Meeting Impression

ERA-EDTA 2017 Congress, Madrid, Spain, Monday, June 5, 2017

MANAGING CKD, DIABETES & CVD: IS EPIGENETICS A NEW WAY FORWARD?

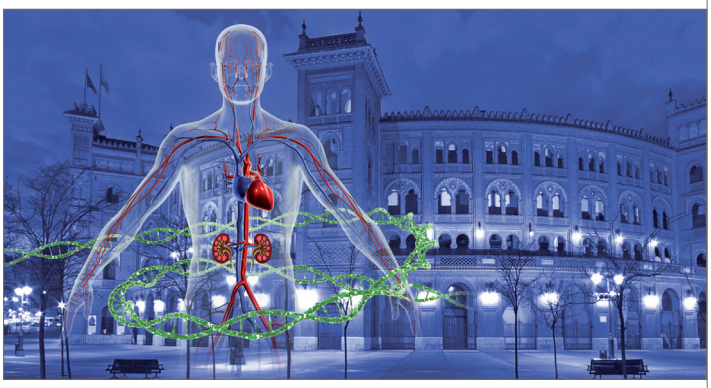
The aim of this educational program was to provide a balanced overview of results with a novel approach to reduce residual CV risk in patients with increased cardiovascular and renal risk, namely BET inhibition. BET refers to a bromodomain and extra-terminal domain of regulatory proteins. Its inhibition modifies gene expression via epigenetic mechanisms. This symposium was held during the 54th ERA EDTA Congress in Madrid, Spain on June 5, 2017. It was chaired by Vincent M Brandenburg, MD (Aachen, Germany) and Carmine Zoccali, MD (Reggio Cal, Italy).

The high risk diabetes patient: What is the need for novel approaches to reduce cardiovascular and renal risk?

Luis M Ruilope, MD – *Hospital 12 de Octubre Madrid, Spain*

Diabetes is associated with a significant loss of life years. For instance, on average, a 50-year old individual with diabetes and no history of vascular disease will die 6 years earlier than someone without diabetes. The main reason of death in diabetes is cardiovascular (CV) death [1]. Renal disease makes the situation worse, as the incidence of myocardial infarction (MI) or all-cause mortality in individuals with diabetes and chronic kidney disease (CKD), is substantially higher than in those with either one of these conditions [2]. This indicates that broader management than only focusing on glycemic control is important in diabetes. Dr. Ruilope therefore discussed relevant clinical issues that need attention in the management of diabetes and CKD.

For example, the need for antihypertensive therapy to reduce CV risk in diabetic patients is well established, but this guidance might not always be implemented. An analysis of the Spanish ABPM registry showed that nighttime blood pressure (BP) was elevated when albuminuria was present (either micro- or microalbuminuria), [3]. Hypertension with nighttime BP is known to be a more detrimental form of hypertension. The significant effect of BP-lowering therapy on nighttime hypertension was shown in the ARTS-DN ABPM substudy, in which the effect of finerenone, a nonsteroidal mineralocorticoid receptor antagonist (MRA), was tested in type 2 diabetes patients with albuminuria. Treatment with finerenone yielded a drop in nighttime BP by 12 mmHg, while the effect on office BP was much lower, with only a 4 mmHg reduction. This underscores the need for a change in BP measurement method in diabetic patients to identify these patients, as nighttime high BP is not always detected and properly treated in daily clinical practice.



Another important problem in diabetes practice, is the acquired insensitivity from effective treatment by renin–angiotensin system (RAS) blockade, since over time, the protective effect of treatment with an ACE inhibitor or an angiotensin receptor blocker (ARB) may diminish. For instance, in the LIFE study , it was noted that with time, the effect of losartan on preventing albuminuria, was diminished in patients with hypertension with left ventricular hypertrophy [4]. It is possible that as a consequence, aldosterone secretion increases and contributes to progression of CV and renal disease. Indeed, in a Spanish cohort, 16.1% of patients chronically treated with an ACE inhibitor and ARB, developed high albuminuria about 3 years after presenting at the clinic, and 1% very high. In diabetic patients, this was much higher, with 25.3% developing microalbuminuria and 4.6% developing macroalbuminuria. Thus, the ACE inhibitor and ARB were not protecting the patients enough against albuminuria. Development of albuminuria is accompanied by a higher number of CV events and death.

