ABSTRACT

Nuclear factor erythroid 2-related factor 2 (Nrf2) is a master regulator of the cellular response to oxidative insults by regulating the transcription of a series of antioxidant-related genes. Nrf2 is normally sequestered by Kelch-like ECH-associated protein 1 (Keap1) for targeted proteasomal degradation. Upon stimuli of oxidative stress, Nrf2 gets activated by dissociating from complex and translocates into the nucleus, binds to antioxidant-responsive elements, and induces expression of a battery of detoxification and antioxidant genes.

In addition to its role in combating oxidative stress, we studied the crucial role of Nrf2 by its activator pterostilbene (PTS) on the cytoprotection of pancreatic β-cells against pro-inflammatory cytokine stress. Nrf2 activation potential of PTS was assessed by its nuclear translocation and its associated phosphorylation using western blot. Additionally, promoter driven expressions of ARE-mediated downstream genes were demonstrated via cell-based assays using reporter gene constructs (NQO1-ARE-LUC and GST-ARE-LUC) in pancreatic β-cells. Further, qPCR analysis was performed to study the expression levels of cytoprotective genes in pancreatic β-cells against cytokine stress.

In order to confirm the anti-apoptotic property of PTS against pro-inflammatory cytokine stress, the indicative of early apoptotic events were measured by the binding of Annexin-V using flow cytometry. Further, qPCR demonstrated the anti-apoptotic role of PTS by measuring the ratio of apoptotic proteins and anti-apoptotic, BAX/Bcl-2 and caspase-3 activation. Additionally, PTS also improved insulin stimulatory activity against cytokine exposed, static glucose treated MIN6 cells as assessed by glucose-stimulated insulin secretion assay. Collectively, in vitro studies demonstrated that the Nrf2 activation by PTS as a promising strategy to protect pancreatic β-cells against pro-inflammatory cytokine-induced toxicity.
To investigate the anti-inflammatory effect of PTS in streptozotocin (STZ)-induced diabetic mice through Nrf2 signaling. PTS administration (5 and 10 mg/Kg BW) for six weeks to diabetic mice significantly reduced glucose and improved insulin levels in the circulation. Histopathological examination of pancreatic tissues showed restoration of pathological changes observed in diabetic groups such as shrunken islets and reduction in islet area by PTS treatment. PTS also suppressed both circulatory and pancreatic pro-inflammatory cytokines such as TNFα, IL-1β and IFNγ as well as the levels of pancreatic inflammatory mediator inducible nitric oxide synthase (iNOS) in diabetic animals. In addition, PTS significantly increased the levels of Nrf2 and its target genes in the pancreatic tissues as demonstrated by immunoblot and qPCR analysis.

Further studies on the profiling of proteins in the target organ, pancreas using proteomics approach could provide valuable insight into the molecular mechanisms underlying the effect of PTS against diabetes. The differentially regulated proteins in the pancreas were screened using label-free quantification by LC-MS/MS. We found 504 differentially expressed proteins in pancreas between the diabetes and PTS administered diabetes mice; which are mainly involved in various metabolic processes and particularly predominant regulators for oxidative stress, ER stress, glyoxylate metabolism and several small clusters of chaperones and transporters. The identified protein-protein interaction was studied using STRING database. Importantly, stress sensors such as PARK7, PDIA3, SOD, chaperones and transporters that provoke apoptosis signaling were highly elevated in pancreas of diabetes group and was found to be reduced by PTS treatment. Further, differentially regulated proteins identified by proteomic analysis were validated using immunoblot assay. Collectively, based on this comparative proteomic analysis, it was clear that, PTS attenuates oxidative and ER-stress mediated changes, reduced the precursors for glyoxylate metabolism by up-regulating its detoxifying enzymes such as glyoxylase-I and II in pancreas of STZ-induced diabetes mice.
This study further extend to highlight the importance of Nrf2 and its association with cytokines in newly diagnosed patients with diabetes (DM) among South Indian population. The circulatory levels of Nrf2 were determined in 150 healthy controls and 180 DM patients. The Nrf2 levels were found to be low in patients with DM when compared with controls. Further we observed that circulatory Th1/Th2 and oxidative stress markers were significantly elevated, whereas Nrf2 downstream targets were decreased in peripheral blood mononuclear cells (PBMC) of DM subjects. To further study the association of Nrf2 with cytokines, PBMCs were isolated from controls were exposed to high glucose as well as cytokines and Nrf2 activation potential was measured using immunoblot analysis. The low levels of Nrf2 was observed in PBMC exposed with both high glucose and cytokines when compared with untreated PBMC. Further, the circulatory Nrf2 levels showed a positive correlation with Th2 cytokines and negative correlation to Th1 cytokines, which favors the Th1 dominance. This study identifies Nrf2 play a crucial role in maintaining Th1/Th2 cytokine balance, during the progression of DM.

To conclude, Nrf2 activation by PTS has a multifunctional role in gene regulation in diabetes. This study highlights the anti-diabetic efficacy of PTS by Nrf2-signaling pathway that could be considered as a plausible therapeutic value. Further these findings demonstrate that PTS might be employed as a plausible candidate to protect against the oxidative stress and cytokine stress- induced apoptosis. However, future clinical trials in particular with diabetes patients are warranted to further confirm our findings.