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

The aim of this educational program was to provide a summary of the epidemiology and pathophysiology of patients at high cardiovascular (CV) risk with diabetes (DM), and to understand the origin of the high residual CV risk in patients with DM. Moreover, an objective was to review how BET inhibition could serve as a novel strategy to improve outcomes in CVD. BET refers to a bromodomain and extra-terminal domain of regulatory proteins. Its inhibition modifies gene expression via epigenetic mechanisms. Current clinical research programs evaluating the role of epigenetic regulation of gene expression in CVD management were reviewed. This symposium was held during the ESC Congress in Barcelona, Spain on August 26, 2017. It was chaired by Kausik Ray (Imperial College London, UK) and Lina Badimon (Cardiovascular Research Center, Barcelona, Spain).

TOPICS

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Prof. Kausik Ray

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Managing high risk diabetes patient with cardiovascular disease: What works, and what else can we do? 

Prof. Kausik Ray, MD - Imperial College London, United Kingdom

Professor Ray set the stage by emphasizing that DM is a global and growing public health challenge. Outcomes in patients with DM remain poor as compared with those not affected by DM, despite the development of many good treatments. DM doubles the risk of coronary heart disease (CHD), increases the risk of cerebrovascular disease by about 80% and death by vascular causes is also increased. Ray thus concludes that DM is a vascular disease. Since many first events are fatal, it is important to treat DM quite aggressively directly after diagnosis. A young patient (about 40 years of age) with DM loses about 6 or 7 years of life, and half of those early deaths can be attributed to vascular deaths. With aging, the number of life years lost by DM is lower, because life expectancy is lower. Thus, age may affect treatment strategy. Patients of about 60 years old with DM and pre-existing CVD may lose an additional decade of life, beyond what they would lose just with DM.

The mainstay of treatment of patients with DM to lower their CV risk is lipid lowering, blood pressure (BP) reduction and glucose lowering. Both primary and secondary prevention trials have shown that the relative efficacy of those interventions is similar in those with and without DM, but the absolute benefit is larger in diabetic patients. In the IMPROVE-IT trial, patients with DM showed a larger risk reduction of the composite CV endpoint than those without DM. It should be noted that, despite achieving LDL-c levels below 53 mg/dL, the
7-year event rate was about 40% in these patients, as compared with 30.2% in those without DM with similar LDL-c levels.\textsuperscript{11} Diabetics also benefit from BP-lowering: 10 mmHg reduction in systolic BP (SBP) reduces the rate of all-cause mortality, and both macrovascular and microvascular outcomes.\textsuperscript{12} The question then arises which SBP target should be applied. There are data that suggest that aiming for an SBP lower than what guidelines recommend (140 mmHg), namely 120 mmHg, may be beneficial for at least certain types of events. For instance, the ACCORD trial showed a reduction of stroke events with intensive treatment as compared with standard treatment.\textsuperscript{13} Historically, the effects of glucose-lowering have actually been fairly disappointing, according to Ray. In a meta-analysis by Ray and colleagues of data of patients achieving 1% HbA1c reduction over 5 years, a significant reduction of non-fatal myocardial infarction (MI) was observed, irrespective of how glucose was lowered. But, while a significant reduction of fatal and non-fatal MI was seen, no significant impact on stroke and all-cause mortality was seen.\textsuperscript{14} Although the effect of lowering HbA1c is independent and additive to the effect of lipid-lowering and BP-reduction, the absolute benefit is much less than that of the other two interventions.\textsuperscript{15} Treatment with the newer antidiabetic class of DPP-4 inhibitors does not seem to result in CV benefit,\textsuperscript{16} in contrast to other new antidiabetic drugs, such as the SGLT2-inhibitor empagliflozin and the GLP-1 receptor agonist liraglutide. It should be noted that these agents have mainly been tested in stable CAD patients, instead of in the post-ACS population. The only trial in a post-ACS population with the GLP-1 receptor agonist lixisenatide did not show a CV benefit. Other novel agents include PCSK9 inhibition with monoclonal antibodies and the IL-1β-inhibitor canakinumab. The PCSK9 antibody evolocumab was shown to reduce LDL-c levels to about 30 mg/dL, which translates to about 15% relative risk reduction in the overall study population.\textsuperscript{16} Still, residual CV risk remains, thus targeting additional pathways may pay off. For instance, the vasculature in ACS patients is perturbed; there is inflammation in the vessel wall, systemic inflammation, activation of monocytes and macrophages, as well as perturbed levels of cytokines and chemoattractants. Moreover, the vessel wall has a procoagulant phenotype; there is a tendency towards clot formation and increased production of adhesion molecules. The PROVE-IT trial demonstrated that the higher the concentration of soluble adhesion molecules in the blood, the higher the risk of CV events. High-dose statin therapy attenuated this relationship somewhat, which is known to result in a number of potential beneficial effects on inflammation.\textsuperscript{17} Studies have attempted to establish the difference of DM patients and why their event rates are so much higher. A number of inflammatory markers, namely CRP, MCP-1 and von Willebrand factor, were examined in patients with or without DM in the OPUS-TIMI 16 study. While higher levels of these markers were associated with higher CV risk in both diabetics and non-diabetics, the relationship was stronger in diabetics.\textsuperscript{17} Validation of the CRP data in the TACTICS TIMI-18 suggested that there was a possible interaction between hyperglycemia or dysglycemia and inflammation. Indeed, in individuals with an increase of 1 mmol of glucose and a high CRP level, the impact on CV risk was not additive but multiplicative.\textsuperscript{18} Clearly, there are patients with unmet clinical needs, among them patients at the highest risk, thus those with ACS or DM. Patients with DM often have a low-HDL phenotype, which further increases CV risk. In addition, they often have a heightened inflammation phenotype. The question is whether or how we can improve treatment of these patients. An option to consider may be an approach with a single target and multiple effects, such as the novel epigenetic therapeutic drug apabetalone.

