eicosanoids thromboxane A_2 (TXA_2)¹ and prostacyclin (PGI_2)^{2,3} represent the opposite poles of an intravascular homeostatic mechanism for platelet aggregation.

We found previously that the formation of platelet generated TXA_2 , measured from plasma as its hydrolysis product thromboxane B_2 (TXB_2), was enhanced shortly after ethanol withdrawal in alcoholics.⁴⁻⁶ Little is known about the changes in the prostacyclin system during this period.⁷

The aim of this study was to extend the observations on platelet reactivity and haemostasis in alcoholics during ethanol withdrawal and to include the prostacyclin system. By measuring platelet capacity to generate TXB_2 , as well as body synthesis of PGI_2 —reflected by urinary 2,3-dinor-6-keto prostaglandin $F_{1\alpha}$ (2,3-dinor-6-keto $PGF_{1\alpha}$), the major PGI_2 metabolite—we hoped to obtain an indication of the balance between the proaggregatory TXA_2 and antiaggregatory PGI_2 .

Material and methods

Eleven normotensive male alcoholics, aged 28 to 50 years (mean 41 years) were studied. All had been admitted to our inpatient detoxification programme. Informed consent was obtained from each.

The men had abused alcohol for 11 to 24 years (mean 16 years). The last binge before admission to the clinic had lasted from 14 to 90 days (mean 42 days). The mean alcohol consumption (hard liquor) had averaged 4.3 (range 1.7–7.2) g/kg of body weight per day. The patients denied drinking additives and taking any drugs during the week preceding enrolment in the study and none had salicylates in their urine the day before blood and urine sampling.

All the alcoholics were smokers, consuming at least 20 cigarettes per day. No changes were made in their smoking habits during detoxification. They were fed a normal hospital diet and received decreasing doses of oxazepam (120, 80, 60, 40 and 20 mg) during the first five days of detoxification. Previous control experiments have shown that this treatment does not influence platelet function.⁴

SAMPLING OF BLOOD AND URINE

Blood and 24 hour urine samples were collected at one, seven, and 21 days after admission. The urine samples were frozen and stored at -70°C before the analysis of 2,3-dinor-6-keto $PGF_{1\alpha}$ (PGI_2-M).

Blood samples were taken after an overnight fast, with minimal stasis, from an antecubital vein into siliconised tubes, each containing one tenth of 3.8% sodium citrate. The amount of anticoagulant was adjusted according to packed cell volume to be the same in each tube. Platelet rich plasma (PRP) and platelet poor plasma were prepared as previously

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described.⁴ The platelet count in PRP was determined with a Linson 431 cell counter (LIC, Sweden). Bleeding time⁸ was measured using the standard Simplate II device by the same examiner on all occasions.

DETERMINATION OF URINARY EXCRETION OF

2, 3-DINOR-6-KETO PGF_{1α}

The amount of PGI₂-M was determined as described previously.⁹ Briefly, an aliquot from each 24 hour urine sample was purified on Sep Pak (Waters Associates, USA) reverse phase cartridges. The metabolite was eluted with methylene chloride/methyl formate (9/1). After partitioning between borate buffer (pH 8) and diethyl ether PGI₂-M was converted to the methoxime benzylester, Tris (trimethylsilyl) ether. Using a deuterium-labelled analogue of PGI₂-M as an internal standard, the amount of PGI₂-M was determined with the aid of a gas chromatograph mass spectrometer, equipped for negative chemical ionisation (Finnigan 4500, USA). Methoxyamine hydrochloride and N,O-bis (trimethylsilyl) trifluoroacetamide were supplied by Pierce, USA, and pentafluoro-benzyl-bromide by Fluka, Switzerland. Diisopropyl-ethylamine and all solvents were purchased from Merck, West Germany. PGI₂-M and its tetradeuterated analogue were gifts from Dr Alan Brash, Vanderbilt University, Tennessee, USA.

Creatinine in urine was determined by standard photometric techniques, and the urinary excretion of PGI₂-M was related to 1 g of excreted creatinine.

PLATELET STIMULATION

For platelet stimulation in PRP, 8 μM adenosine diphosphate (Boehringer Mannheim, West Germany) was used. The stimulation was allowed to proceed with continuous magnetic stirring for five minutes at 37°C on a Platelet Aggregation Profiler, model 3 (Bio-Data Corporation, USA). Thereafter, 1 M hydrochloric acid (final concentration 0.1 M) was added and the samples were immediately frozen and stored at -70°C.

