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PLATELET AND MONOCYTE VARIABLES IN HOMOCYSTINURIA DUE TO CYSTATHIONINE-β-SYNTHASE DEFICIENCY

ANTONIO COPPOLA, ROSINA ALBISINNI, PASQUALE MADONNA, ANNA PAGANO, ANNA MARIA CERBONE, GIOVANNI DI MINNO

Department of Clinical and Experimental Medicine, University of Naples "Federico II", Naples, Italy

ABSTRACT

To gain insight into the mechanisms responsible for enhanced thromboxane (TX) A_2 biosynthesis in homozygous homocystinuria due to cystathionine-β-synthase deficiency (CBSD), we measured a series of platelet and monocyte variables in 9 homozygous and 8 obligate heterozygous CBSD patients and evaluated their relationships to thromboxane formation, as reflected by urinary excretion of its major metabolite, 11-dehydro-TXB_2 (TXM). Consistent with our previous data, homozygous CBSD patients showed abnormally high TXM excretion (1175±236 pg/mg creatinine vs. 284±39 in control subjects; p<0.001). Significantly higher TXM excretion was also found in obligate heterozygotes (755±450 pg/mg creatinine; p<0.05 vs. control subjects). All platelet and monocyte variables fell within the normal range in CBSD patients and none showed a correlation with TXM excretion (p always >0.05). Our results argue against abnormalities of platelet and monocyte function being responsible for the abnormally high *in vivo* TXA_2 biosynthesis in homocystinuria due to CBSD.

Key words: homocystinuria, cystathionine-β-synthase deficiency (CBSD), thromboxane metabolism, 11-dehydro-TXB2 (TXM), platelets, monocytes

A tendency to premature atherosclerosis and arterial and venous thrombosis are major clinical problems in the management of patients with homocystinuria due to homozygous cystathionine-β-synthase deficiency (CBSD).[1,2] A large series of studies has been devoted to identifying the hemostatic mechanisms involved in this tendency.[3] Enhanced platelet biosynthesis of TXA_2 is an important contributor to the thrombotic tendency associated with several cardiovascular risk factors.[4] We have demonstrated abnormally high *in vivo* TXA_2 biosynthesis in homozygous homocystinuric patients, as evaluated by urinary excretion of its major metabolite, 11-dehydro-TXB_2 (TXM). Such abnormal biosynthesis is likely to reflect *in vivo* platelet activation.[5] *Ex vivo* studies have revealed abnormalities of platelet function in clinical settings with abnormally high TXM excretion and a thrombotic tendency *in vivo*.[6] On the other hand, monocytes have been shown to form thromboxane and to generate free radicals, which in turn may trigger platelet activation.[7] Therefore we measured a series of platelet and monocyte variables and evaluated their relationship to TXM excretion in homozygous CBSD patients. Heterozygosity for CBSD is a rather common condition (1-2% of general population) characterized by mild hyperhomocysteinemia and a similar tendency to atherosclerosis and thrombosis.[2] We extended our studies to some obligate heterozygous patients to compare platelet and monocyte variables and TXM excretion in these two settings.

Patients and Methods

Nine homozygous CBSD patients (4 females and 5 males, 14-39 years old, belonging to 6 unrelated families), eight obligate heterozygotes (4 males, 4 females, 40-60 years old, parents of 4 homozygous patients) and ten matched control subjects were studied. Three heterozygotes showed mild hypercholesterolemia and one had high plasma fasting glucose levels. Two of the heterozygotes suffered from arterial hypertension. None of the patients showed clinical signs of vascular disease, whereas duplex scanner analysis revealed early signs of atherosclerosis (iliac arteries) in 3 out of the nine homozygous patients and in 3 out of the eight obligate heterozygotes.

None of the subjects took any medication for at least 1 week before blood and urine sampling. Blood was collected at 9-9.30 a.m., after overnight fasting, into 3.8% trisodium citrate 1:10 and immediately processed. Urinary samples were obtained from 12-hr overnight collections, immediately frozen and kept at –70°C until extracted. The urinary 11-dehydro-TXB_2 assay was carried out as previously reported.[5] Aggregation of platelets by ADP, collagen and arachidonic acid, TXB_2 synthesis and ATP secretion in response to thrombin were performed as described in earlier studies.[8] Mononuclear white blood cells were isolated by a Ficoll gradient and their adhesion to glass, expression of pro-coagulant activity (PCA) in basal conditions and after incubation with *E. coli* lipopolysaccharide, PAI-2 antigen and activity, leukotriene B_4 synthesis by unstimulated cells and in response to ionophore A_{23187} were carried out as reported elsewhere.[9]

Results and Discussion

Consistent with our previous findings,[5] when compared to controls, homozygous CBSD patients had significantly (p<0.001) higher TXM urinary

Correspondence: Prof. Giovanni Di Minno, Dip. di Medicina Clinica e Sperimentale, Università "Federico II", via S. Pansini 5, 80131 Naples. Tel. international +39.81.7462060. Fax. international +39.81.5466152.

