Streszczenie w języku angielskim

Effects of empagliflozin therapy on circulating non-coding RNAs associated with sirtuin pathways in patients after myocardial infarction

Myocardial infarction (MI), one of the major clinical manifestations of cardiovascular disease (CVD), leads to permanent myocardial damage, left ventricular remodeling, and consequently to the development of heart failure (HF) of ischemic etiology. One of the key mechanisms responsible for myocardial injury following infarction is ischemia/reperfusion (I/R) injury, which involves a network of molecular processes, including oxidative stress, mitochondrial dysfunction, activation of inflammatory pathways, and cardiomyocyte apoptosis. Among the molecular regulators involved in these processes are sirtuins (SIRT), which belong to the family of nicotinamide adenine dinucleotide (NAD^+)-dependent enzymes. Their role involves the regulation of cellular metabolism and epigenetic mechanisms through effects on oxidative stress, cellular senescence, mitochondrial function, and the modulation of inflammatory pathways activated during myocardial injury.

An increasing body of evidence indicates that gene expression in CVD is extensively regulated by non-coding RNAs (ncRNAs), including microRNAs (miRNAs) and long non-coding RNAs (lncRNAs). These molecules act as post-transcriptional regulators capable of modulating the expression of proteins involved in myocardial injury and cardiac remodeling, and growing evidence indicates that they may contribute to the pathophysiology of CVD.

In parallel, modern pharmacotherapy for cardiometabolic diseases has evolved substantially with the introduction of novel drug classes originally developed for the management of type 2 diabetes (T2D). Sodium-glucose cotransporter 2 (SGLT2) inhibitors have demonstrated significant cardioprotective effects independent of their glucose-lowering properties. Clinical studies have shown that these therapies reduce the risk of CV events and improve outcomes in patients with diabetes and those at high CV risk. However, despite robust clinical evidence, the molecular mechanisms underlying their CV protective effects remain not fully understood; one hypothesis is that SGLT2 inhibitors modulate SIRT pathways. Moreover, interindividual variability in treatment response remains a major challenge in clinical settings. A deeper

understanding of these mechanisms may facilitate the identification of novel therapeutic targets and support the development of personalized treatment strategies.

Therefore, the main aim of my doctoral thesis was to evaluate the role of circulating ncRNAs associated with SIRT pathways in patients after MI treated with SGLT2 inhibitor - empagliflozin, and to investigate their utility as novel predictive biomarkers of drug response. This work integrates clinical, molecular, and bioinformatic approaches to identify the empagliflozin-ncRNA-SIRT axis involved in MI-related processes.

The central part of the thesis consists of an original study analyzing the molecular effects of empagliflozin in patients after MI. In the first stage of the study, miRNAs associated with SIRT pathways were identified using bioinformatic tools. The results of these *in silico* analyses enabled the development of a grant proposal submitted to the PRELUDIUM competition of the Polish National Science Centre, which was funded on the first submission (2022/45/N/NZ7/0246). Subsequently, the expression of top miRNAs and SIRT was validated in plasma samples obtained from patients after MI participating in the randomized clinical trial: *Empagliflozin in acute myocardial infarction: the EMMY trial*, in which patients received empagliflozin or placebo. The expression of selected miRNAs and SIRT1-7 was assessed by quantitative real-time polymerase chain reaction (qRT-PCR) before treatment initiation and after 26 weeks of therapy in 227 patients. After 26 weeks of treatment, decreased SIRT4 expression ($p=0.018$) and increased SIRT6 expression ($p=0.006$) compared with the placebo group were observed. Furthermore, baseline expression levels of SIRT2 and SIRT4, together with miR-182-5p and miR-302a-3p, demonstrated high predictive accuracy as a panel for empagliflozin response, as assessed by changes in left ventricular ejection fraction (AUC: 0.890; 81% sensitivity; 90% specificity). These findings highlight the potential of these biomarkers for stratifying responders and non-responders to empagliflozin in patients after MI. The study also indicates possible epigenetic mechanisms underlying the effects of empagliflozin and highlights the importance of molecular biomarkers in developing personalized therapeutic strategies for CVD.

The second publication is an original bioinformatic study that broadens the molecular context of the dissertation through *in silico* analysis. This study demonstrated that modern cardiometabolic therapies act within partly overlapping regulatory networks involving glucose homeostasis, energy metabolism, inflammatory responses, vascular function, and cellular stress

responses. These findings provide a system-level interpretative background for the observations obtained in the clinical study.

The third part of the thesis consists of a review article focused on the role of ncRNAs regulating SIRT signaling pathways in myocardial I/R injury. This publication summarizes current experimental evidence on the regulation of SIRT by miRNAs and lncRNAs and their impact on key cellular processes involved in I/R. The literature analysis indicates that ncRNAs can modulate multiple biological processes, including apoptosis, oxidative stress, mitochondrial dysfunction, and inflammatory response, which are critical in the pathophysiology of I/R injury, presenting them as possible treatment targets and novel prognostic and diagnostic biomarkers in MI.

In summary, the present dissertation provides novel data on the importance of the ncRNA–SIRT axis in myocardial response to empagliflozin treatment after MI. The obtained results underscore the importance of epigenetic regulatory mechanisms in CVD and point to the potential role of circulating ncRNAs as biomarkers of drug response. This work also shows that integrating molecular, clinical, and bioinformatic research contributes to a better understanding of the complex pathophysiological mechanisms of cardiometabolic diseases and may support the identification of new therapeutic targets and the development of personalized medicine strategies aimed at improving patient prognosis.

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