SOCIETÀ ITALIANA DI FARMACOLOGIA

MODELLO PER INVIO RELAZIONE DI METÀ E FINE PERIODO

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TIPOLOGIA DI BORSA RICEVUTA: BORSA DI STUDIO PER SOGGIORNO ALL`ESTERO

TIPOLOGIA DI RELAZIONE: RELAZIONE DI META`PERIODO

TITOLO DELLA RELAZIONE: INFLAMMATORY ACTIVITY IN VARIOUS COMPARTMENTS OF ADIPOSE TISSUE IN PATIENTS WITH CORONARY HEART DISEASE

RELAZIONE:

Background

Coronary heart disease (CHD) represents one of the leading death causes in developed countries, in which atherosclerosis constitutes the most relevant underneath pathological underneath [1]. Chronic inflammatory processes represent a key player in initiation and progression of atherosclerosis [2], associated and influenced by other well-known risk factors, including obesity [3]. Unhealthy obesity is indeed accompanied by adipocytes' hypertrophy, insulin resistance and the instauration of a pro-inflammatory environment [4].

The heart is surrounded by epicardial and pericardial adipose tissues (EAT and PAT, respectively), considered as distinguished AT compartments [5]. Specifically, EAT covers about 80% of the heart surface and is localized between the myocardium and the visceral pericardium and surrounds the great coronary vessels and atrioventricular grooves [6]. EAT therefore shares the microcirculation with myocardial tissue, secretes different molecules through paracrine and vasocrine mechanisms and protects coronary arteries [5–7].

On the other side, PAT is located amongst visceral and parietal pericardium and, differently from EAT, it receives blood supply from non-coronary circulation and its role as a source of cardiac biochemical mediators

is still a matter of debate [8]. Concerning their functions, EAT exerts different anti-atherogenic and anti-inflammatory activities and seems to be involved in the regulation of cardiac metabolism and thermogenesis [9], while PAT seems to exert lower inflammatory properties [10]. Interestingly, when the physiological lipid storage capacity of subcutaneous adipose tissue (SAT) is reached, the further caloric overload causes fat accumulation in visceral adipose tissue (VAT) and ectopic AT, including those located in the heart [11]. Consistently, EAT volume is increased in CAD patients [12,13], although not confirmed by a separate study [14], and may be a predictor for cardio-metabolic risk [15].

Interestingly, AT has been recognized as a biochemically active organ, able to secrete a wide variety of adipokines, cytokines and chemokines [16,17], thus being at the crossroad between metabolism and immunity. Together with adipocytes, indeed, different resident immune cell populations can be found in AT, including T and B cells, neutrophils, natural killer cells and adipose tissue macrophages (ATMs), with the latter representing the dominant and most relevant key players in AT inflammation due to their high degree of phenotypic plasticity [18]. Interestingly, the extent of macrophage infiltration and their polarization may vary in lean and obese AT, estimated to range from 10% in lean to 40% in obese mice [19], thus contributing to low-grade chronic inflammation that negatively affects AT secretome, with profound effects on peripheral tissues [17]. ATMs may be classified based on their inflammatory profile, and it is well accepted that ATMs in lean tissues mainly exhibit an alternatively activated M2 profile, with an anti-inflammatory signature, known to properly maintain the insulin pathway, angiogenesis, tissue repair [20]. On the other side, in obese tissues ATMs are mainly polarized towards a classically activated M1 profile, known to produce pro-inflammatory cytokines, able to inhibit the correct insulin signalling in adipocytes [21]. However, different evidence demonstrates a more complex scenario that goes beyond the static M1/M2 macrophages classification, as macrophage polarization *in vivo* should be considered as a strongly dynamic process [22]. At this regard, it has been demonstrated that EAT isolated from patients with coronary artery disease (CAD) exhibited an altered M1/M2 polarization [23], thus possibly suggesting that ATM phenotype might affect cardiovascular disease.

