Abstract

Diabetes mellitus (DM) is an endocrine disorder characterized by hyperglycemia, insulin resistance, pancreatic β-cell failure, reduced incretin effect, and is also associated with disturbed circadian rhythm. Glucotoxicity (high glucose) along with lipotoxicity (high free fatty acids) induces nitro-oxidative stress, which activates pro-inflammatory cytokines eventually leading to insulin resistance. Many pro-inflammatory cytokines such as tumor necrosis factor-alpha (*TNF-α*), interleukin 1-beta (*IL1-β*), and also melatonin receptor 1B (*MTNR1B*) genes polymorphisms are found to be associated with increased risk of type 2 diabetes (T2D). Progressive deterioration of β-cell function and mass is a crucial parameter in the development of type 1 diabetes (T1D) and T2D. Therefore, it is pertinent to develop novel therapeutic approaches that could stop or even reverse deterioration of β-cell function and mass. We aimed to investigate the association of *TNF-α* and *MTNR1B* polymorphisms along with plasma TNF-α protein, free fatty acid (FFA) and melatonin levels; and *TNF-α* transcript levels in peripheral blood mononuclear cells (PBMCs), with metabolic profile and T2D risk in Gujarat population. Further, the therapeutic potential of melatonin (M); dipeptidyl peptidase-IV (DPP-IV) inhibitor, sitagliptin (S); and the combination (S+M) treatment was evaluated in T1D and T2D *in vitro* and *in vivo* experimental mouse models, and humanized euglycemic mouse model of islet transplantation.

Our results on population studies suggest that genetic variants of *TNF-α* & *MTNR1B* along with significantly reduced plasma melatonin ($p<0.001$), elevated *TNF-α* transcript ($p<0.001$) and protein levels ($p<0.05$) with a concomitant rise in plasma FFA levels ($p<0.05$) alter metabolic profile which could be a potent risk factor towards T2D in Gujarat population. Our *in vitro* studies suggest that monotherapies and combination therapy significantly increased pancreatic β-cell proliferation under glucotoxicity (S, M, S+M, $p<0.001$) and gluco-lipotoxicity (S, $p<0.05$; M, $p<0.05$; S+M, $p<0.001$). Our *in vivo* studies suggest that monotherapies and combination therapy significantly bring about glucose homeostasis in young (S, $p<0.05$; M, $p<0.05$; S+M, $p<0.01$) and old (S, $p<0.15$; S+M, $p<0.01$) streptozotocin-induced T1D mouse models by inducing β-cell proliferation (young: S+M, $p<0.001$; old: S, S+M, $p<0.001$) and α- to β-cell transdifferentiation (young: S, $p<0.05$, M, S+M, $p<0.001$; old: M, $p<0.001$, S+M, $p<0.01$), besides reducing β-cell apoptosis (young & old: S+M, $p<0.001$). Furthermore, our *in vivo* studies also suggest that monotherapies and combination therapy significantly ameliorated high fat diet (HFD)- induced T2D manifestations by improving metabolic profile (S, M $p<0.01$, S+M, $p<0.001$), glucose and lipid metabolism (S, M, S+M, $p<0.001$), increasing β-cell mass and islet number (S, M, S+M, $p<0.001$), increasing insulin and leptin sensitivity (S+M, $p<0.05$) in the peripheral tissues, and elevating mitochondrial biogenesis (S, M, S+M, $p<0.001$) and respiration (M, $p<0.01$, S+M, $p<0.001$). Combination therapy also increased human β-cell proliferation in humanised mouse model of islet transplantation ($p<0.001$).

In conclusion, *TNF-α* and *MTNR1B* are implicated in the pathogenesis and increased risk of T2D in Gujarat population. Sitagliptin and melatonin combination therapy shows greater therapeutic benefits in ameliorating T1D and T2D manifestations in experimental diabetic *in vitro* & *in vivo* mouse models, and in humanized euglycemic mouse model of islet transplantation. Although the combination therapy is potent in inducing human β-cell proliferation, it is essential to explore the mechanism of action of melatonin in terms of circadian pattern (nocturnal/diurnal), time of administration, and its dosage (acute/chronic) with respect to glucose challenge, for its use in translational research.