Residual risk is high in diabetic patients with CKD. This unmet need may be addressed by novel epigenetic therapeutic approaches.

Thus, when CVD is present and renal function is normal, BP control, lipid control and RAS blockade are an appropriate and simple but comprehensive approach to better

protect diabetic patients. When CKD is present, resistant hypertension can co-exist, and also nighttime BP can be elevated and needs to be corrected. Concerning lipid control in CKD, only data from the SHARP study is available on use of low-dose simvastatin treatment. With regard to RAS blockade, the possibility of developing hyperkalemia can interfere with the use of ACE inhibition or ARBs. This appears to be the case in up to 30% of patients, either due to actual development of hyperkalemia or to the physician's concern of the patient developing it. The new non-steroïdal MRAs may potentially be of use, due to their lower risk of hyperkalemia. Moreover, the availability of potassium binders may help in prolonging blockade of the RAS system [5].

Then, naturally, glycemic control is important in diabetes. The new glucosuric SGLT2 inhibitors and GLP-1 receptor agonists have been shown to have a positive effect on CV risk. Ruilope showed a graph from the EMPA-REG OUTCOME study, in which an impressive reduction of hospitalization for HF was seen (-38%) with treatment with empagliflozin. The residual risk is, however, still high. Thus, there is an unmet need in patients with diabetes and CKD. Many genes and proteins have been found to be dysregulated in this patient group, which can drive CVD risk. Novel epigenetic therapeutic options are in development. BET proteins are a new molecular target, through which key pathways and markers can be differentially regulated.

Epigenetics as a novel strategy in cardiovascular and renal risk reduction: A closer look at BET as pathway for inhibition

Marta Ruiz-Ortega, PhD – *Fundación Jiménez Díaz, Madrid, Spain*

Epigenetic changes are defined as heritable changes in gene expression that do not involve changes to the underlying DNA sequences. The epigenome refers to the interplay between the genotype and the environment, which together determine the phenotype. Differences in the epigenome can explain differences between identical twins, but also disease state in patients. The clinical manifestation of type 2 diabetes is dependent on genetics, the environment (exercise, nutrients, intrauterine environment) and epigenetics [6].

Several types of epigenetic modifications exist, often involving a modification of chromatin structure. The long DNA strand is folded in the nucleus into chromosomes, by wrapping around so called nucleosomes. Nucleosomes are composed of various histone proteins. These histones have protruding tails, on which chemical modifications take place. The possible chemical reactions at the histone tails include acetylation, methylation, ubiquitination and phosphorylation. These histone modifications can alter chromatin structure, attract proteins of the transcription machinery and thereby affect gene transcription.

Acetylation takes place at lysine residues. The fact that this is a reversible reaction, can be benefitted from for therapeutic strategies. This is where the bromodomain and extraterminal domain (BET) protein family comes in. These proteins can bind to acetylated lysine residues on histone tails. Binding of BET proteins to histone tails can affect gene transcription. Multiple BET proteins exist, of which BRD4 is the most important one. BET proteins are considered epigenetic readers. For instance, BRD4 can bind a histone tail located at a superenhancer region. It recruits other proteins necessary for chromatin remodeling. Moreover, it interacts with the transcription machinery and recruits for instance polymerase. The combination of these processes results in activation of gene transcription.

Apabetalone (or RVX-208) is an inhibitor of BET proteins. It is a novel small molecule that plays a role in modulating multiple biological pathways with a net anti-inflammatory effect [7]. BET inhibitors displace the BET protein from the lysine residue. The first studies with BET inhibition have been performed in cancer. JQ1, another BET inhibitor, induced changes in gene expression, in particular in the *c-myc* gene. To date, one study has been conducted in diabetes, with apabetalone.