Aim of the project

This study aims in investigating the polarization status of ATMs in EAT, PAT and SAT from patients with CHD and from controls, due to its critical involvement in the maintenance of AT homeostasis and its potential impact on CHD development. To this purpose, differences in gene expression of CD206, a cell marker typically associated to M2 macrophages [23], of Nitric oxide synthase 2 (NOS2), used to identify pro-inflammatory M1 macrophages [24], and of L-Galectin 9 (L-Gal9), possibly involved in the regulation of macrophage polarization [25], will be investigated, together with their relationship with circulating levels and anthropometric measures.

Patients and methods

52 patients with CHD subjected to coronary artery bypass grafting surgery and 22 subjects undergoing valve replacement, considered as controls (CTRLs), were recruited at the Oslo University Hospital, Ullevål, Oslo (Norway). Before the surgery, all patients gave written informed consent and the experimental protocol was approved by the Regional Ethics Committee of North Norway (#2016/411), following the Declaration of Helsinki. Briefly, during the open-chest surgery, biopsies from SAT (pre-sternally at the middle of the sternum), PAT (ventrally to the pericardium next to the aorta) and EAT (area between the right coronary artery and the pulmonary artery) were isolated and immediately deep-frozen at -80^{O}C until RNA extraction, while at the start of anaesthesia arterial blood samples were collected.

Total RNA was extracted from SAT, PAT and EAT (RNeasy Lipid Tissue Mini Kit - Qiagen, GmbH, Germany), following the manufacturer`s instructions. cDNA was retro-transcribed starting from 5ng/ml of RNA for each sample using the qScript™ cDNA superMix commercial kit (Quanta Biosciences, USA). L-Galectin 9, CD206 and NOS2 gene expression was determined in each AT sample using the TaqMan® Universal PCR Master Mix on a ViiATM7 machine (Applied Biosystems, CA, USA). The ΔΔCt method was applied to determine the mRNA levels in each reaction, using the β2-Microglobulin as normalizer internal gene, and expressed as a relative quantification (RQ) to a reference sample.

Statistical analyses were performed through the SPSS software version 28 (SPSS Inc., IL, USA). A value of $p<0.05$ was considered as statistically significant and Bonferroni correction for multiple comparison was applied as specified.

Preliminary results

Patients' characteristics were analysed first, observing that among CHD patients males were most represented (77% in CHD vs 50% in CTRLs), while the BMI median values were 27.3 and 28.4 kg/m^2 in CHD and CTRLs subjects, respectively. In CHD subjects, the most relevant comorbidities detected were hypertension (53.85%), angina pectoris (46.15%), diabetes (26.92%) and dyslipidaemia (23%) and 38% of them had a previous acute myocardial infarction (AMI) and percutaneous coronary intervention (PCI). The most commonly prescribed drugs in CHD patients were aspirin (86.5%) and statins (71.15%).

L-Gal 9, CD206 and NOS2 gene expression in the 3 AT compartments was overall similar between CHD patients and CTRLs.

Focusing on the specific AT compartments, in CHD patients CD206 gene expression was significantly higher in PAT as compared to the both SAT and EAT ($p=0.003$ and $p=0.006$, respectively), while NOS2 expression was overall similar among the three considered AT compartments. Conversely, in CTRLs subjects, while CD206 expression was unaltered, NOS2 showed the lowest expression in EAT as compared to SAT ($p=0.007$). L-Gal 9 gene expression was similar in the three AT compartments both in CHD and in CTRLs subjects. These observations may suggest that the lower EAT CD206 expression in CHD patients possibly implies a

macrophage reprogramming towards a pro-inflammatory phenotype, also supported by the lower EAT NOS2 expression retrieved CTRLs.

In CHD patients, CD206 SAT expression correlated to that in PAT and EAT (r=0.431, p=0.002; r=0.393, p=0.004, respectively); similarly, NOS2 in SAT correlated to that in PAT and EAT (r=0.556, p=0.007; r=0.542, p=0.007), suggesting an increased overall inflammatory milieu driven by CHD. In EAT from CHD patients, CD206 expression correlated positively to L-Gal9 (r=0.561, p<0.0001). These associations weren´t observed in CTRLs.