DETERMINATION OF TXB₂

Platelet-generated TXB₂ was measured from plasma by double antibody radioimmunoassay technique.¹⁰ Earlier control experiments have shown that TXB₂ is stable in acidified plasma samples for more than six months. ³H-TXB₂ was bought from Amersham International (Amersham, UK) and the TXB₂ antiserum from Institut Pasteur, Marnes-la-Coquette, France.

STATISTICAL METHODS

Student's *t* test for paired observations was used for statistical analysis. The correlation coefficients between eicosanoid production and the age of the patients were also calculated.

Results

After stopping drinking almost all the alcoholics had reduced platelet counts (figure). During the first week of detoxification the platelet count increased significantly. None of the subjects, however, developed thrombocytosis. On admission to the study bleeding times were slightly prolonged compared with the normal values in our hospital, but one week later they shortened considerably.

The figure shows the formation of TXB₂, calculated per 10⁷ platelets. One of the alcoholics gradually decreased his alcohol consumption over a period of five days before admission, and his platelets had developed an increased capacity to produce thromboxane. Therefore, although the mean TXB₂ concentration of the alcoholics was low on admission to the clinic, it was somewhat higher than in our earlier reports.⁴⁻⁶

The product of the platelet count in the whole blood and platelet TXB₂ formation per 10⁷ platelets provided an indication of the capacity of a unit (one litre) of circulating blood to produce TXB₂ (figure). On the day after admission the capacity to form TXB₂ per litre of blood was normal.⁵ One week later the capacity of platelets to form TXB₂ was increased. More

Figure Changes in haemostatic variables after ethanol withdrawal in alcoholics. Each point represents mean (SEM) of 11 subjects. Asterisks indicate significance of changes from 0 weeks using Student's *t* test for paired observations: **p* < 0.05, ***p* < 0.01.

than four-fold the amount of TXB₂ was produced when calculated per 10⁷ platelets. A 10-fold mean increase could be observed when platelet TXB₂ formation was expressed per litre of whole blood.

The urinary excretion of PGI₂—M was raised on admission. After the first week of detoxification it had decreased significantly ($p < 0.05$). After three weeks of abstinence platelet count, urinary excretion of PGI₂—M, and the skin bleeding time had returned to normal and remained constant. Moreover, TXB₂ formation, calculated per 10⁷ platelets or per litre of blood, had decreased. By this time all the variables had reached the values we previously observed in healthy non-alcoholic male volunteers after an overnight fast.¹¹

To test the possibility that older subjects might have different eicosanoid formation than younger ones the data were separately analysed with regard to age. No significant correlations were found between the age of the patients and the number of eicosanoids produced.

Discussion

The balance between the two principal metabolites of arachidonic acid, proaggregatory and vasoconstrictory TXA₂, and antiaggregatory and vasodilatory PGI₂ seems to be important for primary haemostasis *in vivo*.^{3,12}

The measurement of the inactive metabolite TXB₂ in plasma, after stimulation of platelets in PRP, is a reflection of platelet capacity to synthesise TXA₂.^{1,10} Furthermore, the stimulated TXB₂ formation, calculated per litre of blood, should be a measure of the TXA₂ synthesis potential in the circulating blood.

The concentrations of PGI₂ or its metabolites in plasma are very low.¹³ Urine is the major route of excretion of prostacyclin metabolites in man. The major metabolite is 2,3-dinor-6-keto PGF_{1α} which, however, accounts for less than 10% of the PGI₂ metabolised in the body.¹⁴ Nevertheless, the excretion of PGI₂—M in relation to urinary creatinine is a useful measure of individual changes in the total body synthesis of PGI₂.¹⁵

During the alcohol withdrawal period, we found a large increase in TXB₂ production in platelets stimulated by adenosine diphosphate *ex vivo*. The effect was particularly obvious when expressed as TXB₂ produced per litre of whole blood. These findings agreed with our earlier observations in alcoholics, showing that platelets stimulated by adenosine diphosphate develop enhanced capacity to form TXB₂ one to two weeks after drinking has stopped.^{4–6}

The results of the present study suggest that changes may occur even in the endogenous synthesis of PGI₂. The excretion of PGI₂—M was raised one day after stopping drinking, but in contrast to the

production of platelet TXB₂, it decreased with abstinence.

It is possible to speculate why these changes in the thromboxane-prostacyclin system occur during alcohol withdrawal. It is well known that the platelet count is depressed after a period of prolonged drinking, and this has been attributed to the toxic effects of ethanol on platelets and bone marrow.¹⁶ Moreover, changes in dietary habits, together with intestinal malabsorption and disturbances in the utilisation of nutrients,¹⁷ may contribute to the impaired activity of megakaryocytes.

