The transcriptional profile analysis of bone marrow-derived CD34$^+$ stem cell in diabetic patients reveals a primitive signature of their dysfunction

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Objective

Diabetes mellitus (DM) is characterized by chronic elevation of pro-inflammatory mediators that play an important role in the development of micro- and macro-vascular complications typical of DM. The depletion and dysfunction of circulating CD34$^+$ hematopoietic stem cells (HSC) represent an efficient marker of cardiovascular risk in DM. HSC participate in cardiovascular homeostasis and generate different types of blood cells including lymphoid and myeloid cells. A novel paradigm suggests that hyperglycemia might prime the pro-inflammatory state at HSC level in the bone marrow (BM). Here, we aimed at evaluating the impact of DM on transcriptional profile in BM-derivedHSCs.

Methods

BM-derived CD34$^+$ cells were purified from sternal biopsies from patients undergoing coronary bypass surgery with or without DM (7 CAD-DM and 7 CAD patients). Total RNA was sequenced using a minION (OxfordNanopore Technology). The obtained reads were aligned to the human genome, then gene expression quantification was performed by featureCount while the statistical analysis was carried out by the 'limma' R package; functional pathways analysis was executed by the GSEA tool.

Results

We identified 12185 expressed genes whose 75% were protein-coding genes, 22% non-coding RNA and 3% were putative novel genes not yet annotated. Among those differentially expressed, 117 genes were up-regulated (mean logFC > 1.3 and $p < 0.05$) while 278 were down-regulated (mean logFC < -1.3 and $p < 0.05$) in CAD-DM compared with CAD. GSEA analysis revealed that up-regulated genes were mainly related to transcription and translation biological processes while down regulated genes were mainly associated with chemotaxis and immune-inflammatory response. Specifically, CAD-DM CD34$^+$ cells displayed reduced expression of genes regulating antibacterial and antivirus host defense such as FPR2, IRF2 and TLR1 as well as macrophage differentiation and lymphocyte emigration, proliferation and differentiation such as CSF1R, TNFSF13B, RAB27A, ADAM28 and CD276. Surprisingly, this defense mechanism impairment was associated with an intrinsic pro-inflammatory phenotype of the cells that displayed increased expression of inflammatory genes such as IL1β, MCP-1, IL18RAP CD14, NFKB1, TNFRSF10D andADAM17.

Conclusions

DM induces pro-inflammatory transcriptional alterations in HSCs cells suggesting a possible role in the pathobiology of DM complications.

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Do P2Y12 receptor antagonism and NLRP3 inhibition exert additive cardioprotective effects against ischemia/reperfusion injury?

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Introduction

P2Y12 receptor antagonists, including Ticagrelor, are routinely used in patients with acute coronary syndromes. Inhibition of the P2Y12 receptor somehow triggers cardioprotective signaling *via* a platelet-dependent mechanism. Recently, we observed that the NLRP3 inhibitor, INF4E, significantly reduce infarct size in isolated hearts. Here we hypothesized that INF4E can add its protection to that from the Ticagrelor with the involvement of Reperfusion Injury Salvage Kinase (RISK)pathway.

Materials and methods

Ticagrelor (150 mg kg^{-1}) was administered through oral gavage to rats for three consecutive days. At the end of oral treatment, hearts were isolated and subjected to a protocol of ischemia/reperfusion (30min ischemia/60min reperfusion; I/R). In hearts of animals pretreated or not with Ticagrelor, INF4E (50 μM) was infused just before the I/R protocol, as in our previous study. For comparative purpose, isolated hearts were pre-treated with Ticagrelor (3.70 μM) and subjected to I/R. At the end of reperfusion, we assessed infarct size with nitro-blue-tetrazolium technique. We also assessed the expression of NLRP3 and RISK elements by western blot analysis.

Results

Pre-treatment with Ticagrelor significantly reduced infarct size (49 ±3%) when compared to control I/R group (65±3%). Similarly, acute administration of INF4E just before the I/R injury resulted in a significant infarct size reduction (42±6%). The formation of the NLRP3 inflammasome complex was induced by myocardial I/R and attenuated by INF4E acute treatment, not being affected by acute or repeated exposure to Ticagrelor. The beneficial effects induced by either P2Y12 antagonism or NLRP3 inhibition were associated with a marked improvement of the protective RISK pathway. In contrast, no protective effects were recorded when Ticagrelor was administered in the heart before ischemia. No synergist effects were recorded when Ticagrelor was co-administed with INF4E before the induction of ischemia. The acute exposure to INF4E of hearts of animals pretreated with Ticagrelor showed a slight, not statistically significant, additive cardio protective effect.

Discussion

INF4E is protective when administered to blood-free, isolated hearts suggesting that the lethal inflammasome is mainly located in cardiac tissue rather than the blood. On the contrary, Ticagrelor induces cardioprotection when given to the whole animal only, indicating that its direct target is not in the heart. Nevertheless, the co-infusion of the two inhibitors has a little, if any, adjunctive cardioprotective effect. It is likely that we do not see a clear additive effect as both INF4E and Ticagrelor activate RISK pathway.

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