**Promise of epigenetic modulation as a target in atherosclerotic patients**

**Prof. Erik Stroes, MD, PhD – Academic Medical Centre, Amsterdam, The Netherlands**

Professor Stroes aimed to describe the overriding role of inflammatory activation in the process of atherogenesis, and how epigenetic targeting might counterbalance these effects. In all stages of the atherosclerotic process, from recruitment of monocytes through destabilization of plaque, inflammation plays a central role. Degradation of the fibrous cap can result in a full occlusion of the vessel, and consequently MI. It has been shown that the ipsilateral side, where an inflamed plaque resides, gives rise to stroke, while the contralateral side did not show inflammation of the arterial wall.\textsuperscript{19} A more recent study using 18F-FDG PET imaging demonstrated that in the presence of risk factors for atherogenesis, and in case of overt atherosclerosis, an inflamed arterial wall is seen.\textsuperscript{20} CV patients can be characterized by a profound proinflammatory state that is observed at various levels; in the vessel wall, plasma and bone marrow.

Inflammatory cells do not only get activated once they arrive at the plaque, but activated monocytes are already found in the circulation of patients with elevated Lp(a). It has been described that the stickiness of their white blood cells is...
increased compared with controls.\textsuperscript{21} Even in bone marrow, increased cellular activity is seen in patients who were treated for several months following an ACS; hematopoietic stem and progenitor cells (HSPCs) of CVD patients had a higher progenitor potential than HSPCs of controls. This suggests that the bone marrow senses the ACS event and contributes to a prolonged inflammatory state.\textsuperscript{22} Thus, CV patients can be characterized by a profound proinflammatory state that is observed at various levels; in the vessel wall, plasma and bone marrow.