The expression of monocytes/macrophages, T cells and endothelial cells most representative markers, CD163, CD68, CD3 and CD31, respectively, was then analysed in SAT, PAT and EAT of CHD patients to detect the specific cellular subtypes in each AT compartment. In CHD patients, CD206 expression positively correlated to CD163 and CD68, representing monocyte/macrophages, in all AT compartments (all p<0.0001). These observations are supported by the fact that CD68 is a marker commonly used to identify macrophages, regardless of their phenotype, while CD163 is specific for anti-inflammatory M2 macrophages, similarly to CD206.

Concerning the association between gene expression and anthropometric parameters, in CHD patients a positive correlation between CD206 expression and subjects' BMI (r=0.52, p<0.0001) was observed specifically within SAT, while no other correlations were found in other AT compartments. The same association was observed in CTRLs (r=0.49, p=0.021), even though not statistically significant after Bonferroni`s correction. This positive association may be the result of the activation of a compensatory mechanism involving the reprogramming of macrophage toward a more anti-inflammatory phenotype in response to the increasing BMI, occurring specifically in SAT and not in the other AT analysed.

Finally, when specifically considering lipid profile, in CHD patients with LDL-C values above the median value (>1.8 mmol/l), a higher expression of NOS2 in both PAT and EAT was observed as compared to CHD patients with LDL-C <1.8 mmol/l (p=0.041 and p=0.34, respectively). Furthermore, in the same subjects, an increased CD206 expression specifically in PAT was observed (p=0.02), while no differences were observed in CTRLs. These observations may suggest that in patients with CHD characterized by LDL-C levels above the median levels, the increased NOS2 expression might represent an index of increased macrophage reprogramming towards a pro-inflammatory phenotype in both EAT and PAT, paralleled by a compensatory increased CD206 expression occurring only in PAT.

Altogether, these preliminary observations point to a possible macrophage polarization reprogramming towards a pro-inflammatory phenotype in CHD, particularly evident in EAT.

In the second part of the project the circulating levels of L-Gal9, CD206 and NOS2, as well as their association with the specific AT expression and anthropometric parameters will be evaluated, together with PCSK9 gene expression in AT and its circulating levels. These further analyses will be relevant to establish the influence of the extra-AT environment on macrophage polarization in CHD, possibly representing an interesting pharmacological approach to target the abnormal inflammatory AT environment.