The laboratory of Ruiz-Ortega studies whether BET inhibition could be a potential therapeutic target for renal inflammatory disease. They use experimental murine models of kidney disease, for instance unilateral ureteral obstruction. Starting one day before surgery, mice are treated with the BET inhibitor, and the inflammatory response is evaluated at different time points. After two days, mice that had received a high dose of JQ1 showed a diminished inflammatory response, which was maintained up to five days. Inflammatory cell infiltration of monocytes, macrophages, neutrophils and T lymphocytes was clearly blocked by JQ1, as seen on histological sections. The tested low dose showed partial effect. BET inhibition also diminished upregulation of expression of proinflammatory genes, which was also seen at the protein level (e.g. MCP1).

In the angiotensin II infusion-induced renal damage model, presence of inflammatory cells was lower after treatment with the BET inhibitor and upregulation of proinflammatory mediators was reduced. Similar results were observed in the nephrotoxic serum (NTS)-induced glomerulonephritis model [8]. Other authors have also shown reduced inflammation in experimental models of kidney damage, with different BET inhibitors [9]. Thus, these mouse experiments suggest that BET inhibition abrogates experimental renal inflammation.

Ruiz-Ortega and colleagues then wanted to study the molecular mechanisms underlying this beneficial effect of

BET inhibition. They used an *in vitro* model of renal cells that were stimulated with TNF-α towards an inflammatory response. After treatment with the BET inhibitor, they were analyzed with a DNA genomic array. Genes involved in the immune response and the regulation of inflammation were found to be altered (eg IL-6, MCP1 and other chemokines) after BET inhibition in TNF-stimulated renal cells. BET inhibition prevents the binding of BRD4 to the lysine residues located at these genes, thereby impeding gene transcription.

Some of the genes affected by BET inhibition are dependent on the transcription factor p-TEF, while others are not, suggesting that other molecular routes also play a role in the effects of BET inhibition. Most of the other genes are regulated by NF-κB. This transcription factor is very important in renal disease. It regulates many genes involved in the inflammatory response. In patients with diabetic nephropathy, NF-κB is activated in the kidney, associated with the presence of inflammatory cells in different renal structures [10].

NF-κB activation in injured kidney has been observed in different models of renal injury, which could be prevented by treatment with JQ1. Experiments in renal cells stimulated with proinflammatory cytokines showed that JQ1 only affects NF-κB expression at the nuclear level. Similar results of reduced expression of NF-κB and several proinflammatory gene responses to BET inhibition have been shown in differentiated dendritic cells and T lymphocytes.

A phase I clinical trial evaluated the safety and pharmacokinetics of a single dose of apabetalone (100 mg) in patients with severe renal impairment, as compared with healthy controls. Proteomic analysis was performed to identify the main proteins that are affected by BET inhibition. One of the main pathways affected in human CKD was the NF-κB pathway, as it was markedly diminished as compared with controls. Inflammatory IL-6 signaling and the Th1 pathway were also clearly diminished by apabetalone. There are also TNF-α-induced pro-inflammatory genes that were not affected by JQ1, including components of the NF-κB pathway. This means that BET inhibition specifically diminishes activation of the NF-κB pathway, not the NF-κB pathway per se.

The Th17-mediated inflammatory response is involved in various renal diseases, including non-inflammatory pathologies, and BET proteins are involved in Th17 differentiation. In mouse models of renal damage, BET inhibition reduced inflammation by modulation of the Th17 immune response.

BET inhibition acts not only by diminishing inflammation via inhibiting the NF-κB pathway and the Th17 immune response, but also by lowering activity of transcription factors involved in fibrosis.