The diminished capacity of platelets to form TXB₂ could also be a consequence of the decreased availability or synthesis of arachidonic acid that has been described in experimental animals and humans.¹⁸ Furthermore, the ethanol-induced production of lipid peroxides¹⁹ and possible stimulation of cyclooxygenase by these compounds,²⁰ as well as the availability of the inhibitors of lipid peroxidation, might have influenced the ability of platelets to produce thromboxane after a period of heavy drinking. According to our observations, lipid peroxides, measured as malondialdehyde, might be increased in plasma (unpublished observations), whereas the concentrations of vitamin E, an important free radical scavenger and inhibitor of lipid peroxidation in the membrane,²¹ were found to be normal in alcoholics after heavy drinking had stopped.²²

After stopping drinking an increased platelet count often occurs.²³ A consistent finding in the previous studies^{4–6} was that the new platelets were hyperreactive to adenosine diphosphate stimulation and form more TXB₂ than the normal population of platelets, a finding which was also confirmed by the results of this study.

The extent of the effect of ethanol ingestion on PGI₂ synthesis is still unknown. According to our own study ingestion of 0.25 g/kg body weight of ethanol did not change the urinary excretion of PGI₂—M, suggesting that the synthesis of prostacyclin under these conditions was not affected.¹¹ Other investigators, however, found an increase of some prostacyclin metabolites in plasma by radioimmunoassay shortly after ingestion of 32 g of ethanol.²⁴ Higher doses of ethanol might have completely different effects, as at least *in vitro* ethanol seems to stimulate the formation of PGI₂²⁵ in a concentration dependent way.

The raised concentrations of urinary PGI₂—M seen after a period of prolonged heavy drinking could also result from enhanced adrenergic tone²⁶ associated with ethanol withdrawal. Our patients clearly showed increased heart rate and blood pressure (data not shown) at the beginning of detoxification.

Whatever the reason, we have firm evidence to sup-

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port an increase in the formation of antiaggregatory PGI₂ with concomitant diminished overall capacity of platelets to form TXB₂. The prostacyclin-thromboxane balance thus favours PG₂ which is consistent with the increased bleeding time.

After one week of withdrawal there was an increase in the capacity of platelets to form TXB₂, while the body formation of PGI₂, as observed by urinary PGI₂-M, decreased. The prostacyclin-thromboxane balance was then shifted to thromboxane, and a decrease in bleeding time occurred. The changes in the balance of these eicosanoids observed in this study could therefore be associated with the regulation of platelet activity.

There is epidemiological evidence from different parts of the world to suggest that alcoholism is a risk factor for several types of thromboembolic and haemorrhagic disease.²⁷⁻²⁹ As the prostacyclin-thromboxane balance might be important in maintaining the integrity and patency of the vascular space the disturbances in this balance during ethanol withdrawal period could be one of the predisposing mechanisms for thromboembolic vascular disease and haemorrhagic complications.

In conclusion, the results of our study indicate that in alcoholics the balance between prostacyclin and thromboxane favours prostacyclin immediately after heavy drinking is stopped but that shortly thereafter the balance favours thromboxane. These changes are of importance for primary haemostasis and could contribute to the aetiology of vascular complications seen in alcoholics.

