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ROLE OF ADVANCED GLYCATION END-PRODUCTS (AGES) IN ISCHEMIA-REPERFUSION INJURY OF THE AGED AND DIABETIC HEART: NEW INSIGHTS FROM MOUSE AND HUMAN STUDIES

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1. Summary

Non-enzymatic advanced glycation is a highly toxic and ubiquitous chemical reaction that induces terminal changes to extracellular and intracellular proteins. The accumulation of glycated proteins and other macromolecules (generically named as advanced glycation end-products or AGEs) has been described in several age-related diseases, including renal failure, neurodegenerative diseases, cataracts and chronic inflammation, among others. However, their pathophysiological role in the aging heart is not known. The aim of this project was to investigate the contribution of AGEs to the functional decline of cardiomyocytes during aging, and their participation in the vulnerability of aged cardiomyocytes to ischemia-reperfusion injury and heart failure, two of the most prevalent conditions in the elderly. The achievement of this aim would make it possible to identify new therapeutic targets.

By using a wide spectrum of technical approaches, including Western blot, immunofluorescence, ELISA and multiplexed, high-throughput quantitative proteomics, we have established for the first time the degree of AGEs accumulation in the heart of young (4-6 months) and old (>20 months) mice, and the cardiac proteins specifically affected by such glycative attack. Because AGEs accumulation can be modulated by some endogenous detoxification systems, mainly the glyoxalase-dependent detoxification (which eliminates some of the chemical precursors involved in the generation of AGEs), we have investigated the impact of aging on glyoxalase activity/expression in the heart of mice and humans of different ages. To elucidate the functional consequences of AGEs in the heart, we have quantified cell calcium handling, oxidative damage, mitochondrial respiration and antioxidant potential in cardiomyocytes and mitochondria isolated from mouse with physiological aging and their young counterparts and myocardial human samples. Finally, to establish the potential cause-effect relationship between AGEs and the aging-dependent cardiomyocyte (dys)function, we have developed a cardiomyocyte-like culture model of AGEs accumulation, in which all the previously used functional assays were tested.

In parallel, as a separate substudy, we have investigated the potential role of AGEs as predictors of ischemic heart disease and modulators of ischemia-reperfusion

injury in patients, and their contribution to cardiac dysfunction. To this end, we have quantified the levels of circulating and tissue AGEs (by non-invasive detection of arm and eye's lens fluorescence) in a cohort of type 2 diabetic patients of different ages. A non-diabetic population matched by age and sex has been used as control group. The correlation between AGEs levels, patient's clinical phenotype and the incidence of silent myocardial infarction (detected by coronary TC/PET) and acute myocardial infarction (determined by the extension of necrosis quantified by MRI) is under analysis.

2. Obtained results

By using isolated cardiomyocytes, mitochondria, sarcoplasmic reticulum (SR) vesicles and myocardial extracts from young (5-6 months) and old (≥20 months) c57bl/6 mice and from young (<75 years) and elderly patients (≥75 years) undergoing cardiac surgery in Hospital Vall d'Hebron, we have demonstrated, for the first time, that aging is associated with a significant increase in the levels of AGE-modified proteins in the heart, both in mice and humans. Contrary to what was expected, immunofluorescence labeling and proteomic analysis established that a significant fraction of the AGEs accumulated during aging is present in the *intracellular space* of cardiomyocytes. Spectrophotometric enzyme assay disclosed a reduction in the efficiency of glyoxalase-1 (Glo-1, the enzymatic system responsible for detoxification of dicarbonyl compounds, like methylglyoxal, that eventually evolves into AGEs), despite preserved expression levels of the enzyme. Impaired Glo-1 activity resulted in less D-lactate production, the final metabolite of the detoxification pathway, in the myocardium of both aged mice and elderly patients. Moreover, levels of glutathione, an antioxidant enzyme that acts as Glo-1 cofactor, were also reduced in the aged murine and human myocardium. These results indicate that beyond the passive accumulation of AGEs in the extracellular matrix, aging heart develops a deficiency in one of the most universal endogenous detoxifications systems of AGE precursors, which in turn underlies the excess of intracellular glycoxidative damage.

