Non-invasive stimulation of the resolution of inflammation in atherosclerosis

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The present project aims for true translation towards novel innovative therapeutic strategies inducing the resolution of inflammation to mitigate atherosclerosis and vascular calcification as part of novel preventive measures to combat the increasing burden of cardiovascular disease in an ageing population.

One of the original concepts of the present project is that in contrast to acute inflammation, the chronic inflammatory reactions in atherosclerosis and vascular calcification result from a failure in the resolution of inflammation (*Bäck et al. Nat Rev Cardiol. 2019*). Although initially considered to be a passive process, it is now widely recognized that the resolution of inflammation is an active process in which specialized pro-resolving "stop signals" for inflammation mediate a return to tissue homeostasis. The hypothesis of the present study is that chronic inflammation during atherosclerosis progression and associated vascular calcification results from a defect of the repair process of inflammation (*Bäck et al. Nat Rev Cardiol. 2019*). ANS measure can mainly be used to assess sympathovagal balance and to quantify the degree of autonomic dysfunction in order to detect inflammation and CVD risk early (**Hupin et al. Front Med. 2021**).
Specifically, we addressed how to non-invasively stimulate an activation of the resolution of inflammation. In the present project, our research group's previous research breakthroughs on lipid mediator regulation of inflammation in atherosclerosis and vascular calcification (*Bäck et al. Nat Rev Cardiol. 2019*) converged with our discovery that CVD-preventing physical exercise restores activity of the sympathetic autonomic nervous system (ANS) (*Hupin et al. Eur Heart J. 2017*) towards the overarching aim to determine how to stimulate the resolution of inflammation by means of non-invasive promotion of parasympathetic activity and to establish the mechanisms involved.

The specific aims were:

- ✓ To show how resolution pathways are regulated by ANS in atherosclerosis and vascular calcification.
- ✓ To identify the mediators and receptors transducing the ANS-regulated resolution of atherosclerotic inflammation.
- ✓ To establish the mechanism involved using innovative *in vitro* studies in human cells and tissues.
- ✓ To determine if ANS-stimulating the resolution of inflammation reduces atherosclerosis and vascular calcification *in vivo*.

1. Translational human tissue studies: inflammatory and ANS pathways in CVD pathologies

One of the major outcomes of our research group's previous research efforts has been the generation of a human biobank for morphological and transcriptional analysis of cardiovascular tissues. Specifically, we used human aortic valve tissue as a unique and innovative model of the temporal changes taking place as the CVD progresses from healthy through inflamed and lipid rich tissue towards emerging cardiovascular calcification. This model was established and validated by our group in previous studies (*Artiach et al. 2020*). Containing a total of *294 Affymetrix Human Transcriptome (HTA)* Array completed with Affymetrix miRNA 4.1 arrays in a patient subset, covering in total 67,528 gene transcripts (coding and non-coding) and 2,578 human mature microRNA. Importantly, our bioinformatics analysis was not limited to transcriptomics, but also integrated full clinical information of each patient, including coronary atherosclerosis status, echocardiographic cardiac assessment, inflammatory biomarkers, medications and comorbidities. This biobank is unique and the first of its kind specifically addressing the continuum of CVD

with full coverage of coding and non-coding transcripts. The advanced statistical exploration was made in the software Qlucore Omics Explorer® specifically designed for research in life science allowing a visualization-based data analysis with inbuilt powerful statistics to deliver immediate results and provide instant exploration and visualization of the transcriptomic data. We have also taken the tissue characterization to a further level, by using tissue adjacent to that used for RNA extraction for several approaches. Histology was performed for confirming the correct tissue classification, for immunohistochemistry and cellular localisation of proteins, as well as for additional morphological analysis to distinguish specific phenotypes. For this specific proposal, we used adjacent tissue for morphological neural innervation in relation to transcriptomic inflammatory and ANS and signatures (**Hupin et al.** *original research in progress of submission in a scientific journal*).

2. Experimental interventional study: Assessment of the role of ANS on the resolution of inflammation in human model of vascular calcification

This is a translational, prospective, randomized, sham-controlled trial (protocol submitted in Swedish ethics commitee).

Patients referred for surgical intervention due to aortic valve stenosis, aortic regurgitation or ascending aortic dilatation and included as part of the Disease of the Aortic Valve Ascending Aorta and Coronary Arteries (DAVAACA) ongoing single-center cohort study (Glaser et al. 2021) will be randomly assigned (1:1) to active or sham tVNS. Active tVNS will be performed using a transcutaneous electrical nerve stimulation (TENS) with an ear clip put in the conque aurea of the left ear, which is innervated by auricular branch of the vagus nerve. In the sham group, stimulation will be delivered to the ear lobe, which is devoid of vagal innervation (**Hupin et al.** *protocol in progress of submission in a scientific journal*).

3. Communication and publication

Hupin D, Sarajlic P, Venkateshvaran A, Fridén C, Nordgren B, Opava CH, Lundberg IE, Bäck M. Cardiovascular Autonomic Function Changes and Predictors During a 2-Year Physical Activity Program in Rheumatoid Arthritis: A PARA 2010 Substudy. CVP/CVR Congress 2021. *November 30th. Karolinska institutet, Solna, Sweden.*

Hupin D, Sarajlic P, Venkateshvaran A, Fridén C, Nordgren B, Opava CH, Lundberg IE, Bäck M. Cardiovascular Autonomic Function Changes and Predictors During a 2-Year Physical Activity Program in Rheumatoid Arthritis: A PARA 2010 Substudy. Front Med (Lausanne). 2021 Dec 15;8:788243. doi: 10.3389/fmed.2021.788243.

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