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References

- Hamberg M, Svensson J, Samuelsson B. Thromboxanes: a new group of biologically active compounds derived from prostaglandin endoperoxides. *Proc Natl Acad Sci USA* 1975; **72**:2994-8.
- Moncada S, Gryglewski R, Bunting S, Vane JR. An enzyme isolated from arteries transforms prostaglandin endoperoxides to unstable substances that inhibit platelet aggregation. *Nature* 1976; **263**:663-5.
- Moncada S. Biology and the therapeutic potential of prostacyclin. *Stroke* 1983; **2**:157-68.
- Hillbom M, Kangasaho M, Hjelm-Jäger M. Platelet aggregation and thromboxane B₂ formation after ethanol abuse: is there relationship to stroke? *Acta Neurol Scand* 1984; **70**:432-7.
- Hillbom M, Muuronen A, Löwbeer C, Änggård E, Beving H, Kangasaho M. Platelet thromboxane formation and bleeding time is influenced by ethanol withdrawal but not by cigarette smoking. *Thromb Haemostas* 1985; **53**:419-22.
- Hillbom M, Kangasaho M, Löwbeer C, Kaste M, Muuronen A, Numminen H. Effects of ethanol on platelet function. *Alcohol* 1985; **2**:429-32.
- Pediconi M, Colombo C, Galli G. Ethanol administration in the rat decreases prostacyclin production by isolated brain microvessels. *J Neurochem* 1986; **46**:1458-63.
- Ivy AC, Nelson D, Bucher G. The standardization of certain factors in the cutaneous "venostasis" bleeding time technique. *J Lab Clin Med* 1941; **26**:1912-22.
- Falardeau P, Oates JA, Brash AR. Quantitative analysis of two dinor urinary metabolites of prostaglandin I₂. *Anal Biochem* 1981; **115**:359-67.
- Granström E. Assay methods for prostaglandins and thromboxanes. In: Samuelsson B, Ramwell PW, Paoletti R, eds. *Advances in prostaglandin and thromboxane research*. New York: Raven Press, 1980:69-76.
- Neiman J, Hillbom M, Jones AW, Benthin G, Löwbeer C. Platelet function during acetaldehyde intoxication in healthy male volunteers. *Alcohol Alcoholism* 1987; **suppl** 1:587-90.
- Bunting S, Moncada S, Vane JR. The prostacyclin-thromboxane A₂ balance: pathophysiological and therapeutic implications. *Br Med Bull* 1983; **3**:271-6.
- Blair IA, Barrow SE, Waddell KA, Lewis JP, Dollery CT. Prostacyclin is not a circulating hormone in man. *Prostaglandins* 1982; **4**:570-89.
- Brash AR, Jackson EK, Saggese CA, Lawson JA, Oates JA, Fitzgerald GA. Metabolic disposition of prostacyclin in humans. *J Pharmacol Exp Ther* 1983; **1**:78-87.
- Oliw E, Granström E, Änggård E. The prostaglandins and essential fatty acids. In: Pace-Asciak CR, Granström E, eds. *Prostaglandins and related substances*. Amsterdam: Elsevier, 1983:2-44.
- Cowan DH. Effect of alcoholism in hemostasis. *Semin Haematol* 1980; **17**:137-47.
- Lieber CS. Alcohol-nutrition interaction: 1984 update. *Alcohol* 1984; **1**:151-7.
- Änggård E. Ethanol, essential fatty acids and prostaglandins. *Pharmacol Biochem Behav* 1983; **18**:401-7.
- Shaw S, Jayatilake E, Ross WA, Gordon ER, Lieber CS. Ethanol induced lipid peroxidation: potentiation by long-term alcohol feeding and attenuation by methionine. *J Lab Clin Med* 1981; **98**:417-24.
- Hemler ME, Cook HW, Lands WEM. Prostaglandin biosynthesis can be triggered by lipid peroxides. *Arch Biochem Biophys* 1979; **193**:340-5.
- Gutteridge JMC. The membrane effects of vitamin E, cholesterol and their acetates on peroxidative susceptibility. *Res Commun Chem Pathol Pharmacol* 1978; **22**:563-72.
- Hillbom M, Muuronen A, Neiman J, Björk G, Egberg N, Kangasaho M. Effects of vitamin E therapy on ethanol-induced changes in platelet aggregation, thromboxane formation, factor VIII levels and serum lipids. *Europ J Clin Invest* 1987; **17**.
- Sullivan LW. Effects of alcohol on platelet production. In: Paulus LM, ed. *Platelet kinetics*. Amsterdam: North-Holland, 1971:247-52.
- Landolfi R, Steiner M. Ethanol raises prostacyclin in vivo and in vitro. *Blood* 1984; **64**:679-82.
- Karanian JW, Stojanov M, Salem Jr M. Effects of ethanol on prostacyclin and thromboxane A₂ synthesis in rat aortic rings in vitro. *Prostaglandins Leukotrienes Med* 1985; **20**:175-86.
- Neri Serneri GG, Masotti G, Gensini GF, et al. Prostacyclin and thromboxane A₂ formation in response to adrenergic stimulation in humans: a mechanism for local control of vascular response to sympathetic activation? *Cardiovasc Res* 1981; **15**:287-95.
- Friedman HS. Cardiovascular effects of alcohol with particular reference to the heart. *Alcohol* 1984; **1**:333-9.
- Altura BM, Altura BT. Alcohol, the cerebral circulation and strokes. *Alcohol* 1984; **1**:225-31.
- Hillbom ME. What supports the role of alcohol as a risk factor for stroke? *Acta Med Scand* 1987; **75**-85.

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