A008

PRESENCE OF TISSUE FACTOR AND OTHER COMPONENTS OF ATHEROSCLEROSIS IN HUMAN AORTIC VALVE STENOSIS

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Background — It is now generally accepted that calcific aortic valve disease is an atherosclerotic-like process. Recent studies in an experimental model of aortic valve sclerosis demonstrated the presence of tissue factor (TF), the main contributor to atherosclerotic plaque thrombogenicity, in diseased valve leaflets. We assessed the hypothesis that human aortic valve disease is an atherosclerotic-like process in which TF plays an important role and evaluated the valvular expression and localization of TF and other components of atherosclerosis.

Methods — Calcified aortic valves (n=52) were obtained from patients undergoing aortic valve replacement. Leaflet structure, cellular and lipid infiltration and expression of TF, its inhibitors, VEGF and other components of atherosclerosis were evaluated by histological and immunohistochemical staining. TF, TFPI, osteopontin, MMP-9, TIMP-1 and VEGF antigen were measured by ELISA and TF and alkaline phosphatase activity were determined using chromogenic assays. Finally, we performed semi-quantification of TF transcripts by RT- PCR and further analyzed protein expression by Western blot.

Results — Histological and immunohistochemical staining of the valve leaflets revealed neovascularisation at the centre of the lesions, overall macrophage and myofibroblast infiltration and the abundant presence of MMP-9. On the other hand, TF and TFPI were associated with calcification and extracellular lipid deposits in the fibrosa and the subendothelial layer of the aortic side of the leaflets. Correspondingly, TF antigen and activity were found to be higher in calcified regions of the valve leaflets (733.29 ± 70.49 pg/mg vs 429.40 ± 73.17 pg/mg and 144.75 ± 14.65 pg/mg vs 40.15 ± 6.19 pg/mg respectively ($p<0.0001$)). Similar results were found for osteopontin, MMP-9, TIMP-1 and VEGF. In contrast, TFPI antigen was found to be much lower in these calcified regions (722.54 ± 153.92 pg/mg vs 2459.28 ± 285.36 pg/mg ($p<0.0001$)).

Conclusion — These results demonstrate that aortic valve lesions display several characteristics of atherosclerosis, including TF expression. In addition, we showed that TF is colocalized with calcification and lipid deposition. Further studies are now set up to evaluate the role of TF in aortic valve disease and its association with other components of the atherosclerotic process.

A009

IMPORTANCE OF TWEAK-CD163 SYSTEM IN PERIPHERAL ARTERY DISEASE

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Introduction — CD163 is a macrophage receptor of haptoglogin/haemoglobin complexes responsible for clearance of hemogloin. It has been recently suggested to be a potential scavenger receptor for TWEAK (Tumor necrosis factor-like weak inducer of apoptosis). TWEAK levels were reported to be decreased in carotid atherosclerosis. Our hypothesis is that decreased circulating TWEAK could be paralleled by an increased presence of CD163-expressing macrophage in atherosclerotic plaques. Since peripheral artery disease (PAD) is an important manifestation of systemic atherosclerosis, we have assessed the levels of circulating TWEAK-CD163 in PAD.

Methods and Results — Patients with PAD (n=184) had lower TWEAK (169.2±8.3 vs 211.9±15.4 pg/mL; $p<0.05$) and higher sCD163 (408.1±14.5 vs 317.4±8.4 ng/mL; $p<0.05$) plasma concentration than age-matched controls (n=330). After stratification according to the severity of disease, we observed that TWEAK/sCD163 ratio was significantly decreased in those patients with higher degree of disease (0.39±0.06 vs 0.66±0.08, $p<0.05$) relative to the other groups. Analysis of conditioned medium obtained from cultured human atherosclerotic femoral plaque samples (n=38) and healthy aortas (n=14) revealed that higher amount of sCD163 was released by the atherosclerotic tissue, whereas TWEAK presented the opposite trend.

Conclusions — Our results suggest that CD163/TWEAK plasma ratio could be a potential biomarker of clinical peripheral artery disease. We can hypothesized that decreased levels of circulating TWEAK observed in atherosclerosis may be the result of a trapping by plaque macrophages through their CD163.

A010

INTERFERENCE WITH TOLL-LIKE RECEPTOR 4 PATHWAY MEDIATES THE ANTI-INFLAMMATORY EFFECTS OF ADENOSINE

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Purpose — Adenosine, acting through four types of receptors (A1, A2a, A2b, A3), is anti-inflammatory and cardioprotective. Since Toll-Like Receptor 4 (TLR4), a receptor involved in innate immunity, has recently been shown to mediate adverse left ventricular remodeling after myocardial infarction (MI), we sought to determine whether adenosine acts on the TLR4 pathway.

Methods — Primary human macrophages obtained after in vitro differentiation of blood monocytes isolated from healthy volunteers and patients with acute MI were treated with adenosine (10 μm), adenosine analogs, and/or lipopolysaccharide (LPS, 100 ng/mL). Transgenic mice bearing a cardiac-specific and externally-regulatable overexpression of A1 or A2a receptors were used to determine the receptor involved. Flow cytometry, immunoblotting, quantitative PCR and ELISA were used to