Pharmacological targeting of histon acetylation-based epigenetic mechanisms to reduce renal dysfunction in experimental diabetes

Diabetes is a disease that affects ~9.3% of the adult population worldwide. This complex metabolic disease is a major risk factor when it come to the development of certain micro- and macrovascular diseases that decrease the quality of life as well as increase the risk of major cardiovascular events. According to statistics, around 48% of all diabetic patients are younger than 70 years old. Diabetes is classified into two types: type 1 diabetes or insulin-dependent diabetes characterized by insufficient insulin production, which can appear since childhood and type 2 diabetes, or non-insulin-dependent diabetes due to the ineffective way the cells use insulin, this represents 95% of cases of diabetes globally and it generally occurs in adults. Gestational diabetes can occur during pregnancy and is characterized by hyperglycemic values, above normal but below diagnosable pathological values. One of the chronic complications of diabetes is diabetic nephropathy, which is characterized by inadequate kidney function, leading, in the advanced stages of the disease, to dialysis or kidney transplantation. Patients with diabetic nephropathy have an increased risk of developing cardiovascular disease and a major risk of death. [1]

The main features of diabetic nephropathy are micro-/macro-albuminuria, proteinuria, glomerular basement membrane thickening, podocyte hypertrophy, mesangial cell hyperplasia and hypertrophy, immune system cell infiltration, and increased levels of reactive oxygen species. The inflammatory response is maintained at an increased level by the activation of pro-inflammatory transcription factors, such as nuclear factor kappa β (NF-κB), transcription factor AP-1, C/EBP, and STAT1 that causes increased oxidative stress. [2]

Epigenetic changes increase the expression of molecules that have an important role in the pathophysiology of vascular complications associated with diabetes, such as the NADPH oxidase complex, pro-inflammatory mediators (cytokines, chemokines) and pro-fibrotics (extracellular matrix elements, growth factors) , vasoactive agents (endothelin-1), matrix metalloproteinase 9, vascular cell adhesion molecules (VCAM-1), intracellular adhesion molecules-1 (ICAM-1), E-selectin (E-sel). Therefore, it is very important to investigate new molecular mechanisms in order to have other therapeutic approaches in the treatment of diabetic nephropathy.

In this work, the roles of epigenetic mechanisms involved in nucleosomal histone acetylation in the establishment of oxidative stress, inflammation and renal fibrosis in experimental diabetes were investigated with the purpose of developing new therapies for the treatment of diabetic kidney disease.

The hypothesis of this work consists in the fact that hyperglycemia induces the imbalance of epigenetic mechanisms dependent on histone acetylation, a pathological condition that causes an increase in the specific expression levels of some enzyme systems involved in the production of reactive oxygen species and some pro-inflammatory and pro- fibrotic at renal level.

The objectives of this thesis were:

Objective 1. To investigate the role of histone acetyltransferase p300/CBP-dependent signaling pathways in the establishment of oxidative stress, inflammation and renal fibrosis in experimental diabetes.

Objective 2. Development of an innovative pharmacological strategy based on triterpenes to reduce renal dysfunction in experimental diabetes.

Objective 3. Development of a method for the detection of reactive oxygen species in experimental atherosclerosis by high-resolution molecular imaging in the near-infrared spectral range using functionalized liposomes.

The first part of the thesis, entitled the current state of knowledge of diabetic kidney diseases, provides information on the pathological mechanisms underlying the development of diabetic kidney disease or diabetic nephropathy.

In chapter II, the original scientific contributions aimed at the involvement of histone-acetyltransferase p300/CBP in the pathology of diabetic nephropathy are presented, with special emphasis on the enzyme system that produces NADPH oxidases (vascular sources that generate reactive oxygen species and oxidative stress) and on pro-inflammatory and pro-fibrotic molecules. The potential therapeutic effects of ursolic acid and a standardized bioactive complex consisting of ursolic acid (90.7%) and oleanolic acid (5.8%) in modulating some pathophysiological processes associated with renal dysfunction in experimental diabetes were also investigated. Increased production of reactive oxygen species and increased oxidative stress is a feature of cardiovascular diseases. In this context, studies have been carried out that form the basis of the development of an innovative method for determining the production of reactive oxygen species *in vivo* in animal models with atherosclerosis using functionalized liposomes for the encapsulation of a redox sensitive compound, ROS BriteTM 700, which through its oxidation emits a specific fluorescent signal in the near infrared spectral range.

Conclusions

Article 1.: Activation of histone acetyltransferase p300/CBP signaling pathway mediates increased NADPH oxidase transcription, inflammation and fibrosis in diabetic kidney.

In conclusion, in diabetic kidneys, activation of histone acetyltransferase p300/CBP leads to increased production of ROS (most likely generated by Nox proteins), inflammation and production of extracellular matrix proteins. Based on the fact that p300/CBP is an important co-transcriptional activator that regulates the expression of a wide range of pro-oxidant, pro-inflammatory and pro-fibrotic genes, we can state that pharmacological modulation of p300/CBP could become a tempting therapeutic option in diabetic nephropathy.

