Increased pro-remodeling and pro-thrombotic responses in the left compared to the right atrial appendage: role of low-grade inflammation and the AT1R/NADPH oxidases/SGLT2 pro-oxidant pathway

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Introduction: In atrial fibrillation (AF), there is a complex interplay between arrhythmia burden and cardiovascular risk factors leading to left atrial remodeling, an increased risk of stroke and heart failure. Most thrombi get formed in the atrial appendage. Several studies suggested that sodium-glucose cotransporter 2 inhibitors (SGLT2i), besides showing major benefits on heart failure, might lower the risk of incident AF. Therefore, we examined the expression level of SGLT2 in human right and left atrial appendages (RAA, LAA) and determined its role in pro-oxidant, pro-fibrotic and pro-thrombotic responses.

Methods: Human RAA and LAA were collected from patients undergoing cardiac surgery at a university hospital. The LAA was cut into a proximal part (low stasis, high shear) and a distal part (high stasis, low shear). The level of reactive oxygen species (ROS) was determined using dihydroethidium, mRNA and protein expression levels by RT-qPCR, and Western blot analysis and immunofluorescence, respectively, and the level of fibrosis by Sirius red staining.

Results: The distal LAA had higher mRNA levels of ICAM-1 and p53, and protein levels of ICAM-1, AT1R, p53, p21 and tissue factor than the proximal LAA. LAA displayed higher mRNA and protein levels of SGLT2 and SGLT1, pro-adhesive, pro-thrombotic, pro-remodeling, pro-fibrotic and senescence markers, and components of the angiotensin system, in addition to IL-1 mRNA levels and p-p65 NF-κB protein levels compared to RAA. These responses were associated with higher levels of oxidative stress, nitrotyrosine and fibrosis. SGLT2 immunofluorescence signals in distal LAA were colocalized with those of CD31, CD68, TNF-α and troponin T. Oxidative stress levels in distal and proximal LAA and RAA were reduced by inhibitors of NADPH oxidases, ACE1 and SGLT2, an AT1R antagonist, and by a TNF-α neutralizing antibody whereas NG-nitro-L-Arginine methyl ester, the non-selective NO synthase inhibitor, inhibited ROS in distal and proximal LAA without affecting RAA. In addition, the level of TNF-α in LAA was positively correlated to the indexed LA volume indicating a link between inflammation and remodeling.

Conclusions: The distal part of the LAA of patients undergoing heart surgery showed greater impaired endothelial function and senescence associated with more pronounced pro-oxidant, pro-remodeling and pro-inflammatory responses than the proximal part and the RAA. These responses were associated with an increased expression level of SGLT 2 in endothelial cells, cardiomyocytes, and macrophages in areas showing an inflammatory response. Moreover, the pro-oxidant signal was sensitive to inhibitors of the local angiotensin system, NO synthase, SGLT2 and TNF-α. Thus, the AT1R/NADPH oxidases/SGLT2 pathway appears as an interesting target to blunt the stimulatory pro-oxidant signal in cardiac tissues affected by low-grade inflammation that leads to remodeling and fibrosis.