References

1. Shao, C., Wang, J., Tian, J. & Tang, Y. Coronary Artery Disease: From Mechanism to Clinical Practice. *Adv. Exp. Med. Biol.* **1177**, 1–36 (2020).
2. Ruparelia, N. & Choudhury, R. Inflammation and atherosclerosis: what is on the horizon? *Heart* **106**, 80–85 (2020).
3. Visseren, F. L. J. *et al.* 2021 ESC Guidelines on cardiovascular disease prevention in clinical practice. *Eur. Heart J.* **42**, 3227–3337 (2021).
4. Ahmed, B., Sultana, R. & Greene, M. W. Adipose tissue and insulin resistance in obese. *Biomed. Pharmacother.* **137**, 111315 (2021).
5. Iacobellis, G. Epicardial and pericardial fat: close, but very different. *Obesity (Silver Spring, Md.)* vol. 17 625; author reply 626-7 (2009).
6. Ansaldo, A. M., Montecucco, F., Sahebkar, A., Dallegri, F. & Carbone, F. Epicardial adipose tissue and cardiovascular diseases. *Int. J. Cardiol.* **278**, 254–260 (2019).
7. Wu, Y., Zhang, A., Hamilton, D. J. & Deng, T. Epicardial Fat in the Maintenance of Cardiovascular Health. *Methodist Debakey Cardiovasc. J.* **13**, 20–24 (2017).
8. Si, Y. *et al.* Pericardial adipose tissue is an independent risk factor of coronary artery disease and is associated with risk factors of coronary artery disease. *J. Int. Med. Res.* **48**, 300060520926737 (2020).
9. Iacobellis, G. & Bianco, A. C. Epicardial adipose tissue: emerging physiological, pathophysiological and clinical features. *Trends Endocrinol. Metab.* **22**, 450–457 (2011).
10. Bragina, A. E. *et al.* Relationship of Pericardial Fat Tissue With Cardiovascular Risk Factors in Patients Without Cardiovascular Diseases. *Metab. Syndr. Relat. Disord.* **19**, 524–530 (2021).
11. Longo, M. *et al.* Adipose Tissue Dysfunction as Determinant of Obesity-Associated Metabolic Complications. *Int. J. Mol. Sci.* **20**, (2019).
12. Shan, D. *et al.* Epicardial Adipose Tissue Volume Is Associated with High Risk Plaque Profiles in Suspect CAD Patients. *Oxid. Med. Cell. Longev.* **2021**, 6663948 (2021).
13. Djaberi, R. *et al.* Relation of epicardial adipose tissue to coronary atherosclerosis. *Am. J. Cardiol.* **102**, 1602–1607 (2008).
14. van Meijeren, A. R. *et al.* Association of epicardial adipose tissue with different stages of coronary artery disease: A cross-sectional UK Biobank cardiovascular magnetic resonance imaging substudy. *Int. J. Cardiol. Hear. Vasc.* **40**, 101006 (2022).
15. Villasante Fricke, A. C. & Iacobellis, G. Epicardial Adipose Tissue: Clinical Biomarker of Cardio-Metabolic Risk. *Int. J. Mol. Sci.* **20**, (2019).
16. Bradley, D., Shantaram, D., Smith, A. & Hsueh, W. A. Adipose Tissue T Regulatory Cells: Implications for Health and Disease. *Adv. Exp. Med. Biol.* **1278**, 125–139 (2021).
17. Kunz, H. E. *et al.* Adipose tissue macrophage populations and inflammation are associated with systemic inflammation and insulin resistance in obesity. *Am. J. Physiol. Endocrinol. Metab.* **321**, E105–E121 (2021).
18. Kane, H. & Lynch, L. Innate Immune Control of Adipose Tissue Homeostasis. *Trends Immunol.* **40**, 857–872 (2019).
19. Weisberg, S. P. *et al.* Obesity is associated with macrophage accumulation in adipose tissue. *J. Clin. Invest.* **112**, 1796–1808 (2003).
20. Ruggiero, A. D., Key, C.-C. C. & Kavanagh, K. Adipose Tissue Macrophage Polarization in Healthy and Unhealthy Obesity. *Front. Nutr.* **8**, 625331 (2021).
21. Eshghjoo, S., Kim, D. M., Jayaraman, A., Sun, Y. & Alaniz, R. C. Macrophage Polarization in Atherosclerosis. *Genes (Basel).* **13**, (2022).
22. Viola, A., Munari, F., Sánchez-Rodríguez, R., Scolaro, T. & Castegna, A. The Metabolic Signature of Macrophage Responses. *Front. Immunol.* **10**, 1462 (2019).
23. Hirata, Y. *et al.* Coronary atherosclerosis is associated with macrophage polarization in epicardial adipose tissue. *J. Am. Coll. Cardiol.* **58**, 248–255 (2011).
24. Kieler, M., Hofmann, M. & Schabbauer, G. More than just protein building blocks: how amino acids and related metabolic pathways fuel macrophage polarization. *FEBS J.* **288**, 3694–3714 (2021).
25. Lv, R., Bao, Q. & Li, Y. Regulation of M1-type and M2-type macrophage polarization in RAW264.7 cells by Galectin-9. *Mol. Med. Rep.* **16**, 9111–9119 (2017).

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Firma

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