Malondialdehyde-Like Material and Beta-Thromboglobulin Plasma Levels in Patients Suffering From Transient Ischemic Attacks

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SUMMARY Beta-thromboglobulin (betaTG) and malondialdehyde-like material (MDA-LM) plasma levels were studied in patients affected by transient ischemic attacks (TIA) after 2-4 months from the last episode. BetaTG and MDA-LM values were significantly higher in TIA patients than in 20 controls matched for age. No correlation between MDA-LM and betaTG was seen. This study suggests that in vivo platelet activation and, likely, increase of platelet cyclo-oxygenase activity can be detectable in TIA patients.

PLATELET HYPERAGGREGATION AND high beta-thromboglobulin (betaTG) plasma levels, a sign of in vivo platelet activation, have been reported in patients with transient ischemic attacks (TIA). High plasma levels of malondialdehyde-like material (MDA-LM) have been also found in TIA and stroke patients. The interpretation of these last results is not easy mainly because the origin of plasma MDA-LM is not clear yet. Some Authors have considered this parameter as a sign of platelet activability, i.e. a sign of cyclooxygenase and thromboxane synthetase activities, while other investigators retained that plasma MDA-LM is not necessarily expression of platelet activability since MDA-LM can derive from various tissues and during nonenzymatic autoxidation of polyunsaturated fatty acids.

In this preliminary report we describe 20 patients suffering from recurrent TIA, showing along with in vivo platelet activation high values of plasma MDA-LM.

Patients and Method

Twenty patients, (14 males and 6 females, age 37-73 years old) who had a definite episode of acute neurological impairment of vascular origin lasting less 24 hours and leaving no sequelae, have been included in this study. The patients had the last TIA 2-4 months before and did not take any drug interfering with platelet function or oxidative process from at least one month. 4 females and 2 males suffered from recurrent TIA; the other patients had only one attack before our investigation. A complete clinical assessment was made, with special regard to such risk factor as diabetes, hypertension, smoking and dislipidaemia. Routinary investigation included ECG, chest-X ray, blood levels of cholesterol, HDL-cholesterol, triglycerides, uric acid, glucose, urea and creatinine. All patients performed an Eco-Doppler investigation of cerebral arteries. Six patients were smokers, four patients were affected by hypertension, three were hypercholesterolemic and one was diabetic. Ten patients showed abnormalities on Eco-Doppler investigation. Six patients did not show any of the above mentioned risk factors or laboratory abnormalities.

MDA-LM evaluation was made by Smith’s method partially modified by us: venous blood was withdrawn from subjects fasting at least 12 hours, mixed with 0.13 mol/l Na-citrata in a ratio of 9:1. Platelet poor plasma was obtained after blood centrifugation for 15 min at 2000 × g; plasma MDA-LM was assayed treating 1 ml of platelet poor plasma with 1 ml 20% w/v trichloroacetic acid in 0.6 M HCl. Samples were mixed and centrifugated at 2000 × g for 15 min. 0.3 ml of a pH 7 solution of 0.12 M thiobarbiturate, prepared by dissolving thiobarbituric acid (BDH Chemicals Ltd) in 0.26 M 2-amino-2-hydroxymethyl-1-propandiol (Tris), was added to 1.5 ml of supernatant sample. This mixture was boiled for 15 min in a water bath; afterwards it was cooled to room tempera-
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ure and centrifuged at 2000 x g for 15 min. Optical density of this solution was read at 532 nm on Beckman spectrophotometer (mod. 34). The evaluation of MDA-LM was made using malondialdehyde tetramethyl acetal (Eastman, Kodak Co. Rochester, N.Y.) standard curve (stock solution of 10 mM was made by hydrolyzing 8.2 μl of malondialdehyde with 4 drops of concentrated HCl and then diluted with redistilled water to a final volume of 5 ml). MDA-LM values were given in μmol/l. The day-to-day coefficient of variation of MDA-LM was 10%.

BetaTG plasma levels were studied by RIA method as previously described14 in 10 patients only. 20 normal volunteers (age: 36-76 years old) were also studied as control.

Mean, SD and Student's ‘t’ test were used as statistical analysis.

Results

TIA patients had MDA-LM values significantly higher than normal controls (fig. 1). 11 patients showed MDA-LM values higher than 0.877 μmol/l (mean + 2SD of normal controls) while MDA-LM values higher than 0.877 μmol/l were seen in 1 normal control only.

