Involvement of hydrogen sulfide pathway in human platelet aggregation in hyperhomocysteinemia

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Hyperhomocysteinemia is a risk factor for neurovascular and cardiovascular disease (Boushey et al. 1995; Austin et al. 2004; Towfighi et al. 2008). Many clinical and epidemiological studies have demonstrated a positive correlation among homocysteine (Hcy) plasma levels and cardiovascular disorders [4] leading to the general conclusion that Hcy is a pro-thrombotic factor (Lee et al. 2003; Undas et al. 2005). Hcy is metabolized to methionine by the action of 5,10 methylenetetrahydrofolate reductase (MTHFR) (Selhub 1999). Alternatively, by the transulfuration pathway, homocysteine is transformed to hydrogen sulfide (H_2S), through multiple steps involving cystathionine β-synthase (CBS) and cystathionine γ-lyase (CSE) (Kimura 2011). To date, the influence of H_2S on platelet function has been poorly explored. Here we have evaluated the involvement of H_2S in the thrombotic events associated to hyperhomocysteinemia. To this purpose we have used platelets harvested from healthy volunteers or patients firstly diagnosed with hyperhomocysteinemia (MTHFR++ carriers). Sodium hydrogen sulfide (NaHS) or L-cysteine were used as exogenous or endogenous source of H_2S, respectively. NaHS (0.1-100μM) or L-cysteine (0.1μM-100μM) preincubation of platelet harvested from healthy volunteers, significantly increased aggregation induced by thrombin receptor activator peptide-6 amide (TRAP-6, 2 μM) in a concentration-dependent manner. This increase was significantly potentiated in platelet harvested from MTHFR++ carriers and it was reverted by the inhibition of either CBS or CSE. In MTHFR++ carriers the content of H_2S was significantly higher in either platelets or plasma. Interestingly, thromboxane A_2 production was markedly increased in response to both NaHS or L-cysteine in platelets of healthy volunteers. The inhibition of phospholipase A_2, cyclooxygenase or thromboxane receptor blockade markedly reduced the effects of H_2S. Finally, phosphorylated-phospholipase A_2 expression was significantly higher in MTHFR++ carriers when compared to healthy volunteers. In conclusion H_2S pathway is involved in the pro-thrombotic events in hyperhomocysteinemic patients through the arachidonic acid cascade.

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