Now, can this process be targeted with therapy? The CANTOS trial evaluating treatment with an IL-1\textbeta antibody in post-ACS patients with elevated CRP levels as a marker of inflammation, has met its primary endpoint of reduction of MI, stroke and CV death. A related question is which effects the induced immunosuppression has on patients; in other words do we put patients at risk? This is where epigenetic therapy comes into play. Using an analogy of reducing fuel use in an airplane, Stroes explained that epigenetics refers to modifying, or tweaking, gene expression in a more subtle intermediate manner than turning expression on or off. In epigenetic processes, gene expression is modified without alteration of the genetic code itself. He illustrates this by using a movie as a metaphor of human life. Our DNA is the script, which is rigid, genetic regulation is the screen writing, and epigenetics is the director who will ultimately control and direct how the script is read.

Normally, DNA is tightly packed in chromosomes in the cell nucleus, which makes it hardly accessible for transcription factors. The DNA is wrapped around protein complexes called nucleosomes, which consist of various histone proteins. This level of organization is referred to as chromatin. These histones have protruding tails that can be chemically modified. Modification of the DNA or the histones can result in local opening of the chromatin structure, which makes the DNA more accessible for transcription factors. Similarly, the chromatin can be compacted, which generally results in gene silencing.

Various types of histone modifications can take place, including methylation and acetylation. Multiple enzymes are involved in placing or taking away these histone marks, therefore sometimes referred to as ‘writers’ and ‘erasers’, thereby regulating gene transcription. Depending on the location, histone methylation can lead to gene activation or repression, while histone acetylation always results in activation of gene transcription by opening up the chromatin, allowing access of transcription factors.

In advanced human plaques, many histone modifications have been described. An overrepresentation of activating methylation marks is seen, and a decrease of repressing methylation marks. This might be related to the activated monocytes seen in patients with elevated Lp(a).\textsuperscript{21} Indeed, increased trimethylation of histone 3 at lysine 4 (H3K4Me3), a mark associated with open chromatin, is seen in the promoter regions of genes that encode proinflammatory cytokines (unpublished data, Van der Valk et al). The histone modifications can in turn be recognized by the so-called epigenetic readers, among which is the family of bromodomain and extra-terminal domain (BET) proteins. Multiple BET proteins exist, of which BRD4 is the most important one. BRD4 binds DNA polymerase. Inhibiting the BET protein BRD4 will inhibit DNA transcription into mRNA. This has a variety of downstream effects, and BET inhibition has received a lot of attention in oncology. Focusing on the inflammation cascade, it has been shown that inhibiting BET proteins with siRNA in epithelial cells leads to inhibition of adhesion of monocytes to the endothelium.\textsuperscript{24}

**The rationale of epigenetic modulation to reduce ‘redundant’ inflammation, without risking overall immune suppression**

Apabetalone (or RVX-208) is a selective BD2 inhibitor. BD2, together with a BET protein, normally recognizes acetylated histone tails. When apabetalone binds BD2, it prevents recognition of the histone mark, thereby preventing gene transcription. Treating endothelial cells with apabetalone results in inhibition of expression of the VCAM-1 adhesion molecule and in lower adhesiveness of monocytes to the endothelial cells (Resverlogix, data on file). Moreover, adding a BET inhibitor to macrophages in vitro has been shown to decrease their proinflammatory activity.\textsuperscript{25} It has been studied in mice whether these effects translate into a decrease of atherogenesis. Indeed, after treatment with apabetalone, hyperlipidemic ApoE\textsuperscript{-/-} mice showed a 40% reduction of atherogenesis.\textsuperscript{26} When apabetalone is added to human peripheral blood monocytes from healthy volunteers, downregulation of a wide array of proinflammatory cytokines and chemokines and their receptors is seen within 24 hours (Resverlogix, data on file), which suggests a possible anti-inflammatory potential in humans. The safety data of for example the ASSURE study, suggest a decreased proinflammatory state in CVD patients treated with apabetalone. For instance, CRP is lowered by about 21% and TNF by 14%.\textsuperscript{27} Thus, these experimental findings might suggest that epigenetic therapy by means of BET inhibition may reduce inflammation, and potentially thereby reduce atherogenesis. The rationale of epigenetic modulation is to reduce ‘redundant’ inflammation, without risking overall immune suppression. The first safety data in human patients are reassuring.
Insights from the first trials in epigenetics in human: What is the way forward?