Fibrosis is another important aspect of renal disease, because increased extracellular matrix deposition occurs in response to chronic inflammation. BET inhibition has been found to downregulate many genes involved in extra cellular matrix regulation and indeed, it reduced experimental renal fibrosis [9,11]. Research of Ruiz-Ortega's laboratory has revealed that the reduced fibrosis in NTS nephritis is mediated by diminished activation of SOX9 activation, a transcription factor involved in the regulation of type IV collagen.

Thus, BET inhibition acts not only by diminishing inflammation via inhibiting the NF-κB pathway and the Th17 immune response, but also by lowering activity of transcription factors involved in fibrosis. Data are also available that showed that BET inhibition reduced renal damage (restoration of serum creatinine, and tendency of restoration of urinary albumin) in experimental glomerulonephritis mouse models, thus renal function was ameliorated with JQ1.

BET inhibition in renal and cardiovascular disease: What is the clinical roadmap?

Kamyar Kalantar-Zade, MD – *UC Irvine School of Medicine, Irvine, CA, USA*

Kamyar Kalantar-Zade elaborated on how BET inhibition may play a role in addressing the unmet need in diabetes and CKD. As opposed to traditional pharmacological therapies that act at the post-translational model, apabetalone affects transcription, and can affect several pathways simultaneously. This might be interesting in light of the multifactorial aspect of CKD. In addition to inflammation and oxidative stress and complement activation, platelet activation, endothelial dysfunction and vascular calcification are seen. These processes might be related to epigenetic alterations, which is the rationale to why BET inhibition has the potential to favorably affect CKD.

In human cells, BET inhibition with apabetalone countered extracellular calcium deposition in a dose-responsive manner [12]. In response to apabetalone treatment, expression of genes related to vascular calcification was downregulated in multiple cell types, including alkaline phosphatase (ALP), osteopontin and osteoprotegerin. In human coronary artery vascular smooth muscle cells, apabetalone inhibited induction of gene expression of osteogenic markers, again including ALP and osteoprotegerin [12]. ALP is also downregulated by apabetalone in primary human hepatocytes [12].
ALP is a hydrolase enzyme that removes phosphatase groups (dephosphorylation) from a diverse group of molecules, including nucleotides, proteins and alkaloids. ALP is present in all tissues, but at higher concentrations in liver, bone, intestinal mucosa and placenta, and tissue-specific isozymes exist.

In a recent review, the concept of ALP molecules leaving the cell and acting as ectoenzymes to lead to vascular

calcification, was presented [13]. ALP has recently also been recognized as a prognostic biomarker in CKD. Higher levels have been found to be associated with increased mortality in the general population and in survivors of MI and CKD patients. In another study, the highest quartile of serum ALP was associated with higher prevalence of MI, stroke, congestive heart failure and diabetes [14]. In patients with end-stage renal disease, higher ALP levels were associated with increased mortality [15]. Also in the general population, positive correlations between ALP tertiles and death, coronary heart disease mortality, congestive heart failure an MI were observed [16].

Parathyroid hormone (PTH) is a marker commonly used by nephrologists, which shows a curvilinear association with the risk of all-cause death. ALP shows a more linear relationship with mortality, and may thus be a more relevant biomarker [17]. Also in patients who have received a kidney transplant, ALP is a stronger and more linear predictor of post-transplantation survival than PTH [18].

Alkaline phosphatase (ALP) expression is affected by BET inhibition and studies have associated elevated ALP to poor CVD outcomes.

Vascular calcification is strongly predictive of death in CKD patients [19]. While the precise mechanistic link between ALP and vascular calcification remains to be elucidated, it is postulated to involve pyrophosphate. ALP is thought to inhibit pyrophosphate, which normally inhibits medial vascular calcification [20]. Indeed, in CKD patients, having ALP >120 IU/L was associated with coronary calcification, consistent across various branches of the coronary artery [21].