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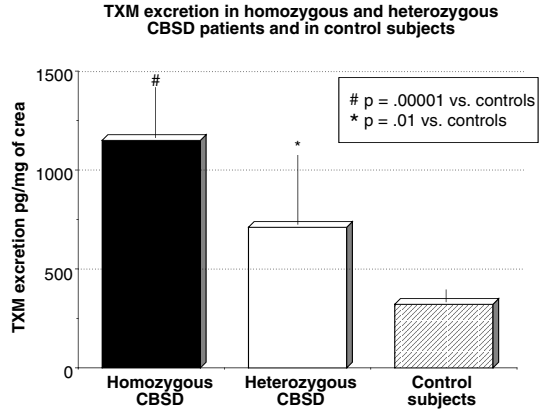

Figure 1. Urinary 11-dehydro-thromboxane B_2 (TXM) in CBSD homozygous and heterozygous patients and in control subjects. Values are expressed as mean ± 1 SD. p, respectively, < 0.001 and < 0.05 when homozygous patients and control heterozygous subjects and controls were compared to controls.

excretion (1175±236 pg/mg creatinine, mean±SD; range 781-1551). TXM levels of all patients were in excess of 2 standard deviations from the control mean (284±39, range: 208-321; Figure 1). Enhanced TXA_2 biosynthesis proved to be unrelated to established cardiovascular risk factors and independent of detectable atherosclerotic disease. Heterozygotes for CBSD presented raised TXM urinary excretion as well (755±450 pg/mg creatinine, range 288-1654; p<0.05 vs. controls; Figure 1); 7 out of the 8 subjects studied showed values in excess of 2 SD from the control mean. No significant differences versus the mean were detected in the hypercholesterolemic, hypertensive or diabetic (only one) patients. Platelet aggregation values were within the normal range in both homozygous and heterozygous CBSD subjects, as were TXB_2 generation and ATP secretion in response to thrombin (Table 1). Thus, similar to homozygous CBSD, obligate heterozygotes show abnormally high TXM excretion that is not associated with abnormalities of *in vitro* platelet function tests. Likewise, monocyte functions in CBSD subjects were entirely normal (Table 1). No statistically significant correlation was detected between any of the parameters studied and TXM excretion. None of the comparisons between homozygous and heterozygous CBSD patients showed any significant differences (p always >0.05).

These data show that, at variance with other clinical conditions with thrombotic tendency and abnormally high *in vivo* TXM excretion, enhanced thromboxane biosynthesis in CBSD is not associated with significant *in vitro* abnormalities of platelet and monocyte function. This discrepancy between *ex vivo* and *in vivo* data further support the possibility that enhanced TX biosynthesis in homocystinuria

Table 1. Platelet and monocyte variables in CBSD patients and control subjects.

Parameter	*Homozygous CBSD*	*Heterozygous CBSD*	*Control Subjects*
Platelet variables			
Platelet aggregation (Ac_{50})			
ADP (µM)	1.0±0.7	1.1±0.6	1.2±0.4
Arachidonic acid (mM)	0.5±0.2	0.5±0.2	0.6±0.2
Collagen (µg/mL)	0.8±0.4	0.7±0.5	0.6±0.3
ATP secretion (µMol/10^{11} plt)	3.6±0.4	ND	3.1±0.8
TXB_2 (pMol/3x10^6 plt)	4.2±0.7	ND	3.9±1.0
Monocyte variables			
Adhesion to glass (%)	73±2.8	ND	79±5.5
PAI-2 Ag (ng/mL)	103±54	96±58	115±59
PAI-2 Act (U/mL)	3.9±1.4	4.4±1.3	4.5±1.5
LTB_4 (pg/mL)			
unstimulated cells	61.9±10.8	ND	55.7±16.5
A_{23187}	514±90.4	ND	603±135
PCA (U/10^5 cells)			
unstimulated cells	7.6±1.6	6.2±3.4	7.9±3.3
LPS	134±38.3	142±35.1	144±47.9

Each value is the mean ± 1 SD of the results obtained in 9 homozygous and 8 obligate heterozygous subjects; p always >0.05 when the two groups of patients or patients and control subjects were compared. ND: not detected; TXB_2: thromboxane B_2; PAI-2: plasminogen activator inhibitor type 2; Ag: antigen; Act: activity; LTB_4: leukotriene B_4; PCA: pro-coagulant activity; LPS: lipoplysaccharide from E. coli.

reflects the activation of platelets and/or other cells in response to stimuli operating *in vivo*, the nature of which remains to be elucidated. Our previous observations on the partial dependence of thromboxane generation on probucol-sensitive mechanisms[5] suggest that the oxidative stress by homocysteine may be responsible, at least in part, for these abnormalities of arachidonate metabolism. Other studies with antioxidant interventions, more potent and more selective than probucol, will help clarify this issue and provide new strategies for preventing the risk of thrombosis in hyperhomocysteinemia.

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