Article 2.: Triterpenic acids inhibit the expression of markers related to inflammation and oxidative stress in human proinflammatory macrophages.

The Salvia officinalis extract samples obtained have a high content of triterpenic acids, mostly ursolic acid.

At concentrations of 1-10 µM, triterpenic acids, namely ursolic, oleanolic, betulinic and the standardized bioactive complex formed by the triterpenic acids AU (90.7%) and AO (5.8%) cause the

reduction of specific mRNA expression levels of some pro-oxidant and pro- - inflammatory agents with an important role in the pathophysiology of cardiovascular complications associated with diabetes.

Treatment of diabetic mice with ursolic acid or the standardized bioactive complex consisting of AU (90.7%) and AO (5.8%) caused significant decreases in glomerular hypertrophy and gene or protein expression levels specific to certain molecules (of oxidative stress, inflammation and fibrosis) that have an important role in the pathophysiology of diabetic nephropathy.

Ursolic acid (UA) causes the reduction of specific gene expression levels of some molecules associated with the pro-inflammatory phenotype (M1) of macrophages in culture.

Article 3.: Detection of vascular reactive oxygen species in experimental atherosclerosis by high-resolution near-infrared emission spectrum imaging using VCAM-1-targeted liposomes that are loaded with a fluorogenic redox-sensitive probe.

The obtained data indicate that encapsulation of RB700 in sterically stabilized endothelium-targeting Lp could become a reliable method to directly quantify and visualize the sites of ROS formation in the vasculature and organs in animal models. This method could be applied not only in experimental atherosclerosis, but also for cancer, diabetes, obesity and neurodegenerative diseases, as all these diseases are characterized by increased ROS formation. Moreover, this new setup for molecular detection of ROS based on RB700-Lp targeting activated endothelium is of particular importance to highlight the intensity and mechanism(s) of oxidative stress production induced by risk factors associated with different pathologies, such as and regarding the development and preclinical testing of new drugs and therapeutic strategies.

The original results obtained in this thesis demonstrate that:

1. Activation of histone acetyltransferase p300/CBP-related signaling pathways mediates glomerular hypertrophy and accumulation of extracellular matrix proteins in the kidney of diabetic mice.

2. Pharmacological inhibition of histone acetyltransferase p300/CBP reduces the level of acetylation of histone 3 at lysine residue 27 (H3K27ac) in the kidneys of diabetic mice compared with diabetic animals.

3. Histone acetyltransferase p300/CBP mediates increased gene and protein expression levels specific to Nox1, Nox2 and Nox4 subunits in the kidney of diabetic mice.

4. Pharmacological inhibition of histone acetyltransferase p300/CBP reduces the formation of reactive oxygen species in the kidneys of diabetic mice.

5. Treatment of diabetic mice with C646, a specific inhibitor of p300/CBP, significantly reduces subtype-specific protein levels of Nox1 and Nox4 at the glomerular level.

6. Pharmacological inhibition of p300/CBP coactivator significantly reduces the activity of NF-kB and STAT transcription factors.

7. The activation of histone acetyltransferase p300/CBP mediates the increase in gene levels of some pro-inflammatory (MCP-1, TNFα, NOS2, ICAM-1, VCAM-1, E-selectin) and pro-fibrotic (collagen IV, fibronectin, laminin) mediators .

8. Treatment of diabetic mice with ursolic acid or with the standardized bioactive complex consisting of ursolic acid (90.7%) and oleanolic acid (5.8%) causes significant decreases in glomerular hypertrophy and gene/protein expression levels specific to molecules associated with oxidative stress, inflammation and fibrosis with an important role in the pathophysiology of diabetic nephropathy.

9. Ursolic acid reduces specific gene levels of the catalytic subunits Nox1, Nox2, Nox4 and Nox5 and the pro-inflammatory mediators MCP-1, TNFα and NOS2 in Mac with a pro-inflammatory phenotype (M1-Mac) and the adhesion molecules ICAM-1 , VCAM-1 and E-selectin.

10. Treatment of diabetic mice with ursolic acid or with the standardized bioactive complex obtained from *Salvia officinalis* leaves containing ursolic acid (90.7%) and oleanolic acid (5.8%) causes a significant decrease in the protein expression of Nox1, Nox2 and Nox4, and production of reactive oxygen species at the glomerular level, as well as the formation of products such as 4-HNE and the expression of VCAM-1 at the glomerular level in the kidneys of diabetic mice.

11. Liposomes sterically stabilized and targeted to the pro-inflammatory adhesion molecule VCAM-1 represent an efficient delivery system for the concentration of the redox indicator RB700 at the level of atherosclerotic lesions in ApoE-/- mice.

Bibliography

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