Hydrogen sulfide pathway as a new target in cardiovascular risk associated to hyperhomocysteinemia

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Homocysteine (Hcy) is a sulfur-containing amino acid, naturally present in the bloodstream. Abnormally high levels of Hcy in the blood represent a medical condition defined Hyperhomocysteinemia (HHcy). HHcy is considered a pro-thrombotic factor (Fay WP. 2012) involved in neurovascular and cardiovascular disease associated with endothelial dysfunction and atherosclerosis (Austin RC, et al., 2004). Hcy is generated by two main routes, re-methylation and trans-sulfuration pathways. The predominant re-methylation process is vitamin B12-dependent and is based on the conversion of 5-methylentetrahydrofolate into tetrahydrofolate operated by 5,10-methylentetrahydrofolate reductase (MTHFR). In the trans-sulfuration pathway, Hcy is converted to cystathionine by cystathionine β-synthase (CBS) in a vitamin B6-dependent reaction. Furthermore Hcy can also be converted into L-Cysteine (L-Cys), which in turn releases hydrogen sulfide (H2S) through the action of CBS and cystathionine γ-lyase. Enzymatic activity of aforementioned pathways determines plasma Hcy concentration. Indeed the homozygosity for the C to T substitution at nucleotide 677 of the gene MTHFR (MTHFR++) is the most common cause of moderate HHcy (Frosst et al., 1995). Besides CBS deficiency (CBS-/-) is associated with severe HHcy and homocysteinuria. Both CBS-/- and MTHFR++ have been strongly associated with HHcy, (Humphrey et al., 2008). Nevertheless, the mechanism through which HHcy can promote vascular diseases and thrombosis remain poorly understood. In these regards, we recently demonstrated that H2S pathway is involved in the pro-thrombotic events occurring in MTHFR++ carriers (d'Emmanuele et al., 2013). Nonetheless, further studies are request because HHcy is part of another unresolved enigma regarding HHcy therapy.

In order to address this issue, we enrolled MTHFR++ carriers and CBS-/- patients and we monitored changes of H2S cascade intermediates before and after therapeutic protocol used in the treatment of HHcy (folic acid 40mg/die for 1 month).

To this purpose we used washed platelets harvested from healthy volunteers or MTHFR++ carriers or CBS-/- before and after treatment with folates. Thrombin receptor activator peptide 6 amide (TRAP6)–induced aggregation was similar in healthy volunteers and MTHFR++ carriers but was significantly higher in CBS-/-. L-cys incubation (0.1-100 μM) significantly increased platelet aggregation induced by TRAP6 in a concentration-dependent manner in healthy volunteers. This increase was significantly potentiated in MTHFR++ carriers. On the contrary, L-Cys did not affect platelet aggregation in CBS-/-. Interestingly, in MTHFR++ carriers L-Cys-induced increase in platelet aggregation was markedly reduced after one moth of treatment with folates showing an aggregation profile similar to healthy volunteers. Contrary to what observed in MTHFR++ carriers, the treatment with folates did not change platelet response in CBS-/- suggesting that there is no clinical benefit of the use of folic acid in CBS-/-.Similarly, the content of H2S in platelets was

significantly higher in MTHFR++ carriers compared with healthy volunteers and after one month of treatment with folates the H2S content was notably reduced. Platelets harvested from CBS-/- produced an higher amount of H2S compared with healthy volunteers but treatment with folates did not modify H2S content.

In conclusion we demonstrated the contribution of H2S pathway in MTHFR++ carriers and in CBS-/-. In addition, the treatment with folates reduced the increase in platelet aggregation induced by L-Cys as well as the increase in H2S content in platelet in MTHFR++ carriers but not in CBS-/- .Therefore, in MTHFR++ carriers, i.e. moderate HHcy, but not in CBS-/- carriers, i.e. severe HHcy, the treatment with folates reduced the H2S levels and in turn the thrombotic events.

References

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