Prof. Stephen Nicholls, MD, PhD – South Australian Health and Medical Research Institute (SAHMRI), Adelaide, Australia

Professor Nicholls shared some of the insights from the first human trials on epigenetic approaches to treat CVD. The need for additional therapeutic strategies above and beyond statin therapy stems from the residual CV event risk that is observed in high risk patients despite effective statin treatment.

Treatment with apabetalone is the first epigenetic approach to treat CVD. The agent was first thought to increase synthesis of ApoA-I in the liver, which appears to associate with an increase in HDL, but also with increased cholesterol influx capacity. A range of molecular studies later also showed that apabetalone beneficially affected several other pathways that have a role in driving the atherosclerotic disease process. Examples include inflammation, complement system, a range of metabolic effects, vascular calcification and coagulation. Thus, apabetalone appears to favorably modify atherogenesis across the whole continuum from early formation of plaque to ultimate rupture of plaque and coagulation causing acute MI.

To date, clinical experience with apabetalone has been obtained in 706 participants of completed trials. Of those, 576 patients had either CAD or dyslipidemia. All patients were well treated according to standard of care. Initial clinical studies with apabetalone were in high risk CAD patients. Three phase II studies have been completed in CAD patients: ASSERT (12 weeks, n=299) examined lipid effects of apabetalone, as did SUSTAIN (24 weeks, n=176), and ASSURE (26 weeks, n=323) evaluated the effects of apabetalone on coronary atherosclerosis using IVUS. The phase III CV outcome trial BETonMACE is currently recruiting.

Upon treatment with apabetalone, an increase of plaque fibrous tissue was seen, a reduction in fibro-fatty tissue and an increase in plaque calcification; all aspects that suggest stabilization of plaque.

The early experience with increasing doses of apabetalone in patients with CAD treated with a statin, showed a significant, albeit modest dose-dependent increase in ApoA-I and HDL-c. Importantly, robust increases were seen in large HDL-c particles, suggesting that the increased cholesterol efflux capacity observed in preclinical studies, can be translated to statin-treated CAD patients. Moreover, in ASSURE these modest changes translated into a trend towards regression of atheroma volume; changes in atheroma volume were as predicted based on the observed metabolic changes. The ASSURE study also looked into the effect of apabetalone on plaque composition with virtual histology. Upon treatment with apabetalone, an increase of plaque fibrous tissue was seen, a reduction in fibro-fatty tissue and an increase in plaque calcification; all aspects that suggest stabilization of plaque.

Small imaging studies suggest that apabetalone may favorably impact both the size and the composition of plaque.

A later study examined attenuated plaque, which probably reflects a more vulnerable type of plaque, most likely containing more lipid and inflammation. Individuals with and without attenuated plaque do not differ much in respect to clinical features. At ESC 2017, it was presented that patients with attenuated plaque do show higher plaque burden as compared with those without attenuated plaque (atheroma volume: 43.8% vs. 37.5%, P=0.007 and total atheroma volume: 245.0 mm3 vs 193.6 mm3, P=0.005). This suggests that those more vulnerable lesions are also the more bulky lesions. The presented data suggest that apabetalone can reduce features of attenuated plaque on serial observations. Based on this small group of patients, these imaging studies suggest that apabetalone may favorably impact both the size and the composition of the plaque.

