Inhibition of PTP1B Ameliorates Post Diabetic Neuro- and Nephropathy by Regulation of NLRP3/AIM2 Inflammasomes complex in Type 2 Diabetic Mice Model

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ABSTRACT

Type 2 Diabetes Mellitus (T2DM) is a chronic metabolic disease, considered the fastest-growing pandemic of the 21st century. Persistent hyperglycemia-mediated meta-inflammation plays a critical role in the pathophysiology of microvascular complications in T2DM. Among various inflammatory pathways, the hyperactivated NLRP3 and AIM2 inflammasomes are considered the major regulators of metabolic inflammation. Protein tyrosine phosphatase 1B (PTP1B) is involved in the inhibition of insulin signaling and the regulation of inflammation. The development of PTP1B inhibitors is a hot research topic for therapeutic interventions. Our compound (5,7-dihydroxy-3,6-dimethoxy-2-(4-methoxy-3-(3- methyl but- 2-enyl) phenyl)-4H-chromen-4-one) isolated from *Dodonaea viscosa*, is a metabolically active polyphenol and a potent PTP1B inhibitor. This study is designed to elucidate the effect of our PTP1B inhibitor on diabetes-induced chronic low-grade inflammation via regulation of NLRP3-AIM2 inflammasome in microvascular complications of T2DM. To investigate the anti-inflammatory effect of our compound, we developed STZ-HFD induced mice model. We observed alleviated BGL and serum ROS levels in the compound-treated group, confirming the hypoglycemic and antioxidant activity. Tissue morphological analysis further confirmed the retrieval of the normal physiology of the brain and the kidney. RT-qPCR data revealed the anti-inflammatory property through significant reduction of PTP1B, inflammasome complexes, and associated downstream pro-inflammatory cytokines. Moreover, we observed reduced pyroptotic and apoptotic flux respectively, and induction of protective autophagy. Our polyphenolic compound ameliorates diabetic inflammation by targeting Mitochondria and Endoplasmic Reticulum stress-associated NLRP3-AIM2 inflammasome activation.

Keywords: AIM2, Dodonaea viscosa, Inflammasomes, NLRP3, PTP1B, T2DM

INTRODUCTION

Type 2 Diabetes Mellitus (T2DM) is considered the fastest-growing pandemic of the 21st century. It is a chronic metabolic and inflammatory disease (Aggarwal *et al.*, 2022). Persistent nutrient overload-induced meta-inflammation plays a critical role in the pathophysiology of microvascular complications in T2DM. Various studies have focused on the involvement of innate immune responses in the pathophysiology of diabetic complications (Miao *et al.*, 2022). Inflammasomes are key components of these responses. They function as unique sensors for metabolic dysregulations. NLRP3 and AIM2 inflammasomes have been known so far for their involvement in diabetes (Poh *et al.*, 2021). Metabolic DAMPs (such as FFAs, and hyperglycemia) act as a priming signal and activates the NF-κB pathway, which leads to enhanced transcription of components of

NLRP3, AIM2 inflammasomes, whereas activation signal trigger the oligomerization of NLRP3 or AIM2 with ASC and pro-caspase 1, leading to activation of the inflammasome complex (Fusco *et al.,* 2020)(Cañizalez *et al.,* 2018). Besides this, protein tyrosine phosphatase 1B (PTP1B) which belongs to the protein tyrosine phosphatase family, is also involved in diabetic pathophysiology (Teimouri *et al.,*2022). It negatively regulates insulin and leptin signaling through dephosphorylating the tyrosine residues of receptors (Bansal *et al.,* 2021). It can also regulate cytokine signaling through the NF-κB pathway. PTP1B expression can be upregulated by IL-6, IL-1β and *TNF-α,* implicating the role of inflammation in the activation of PTP1B. Our compound isolated from *Dodonaea viscosa* is a metabolically active polyphenol and a potent PTP1B inhibitor (Uddin *et al.,* 2018).

OBJECTIVES

This study was designed to investigate the anti-inflammatory effect of our PTP1B inhibitor on diabetes-induced chronic low-grade inflammation via regulation of NLRP3-AIM2 inflammasome in microvascular complications of T2DM.

METHODOLOGY

To investigate the anti-inflammatory activity of viscosol *in vivo*, we developed a low-dose streptozotocin and HFD-induced mice model. The duration of our model preparation was almost 3 weeks. We selected male mice as an experimental model. All mice were acclimatized for 1 week under standard conditions (27°C with 12 hours of light & dark cycles). All mice were randomly divided into three groups, each containing 3 mice. In Group 1 (control), mice were fed with a standard diet and normal water. On Day 11, saline solution was administered at fasting state. In Group 2 (HFD-STZ-induced diabetic group) and Group 3 (compound-treated group) diabetes was induced. Mice were fed on HFD and low-dose STZ was injected intraperitoneally, at a fasting state. After confirmation of T2DM (BGL>250mg/dl), on Day 11 both glucose water and HFD were replaced with a normal diet and tap water, and mice of Group 3 were then injected with the compound. After critical examination and estimation of fasting BGL, mice were euthanized and dissected for further studies. Blood was collected and serum was separated for assessment of biochemical parameters. Organs were extracted for morphological as well as molecular studies. The serum ROS profile of all the groups was estimated by a high-throughput, and cost-effective D-ROM assay. For histological studies, 10% of formalin-treated tissue sections were processed and stained with hematoxylin and eosin dyes. RNA was extracted, and cDNA was synthesized. For expression analysis of targeted genes, real-time PCR was performed. After RT-qPCR, the relative mRNA expression of the target genes was calculated by using the $2^{-\Delta\Delta CT}$ method.

RESULTS

Viscosol successfully restored the normal body weight of all mice, decreased the BGL and serum ROS levels in the compound-treated group, confirming the hypoglycemic and antioxidant activity. Tissue morphological analysis further confirmed the partial retrieval of the normal physiology of the brain and the kidney. RT-qPCR data revealed the anti-inflammatory property through significant reduction of PTP1B, inflammation regulators (NLRP3-AIM2 inflammasomes), and associated downstream pro-inflammatory cytokines. Moreover, we observed reduced pyroptotic and apoptotic flux respectively, and induction of protective autophagy.

CONCLUSION

PTP1B inhibitor (Viscosol) might show anti-inflammatory activity by reversing the NLRP3/AIM2 mediated inflammation in diabetic neuro and nephropathy thus making viscosol a potent therapeutic drug for the treatment of diabetes-linked pathophysiology.

ACKNOWLEDGMENT

This work was supported by University Research Funding (URF 2019-20) of the Quaid-I-Azam University, Islamabad, Pakistan and Princess Nourah bint Abdulrahman University Researchers Supporting Project number (PNURSP2022R213), Princess Nourah bint Abdulrahman University, Riyadh, Saudi Arabia.

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