Remarkably, among the proteins affected by age-dependent glycation, cardiac ryanodine receptor (RyR) from sarcoplasmic reticulum (SR) was identified as a

prominent target of glycative attack, both in mice and humans, as detected by ELISA, immunofluorescence and differential proteomics. Glycation of RyR was found to have functional consequences on SR calcium handling, consisting in an increased RyR-dependent calcium leak. Because SR forms close anatomical and functional units with the surrounding mitochondria, with which they bidirectionally exchange calcium and ATP, we investigated the impact of such SR-dependent calcium leak on mitochondrial fitness and integrity. Our results indicate that during aging, cardiac mitochondria develop an increased calcium precipitation in their matrix, both in mice and humans. Functional mitochondrial analysis indicated that this excessive calcium precipitation has deleterious consequences, including reduced aerobic capacity and fewer respiring mitochondria in the aged heart of mice and humans.

To establish the cause-effect relationship between age-dependent intracellular glycation, altered calcium handling and mitochondrial dysfunction, we chronically exposed cultured HL-1 cardiomyocytes to conditions mimicking aging, i.e., Glo-1 inhibition and excess of methylglyoxal. When cultured HL-1 cardiomyocytes were exposed to these conditions, intracellular AGEs accumulation was developed in 3 days. As previously observed in intact cardiomyocytes, RyR was identified as protein target of glycation in HL-1 cells. Glycation of RyR recapitulated the altered calcium exchange between SR and mitochondria present in the aged heart.

3. Relevance of the study and potential future implications

What is the relevance of the study?

Our data show for the first time a deficient dicarbonyl detoxification capacity in the heart as common hallmark of aging, as it is present both in aged mice and elderly patients. Reduced Glo-1 activity has previously been associated with the severity of atherosclerosis, diabetic cardiomyopathy and coronary artery disease and we now provide evidences of its contribution to the pathophysiology of the aging heart.

We identified RyR as a prominent target of glycative damage in aged cardiomyocytes; glycation of RyR alters SR calcium handling properties. Adequate

RyR function is essential for contractile activity and calcium homeostasis in the heart; alterations in RyR-dependent calcium release have been causally linked to cardiomyocyte dysfunction in heart failure and cell death in ischemia-reperfusion injury. Our results support the concept that endogenous RyR glycation may participate in the increased extension of necrosis during myocardial infarction and the higher incidence of heart failure consistently observed in elderly patients.

Because mitochondrial calcium uptake dynamically regulates energy demand/supply matching and closely depends on calcium transferred from SR, we provide evidence indicating that RyR glycation results in defective SR-mitochondria calcium exchange that eventually leads to mitochondrial damage. The heart is the organ with the highest dependence on mitochondrial aerobic capacity. Mitochondrial damage secondary to calcium overload can not only aggravate ischemia-reperfusion injury but may also underlie the reduced tolerance to exercise and stress of the aged heart.

What are the potential clinical implications?

We identified a previously unknown pathophysiological mechanism of cardiac aging, potentially involved in the transition from healthy towards failing cardiomyocytes during aging**;** this mechanism may also explain the increased vulnerability of the aged cardiomyocytes to ischemia-reperfusion injury.

Preventing the formation of dicarbonyl intermediates, promoting their detoxification, or alleviating the functional consequences of RyR glycation are proposed as novel therapeutic strategies addressed to improve mitochondrial function in the aged cardiomyocytes.

Altogether, these results open a new field of research to identify the best therapeutic strategies capable of preventing intracellular glycation in cardiomyocytes (and potentially other cell types) during aging and/or alleviating its consequences in some pathological contexts, like ischemia-reperfusion injury.

4. Scientific bibliography generated

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