GREMI Prize 2013 - PhD Thesis Award ex-aequo:
Role of prostaglandin E_2 hydrogen sulfide in the physiopathology of the human saphenous veins

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Varicose veins are a form of chronic venous insufficiency, they are characterized by venous backflow and deoxygenated blood stagnation (Jacob *et al.*, 2002). Hypotheses explained that venous hypertension or valves anomalies could be responsible for varicose veins formation (Golledge *et al.*, 2003; Lim *et al.*, 2009). Clinically and histologically varicose saphenous veins reflect a modification of the extracellular matrix and reorganization of the vascular wall (Badier-Commander *et al.*, 2001). Other factors, such as inflammation are still controversial despite the use of anti-inflammatory drugs for pain.

In order to understand the underlying disease mechanism, research has been carried out to study the physiology of healthy and varicose saphenous vein (C2 stage). The major mediators of vascular inflammation such as macrophages, neutrophils, C-reactive protein, pentraxin-3, phospholipase A_2 group IIA (figure 1.) or cyclooxygenase -2, were measured and none of them showed an increased presence or activity in varicose as compared to healthy saphenous veins.

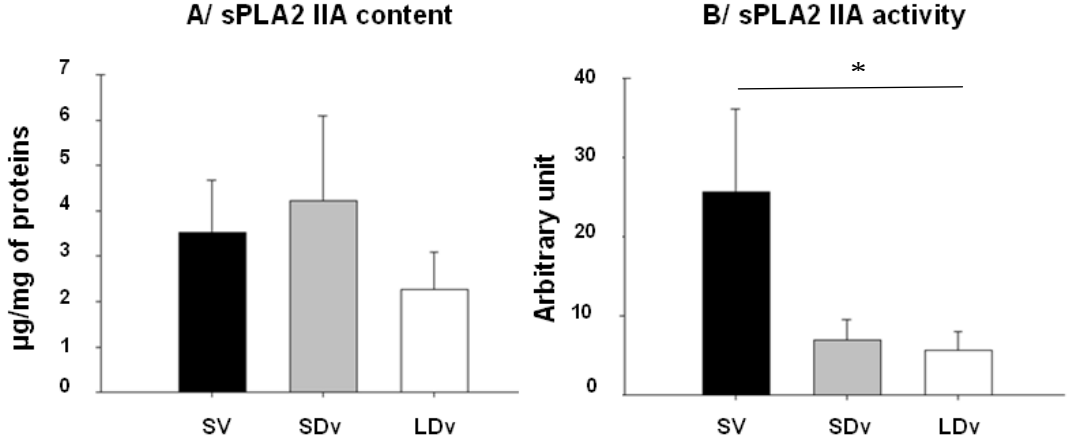

Figure 1. A/ Secreted phospholipase A_2 group IIA ($sPLA_2$-IIA) mass and enzymatic activity in human small and large diameter varicosities (paired SDv and LDv, n=5) and healthy saphenous veins (SV, n=5). $sPLA_2$ was determined in the supernatants after 30 min of incubation of venous preparations.

After assessing the role of inflammatory mediators, the role of prostaglandin (PG) E_2 and hydrogen sulfide (H_2S) in this pathology were investigated. PGE_2, a bioactive lipid, can control vascular tone and induces the activation of matrix metalloproteinase (MMPs) (Lee *et al.*, 2011) and is generally increased in inflammation. Recent studies have shown that PGE_2 production can be regulated by H_2S (Li *et al.*, 2013; Whiteman *et al.*, 2010). This endogenous gas could be involved in regulating the cardiovascular system (Liu *et al.*, 2012) and by the inhibition of PGE_2, could act as an anti-inflammatory drugs.

Our experiments have shown that PGE_2 is significantly produced by vascular wall or the perivascular adipose tissue of human healthy saphenous veins (SV). Furthermore, PGE_2 induces a potent vasorelaxation after the activation of its EP4 receptor. The lack of inflammatory involvement confirms that inflammatory activation is not a part of this disease process. PGE_2 and EP4 receptor were decreased in varicose veins, a study their involvement

in vascular wall remodeling was carried out. The reduced synthesis (decreased mPGES-1) and increased degradation (by increased 15-PGDH) of PGE_2 are responsible for its lower concentration in varicose veins. This dysregulation and a lower density of EP4, are responsible for the down-regulation of active MMP-1 and their endogenous inhibitors, TIMP-1 and TIMP-2. In varicose veins, decreased active MMP-1/TIMP-1 and active MMP-1/TIMP-2 calculated from our results, could explain the accumulation of collagen observed in varicose veins. Finally, our experiments have shown that the endogenous H_2S production is two times higher in varicose veins compared to the SV. Using a H_2S donor (NaHS) an inhibition of PGE_2 synthesis has been shown (Figure 2.) and using an inhibitor of the endogenous synthesis of H_2S induces an increased PGE_2 content.

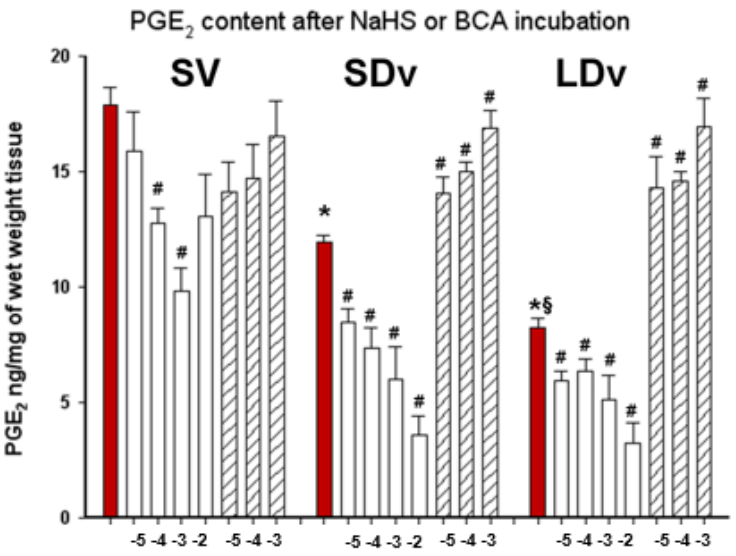

Figure 2. PGE_2 content in human human small and large diameter varicosities (paired SDv and LDv, n=16) and healthy saphenous veins (SV, n=15). Controls are presented by red bars. Treatments with NaHS (concentration are expressed in logarithm M, n=9) are presented by white bars. Treatments with H_2S inhibitor (beta cyano L alanine, BCA, concentration are expressed in logarithm M, n=9) are presented by hatched bars. Values are determined by EIA in supernatants after 24h incubation.

In conclusion, H_2S has an anti-inflammatory effect and could be partially responsible for the vascular wall thickening in human varicose saphenous vein. This mechanism could be explain by the inhibition of PGE_2 production as well as by lower ratios of active MMP-1 / TIMP. This endogenous gas could play a protective role in strengthening the vascular wall to resist venous stasis. This vascular wall remodeling could avoid ectasic segment formation and a venous wall rupture.

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