High betaTG plasma levels were also seen in TIA patients (fig. 2); 5 out of 10 patients showed betaTG values higher than 51 ng/ml (mean + 2SD of normal controls). No correlation between betaTG and MDA-LM values were seen in 10 patients in whom both these parameters have been studied. High values of betaTG and MDA-LM were not dependent upon the presence or less of hypercholesterolemia, hypertension or diabetes. Six out of 10 patients with Eco-Doppler abnormalities had MDA-LM values higher 0.877 μmol/l.

Discussion

TIA is considered a warning sign of an impending stroke especially within the year following the first ischemic attack.12-13 Platelet hyperaggregation14,15 and blood hyperviscosity6 seem to have a pathogenic role in determining TIA. Platelet hyperaggregation and more recently high betaTG plasma levels, have been reported in TIA.2,3 Our data showing high levels of betaTG agree with these reports. However the clinical importance of these data is, up to now, not defined, even though recent report suggests that high betaTG levels could be predictive of further cerebro-vascular accidents.17

The increase of plasma MDA-LM shown by our patients could be also connected with platelet hyperactivity, i.e. with the increase of cyclooxygenase activity, but we have not a clear cut evidence for affirming this. Actually the origin of plasma MDA-LM is not fully understood14-5 and further investigations are necessary for concluding that plasma MDA-LM reflect platelet cyclooxygenase activity. However, the high values of plasma MDA-LM and betaTG seem to be a frequent feature of TIA since half of patients revealed these abnormalities. Besides, patients with Eco-Doppler alterations often had high values of MDA-LM, suggesting a possible connection between the increase of plasma MDA-LM and the atherosclerotic disease.

References

Reduction of Serum Prostacyclin Stability in Ischemic Stroke

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SUMMARY Prostacyclin is a powerful vasodilator and inhibitor of platelet aggregation that has been implicated to play a role in cerebrovascular disease. Prostacyclin is unstable in aqueous solution and stabilized in serum by binding to an unidentified serum protein as measured by gel filtration. In 15 patients with ischemic stroke we measured the serum prostacyclin binding capacity and the rate of degradation of exogenously added prostacyclin. There was a significant reduction in serum prostacyclin binding capacity and a significant increase in rate of degradation in the patients with ischemic stroke as a whole compared to controls, and in patients with persistent deficits compared to those with transient deficits. Decreased serum prostacyclin binding capacity and accelerated rate of prostacyclin degradation in vitro, may reflect an accelerated rate in vivo of prostacyclin degradation, thereby increasing susceptibility to stroke. Since only a small number of patients were investigated, the findings are of a preliminary nature and must be confirmed by further studies with large numbers of patients and appropriate patient controls.

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Although much is known about the biochemistry of prostaglandins and their metabolites,1 the role of these substances in cerebrovascular disease remains speculative. Prostacyclin (PBI2), an arachidonate metabolite, is a powerful vasodilator and inhibitor of platelet aggregation which has been implicated to play a potential role in defense against thrombosis.3 Prostacyclin is unstable in aqueous solution at physiologic pH and temperature, and is stabilized by binding to serum macromolecules.4,5 A reduced serum prostacyclin binding was observed in a patient with chronic thrombotic thrombocytopenic purpura and appeared to increase the risk of microvascular thrombus formation by allowing the rapid degradation of prostacyclin to its inactive metabolite 6-keto prostaglandin F1α (6KPG F1α).6,7 To investigate the potential role of prostacyclin instability in stroke we measured the prostacyclin binding capacity and the rate of prostacyclin degradation in patients with cerebrovascular disease.

Patients and Methods

Patients

Fifteen patients with well documented thromboembolic stroke were included in the study from August to October 1982. Nine patients, 5 male and 4 female, mean age 62 (range 35–78) had persistent deficits (persistent group) and 6 patients, 3 male and 3 female, mean age 48 (range 31–69) had reversible deficits lasting less than 7 days (transitory group). In the persistent group, the ischemic stroke was due to a cardiac embolus in 2, a pure sensory lacune in 2 and the remaining patients had no identifiable source. The platelet count, hemoglobin, hematocrit, red cell count and leukocyte count and differential were normal in each patient. The serum albumin and globulin concentrations were also normal in each patient. None of the patients took aspirin containing drugs in the 2 weeks prior to the stroke. Healthy subjects were included as controls. As the
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