Atherosclerosis and Prostaglandin

145 Evaluation of Platelet Thromboxane Formation Ex Vivo

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Abnormal platelet prostaglandin (PG) and thromboxan (TX) synthesis may contribute to the bleeding tendency in some conditions with congenital or acquired platelet disorders such as in uremia, following renal transplant rejection, and in some patients with autoimmune thrombocytopenia or acute thrombotic thrombocytopenic purpura (TTP), respectively. However, there is difficulty in using measurements of TXB_2 in biological fluids as an index of TXA_2 generation *in vivo*. Thus, in many studies the reported levels of plasma TXB_2 appear to reflect artefacts due to sampling technique and/or methodological problems. A more appropriate approach to evaluate platelet TXA_2 biosynthesis is the measurement of TXB_2 *ex vivo*, such as in whole blood or in stimulated platelet-rich plasma (PRP).

We studied TXB_2 production after incubation of PRP (2.5×10^8 platelets/ml) with thrombin (10 IU/ml) or arachidonic acid (AA, 450/uM). The amount of TXB_2 formed during a 5-min. incubation period was determined by radioimmunoassay according to the method of Smith and coworkers using rabbit anti-TXB_2 and standard TXB_2 from Upjohn Co. (Kalamazoo, MI, USA) and ^3H-TXB_2 (115.9 Ci/mmol) from Dupont

NEN (Boston, MA, USA). The recovery of added ^{3}H-TXB_2 ranged from 80 to 120 %. TXB_2 formation in unstimulated PRP was less than 0.02 nmoles/10^9 platelets.

Platelets from recipients of renal allografts revealed a significantly reduced TXB_2 formation upon stimulation with thrombin at the time of acute transplant rejection ($n = 15$; TXB_2 (mean ± SD): 2.25 ± 1.15 nmoles/10^9 platelets) as compared to controls ($n = 10$; TXB_2: 4.72 ± 1.90 nmoles/10^9 platelets; $p < 0.05$). The same was true in two patients with acute thrombotic thrombocytopenic purpura (TTP) who also showed a significantly reduced thrombin-induced TXB_2 formation (0.32 ± 0.08 nmoles/10^9 platelets; p vs. control < 0.001). Incubation of PRP with exogenous AA failed to restore normal TXB_2 production in platelets from patients with acute TTP. This finding is in contrast to the observation that washed human platelets pretreated with thrombin in vitro form more TXB_2 in response to exogenous AA indicating intact cyclooxygenase and thromboxane synthetase activity.

These data demonstrate that an abnormality of platelet arachidonic acid metabolism exists in acute thrombotic thrombocytopenic purpura and during acute renal transplant rejection, leading to a reduced TXA_2 production. This dysfunction may reflect the previous activation of platelets in vivo. The determination of TXB_2 formation from stimulated PRP is an appropriate tool to study abnormal platelet thromboxane synthesis. However, since these measurements are related to capacity rather than to actual TXB_2 production rates in vivo, the relationship of these measurements to endogenous platelet TXA_2 biosynthesis remains to be determined.