Other studies are now emerging that demonstrate associations between elevated ALP and poor outcomes, all pointing towards a role for ALP as a biomarker for survival and coronary events. The next question is then whether it is possible to lower serum ALP and whether this intervention is associated with better CV outcomes. In fact, the phase II ASSURE and SUSTAIN studies showed that patients treated with apabetalone experienced fewer CV events, independent of having diabetes or inflammation (as measured by CRP) [22, 23]. Patients with a higher ALP level at baseline, exhibited a higher likelihood of MACE (major adverse cardiovascular events), also in an analysis that stratified patients for diabetes status. Patients who received apabetalone showed a clear drop in serum ALP, which was maintained for at least 6 months [24]. Moreover, in all patients of the phase II studies ASSERT, SUSTAIN and ASSURE who suffered from MACE, had significantly higher ALP levels at baseline than those without MACE.
A post-hoc analysis of the phase II studies showed that patients with CKD (eGFR <60) who received apabetalone also showed sustained or even improved (+3.5% vs. -5.9% with placebo) eGFR function through 6 months [24].

These data give rise to the following hypothetical model: elevated ALP is associated with increased CVD mortality, both in the general population and in CKD patients. More specifically, elevated ALP is associated with increased vascular calcification. Vascular calcification in turn is associated with increased mortality, thus providing a biologically plausible link between ALP and mortality. Clinical studies have shown that the BET-inhibitor apabetalone lowers ALP in a dose-dependent and time-dependent manner. Elevated ALP levels may be the consequence of epigenetic disturbances. Apabetalone may act at several levels, including vascular calcification, to diminish effects of high ALP levels.

The phase III trial BETonMACE is ongoing and has included about 50% of patients. It aims to evaluate the effect of apabetalone 200 mg daily, compared with placebo, in addition to standard of care, on MACE in about 2400 diabetic subjects with existing CVD and eGFR <60 at screening. Treatment duration is up to 104 weeks, with an additional 4-16 weeks safety follow-up.

References

1. Seshasai *et al.*, N Engl J Med. 2011; 364: 829-41
2. Tonelli M *et al.*, Lancet. 2012; 380: 807-814.
3. Ruiz Hurtado *et al.*, Diabetes Care. 2016; 39:1729-37
4. Ibsen *et al.*, Kidney Int Suppl. 2004; 92:S56-8
5. Ruiz-Hurtado G & Ruilope LM. Nat Rev Cardiol. 2016;13:603-8
6. Ling *et al.* Diabetes. 2009; 58: 2718–2725
7. Filippakopoulos P *et al.* Nature. 2010; 468:1062
8. Suarez-Alvarez B *et al.* J Am Soc Nephrol. 2017; 28:504-519
9. Xioung C *et al.* Oncotarget. 2017; 7; 43
10. Mezzano *et al.*, Kindey Int. 2003; 64: 64-70
11. Zhoug B *et al.*, Redox Biology 2017; 11: 390-402
12. Kulikowski *et al.*, ERA-EDTA 2017 Madrid, Spain
13. Haarhaus *et al.*, Nat Rev Neprhol. 2017; 13:429-42
14. Krishnamurthy VR *et al.*, Am J Med. 2011; 124, 566.e1-e7
15. Kalantar-Zadeh K *et al.*, Kidney Int. 2006; 70:771-80
16. Tonelli M *et al.*, Circulation. 2009; 120:1784-92
17. Regidor DL *et al.*, J Am Soc Nephrol. 2008;19:2193-203
18. Molnar MZ *et al.*, Clin J Am Soc Nephrol. 2012;7:1859-71
19. Shantouf RS *et al.*, Am J Nephrol. 2010;31:419-25
20. Lomashvilli KA *et al.*, Kidney Int. 2008;73:1024-30
21. Shantouf R *et al.*, Clin J Am Soc Nephrol. 2009;4:1106-14
22. Gilham D *et al.*, Atherosclerosis 2016; 247:48-57
23. Puri *et al.*, J Am Coll Cardiol. 2014; 63:12_S
24. Kalantar-Zadeh *et al.*, Poster ASN 2015, San Diego, CA, USA

MEDCON INTERNATIONAL