Biomarker analyses were also done in these phase II studies. A complementary story unfolds on biomarker effects, that is similar to some of the molecular biology results seen in preclinical studies. For instance, apabetalone reduces alkaline phosphatase activity in a dose-dependent manner in a clinical trial setting, which is thought to be an important parameter of plaque calcification. Moreover, apabetalone was found to reduce levels of osteoprotegerin, another factor important in regulating plaque calcification. Thus, the observed in vitro effects of apabetalone are also seen when humans were treated with it. In various cell models, expression of a range of factors involved in calcification was reduced upon apabetalone treatment. For instance in macrophages, apabetalone appears to have the capacity to reduce the inflammation-driven form of calcification, which occurs early in the atherosclerotic process. This implies that apabetalone has the potential to favorably impact the natural history of plaque, and ultimately CV events.

A downregulation of pathways involved in vascular calcification has also been observed in patients with chronic kidney disease (in a phase I safety and pharmacokinetic study). A single dose administration (100 mg) of apabetalone resulted in downregulation of vascular calcification mediators after 12 hours in these patients. It is also known that apabetalone has favorable effects on vascular inflammation, and it inhibits atherosclerotic disease. In animal models, a reduction is seen in the expression of a range of inflammatory factors that are associated with the formation, progression and ultimately the rupture of atherosclerotic disease. Again, in the clinical studies...
apabetalone reduced a number of inflammatory markers, implying that the findings can be translated from bench to bedside.

Data from these early studies have been pooled, in order to look at early signals regarding CV events. The treatment groups differed slightly, with patients in the apabetalone group more likely to be male, older and to have more evidence of dyslipidemia. The percent changes in biochemical parameters were very similar to that seen in the individual studies. Apabetalone was superior as compared with placebo in terms of raising ApoA-I (6.7% vs. 2.7%, \(P<0.001\)) and HDL-c (6.5% vs. 0%, \(P<0.001\)), as well as the number of total HDL particles (4.8% vs 0.5%, \(P<0.001\)) and large HDL particles (23.3% ± 1.7%, \(P<0.001\)). In addition, hsCRP was significantly more reduced with apabetalone (-21.1% vs -13.3%, \(P=0.04\)), consistent with the reduction in systemic inflammation in apabetalone-treated high-risk patients.

When looking at CV events in pooled data from phase II trials, a lower event rate was seen with apabetalone as compared with the placebo group (44% relative risk reduction, \(P=0.0232\)). Patients with DM showed a greater risk reduction (RRR: 57%, \(P=0.0151\)). Moreover, patients with an elevated CRP level at baseline also show a greater risk reduction (RRR: 62%, \(P=0.0166\)). These data give insight into which patients are more likely to have modifiable risk when treated with this drug. These small, short-term studies were not designed examine these issues, but they give some hope that CV benefits are seen when apabetalone treatment is studied in a larger trial.

Thus far, studies to date showed that apabetalone was well tolerated; across the three studies the most common adverse effects were similar to placebo, and consisted of gastrointestinal disorders and infections. With few exceptions, the adverse events were mild and moderate in severity. The most clinically significant adverse effect was a raise in hepatic ALT/AST levels. The effect of apabetalone on liver transaminases has been studied in detail in the phase II study. The incidence of transaminase elevation >3x ULN is quite stable at 7-8%. No cases of Hy’s law or serious hepatic injury have been reported in over 1000 subjects. Thus, although the transaminase levels increase, no clinical syndrome of liver injury was observed. Moreover, the transaminase elevations resolve rapidly following cessation of the drug. Both early and long-term hepatic safety will be further delineated in longer and larger studies, for instance in the CV outcomes trial that is currently enrolling patients. The BETonMACE evaluates treatment (up to 104 weeks) with apabetalone 200 mg or placebo in addition to standard of care in 2400 subjects at high risk following ACS, after a run-in period of 1-2 weeks with atorvastatin or rosuvastatin. It is an event-based trial that will continue until 250 events have occurred. All patients have T2DM and had a CAD event between 7 and 90 days prior to visit 1, and HDL-c should be <1.04 for males and <1.17 for females. These criteria are based on prior observations, focusing on the population with the possible greatest benefit with this therapy. The primary endpoint will be CV death, non-fatal MI and stroke, and a range of other clinical and biochemical effects will also be assessed throughout the course of the study.

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