ABSTRACT

Background

Obesity, a nutritional disorder, has become one of the major challenges for public health in the 21st century. The serious adverse consequences of obesity are insulin resistance, type-2 diabetes mellitus, non-alcoholic fatty liver disease, cardiovascular complications, osteoarthritis, depression, and also cancer. The fundamental cause of obesity involves an imbalance in the energy homeostasis in the body, basically due to the increased consumption and decreased expenditure of calories, eventually increasing the adiposity. According to the World Health Organization estimates, the prevalence of obesity has tripled in just forty-one years worldwide across all the age groups, reaching pandemic levels. The World Obesity Federation has estimated 2.7 billion adults to be overweight by 2025 from 1.9 billion in 2016. These estimates are alarming because of the decline in the quality of life together with reduced life expectancy due to the serious consequences of obesity. Obesity is a complex multifactorial disorder involving interaction of multiple factors, which includes a calorie-rich diet, inactive lifestyle, hormonal imbalance, genetics, and environment. Due to its progressive increase over the past few decades, it presents a major growing health threat in developed as well as developing countries and has turned out to be a mammoth challenge in the 21st century for the public health.

The expanded energy stores in obesity severely impair the adipose tissue and drive the remodeling of the adipocytes precipitating expansion, low-grade chronic inflammation, and mitochondrial dysfunction in the adipose tissues. This affects the primary function of energy storage in the adipose tissues, and most importantly, the

secondary function of synthesis and release of adipose tissue-specific adipokines. Adipokines are salient signaling molecules mediating the cross-talk between the adipose tissue and other metabolic organs. They regulate different biological processes important for maintaining metabolic homeostasis. With the excessive energy in adipose tissues, the surplus energy is then directed towards the ectopic sites, such as the liver, skeletal muscles, and pancreas, which supplement the disturbance in the existing metabolic homeostasis.

The management of obesity involves lifestyle and behavioral interventions, which are regarded as the keystone of weight reduction, anti-obesity drugs, medical devices, and bariatric surgery. The pharmacotherapy of obesity includes the weight reducing agents, which act by increasing satiety and reducing the food intake, which cannot be an ideal strategy for the treatment of increased weight. Bariatric surgery is costly and recommended for severely obese patients only. In this regard, the other possible mechanism for the management of obesity involves increasing energy expenditure.

5’ adenosine monophosphate-activated protein kinase (AMPK), a serine-threonine kinase, and a heterotrimeric complex is an endogenous and central metabolic sensor. It maintains the cellular energy status at the cellular and whole-body level, by the counter-regulatory mechanisms via switching on the catabolic pathways and switching off the anabolic pathways. Due to its role in energy regulation through the various metabolic pathways, it is recognized as an attractive target for the treatment of obesity and associated type-2 diabetes mellitus, metabolic syndrome, and fatty liver. Also, AMPK alteration occurs in different tissues in obesity, fatty liver, and type-2

diabetes mellitus. Consequently, targeting of AMPK pathway can be of therapeutic advantage in the metabolic complications.

Sitagliptin is an oral hypoglycaemic agent approved by the US-FDA in 2006 for the treatment of type-2 diabetes mellitus. It is commercially available under the brand name Januvia. Its mechanism of action involves increasing the half-life of incretins glucagon-like peptide-1 (GLP-1) and glucagon insulinotropic peptide, by the sustained inhibition of the dipeptidyl peptidase-4 enzyme in the gastrointestinal tract. The incretins mediate insulin release from the pancreatic β-cells and decrease glucagon release from the pancreatic α-cells in a glucose-dependent manner following meal intake. It is either administered as a monotherapy or as add-on therapy with other drugs for the management of type-2 diabetes mellitus. There is no occurrence of hypoglycemic risk with sitagliptin, unlike other anti-diabetic agents. The earlier clinical and pre-clinical studies have highlighted the efficacy of sitagliptin in improving the metabolic complications, including obesity. However, conflicting results are available for them. With this background, the present study is designed to identify sitagliptin for the treatment of obesity, metabolic syndrome, and fatty liver, providing its new indication. Drug repurposing is the identification of an existing and approved drug for a new use, outside the original indication. The repurposing of the approved drug involves lower developmental costs and less time. In addition to this, the pharmacokinetics, side effects, and interactions of the drug are already known because of the existing data of research and development, which could expedite the approval by US-FDA.

Objectives

- To study the effect of Sitagliptin on metabolic syndrome, fatty liver, and hepatic oxidative stress in high-fat diet-induced obese mice model (I).
- To analyze the potency of Sitagliptin on white adipose tissue inflammation, adipocytokine secretion, and hepatic fatty acid metabolism in experimentally induced obese mice with focus on AMPK signaling (II).
- To investigate the effect of Sitagliptin on oxidative stress and mitochondrial biogenesis markers in white and brown adipose tissues in metabolically compromised obese mice (III).

Methodology

The experimental protocol was of sixteen weeks, where the Swiss albino mice were impartially segregated in two groups, one group received control diet (10% fat, 23% protein, and 67% carbohydrate with an energy content of 3.4kcal/g) and normal drinking water (without fructose) (n=6: C group) and other group received high-fat diet (60% fat, 20% protein, and 20% carbohydrate with an energy content of 5kcal/g) and 20% fructose drinking water (HFFW) (n=36) *ad libitum* for eight weeks. After eight weeks, body weight and oral glucose tolerance test (OGTT) were observed, and only those animals with body weight between 11-15% of original and postprandial blood glucose levels between 12mmol/l to 15mmol/l were considered for the further study. Selected animals were segregated and randomly allocated into the five groups (n=6 each): MS group- HFFW only, MET- HFFW and metformin 100mg/kg, MS+SGN10- HFFW and sitagliptin 10mg/kg, MS+SGN20- HFFW and sitagliptin 20mg/kg, and

MS+SGN30- HFFW and sitagliptin 30mg/kg. The respective diets were continued till the sixteenth week. At the end of the experimental protocol, the following parameters were assessed.

- Body weight, organ weight, and cumulative food intake
- Fasting blood glucose (FBG) and OGTT
- Insulin tolerance test (ITT), fasting serum insulin (FSI) and Homeostasis model of insulin resistance (HOMA-IR)
- Serum lipid profile (total cholesterol-TC; triglycerides-TG; high-density lipoprotein-cholesterol-HDL-C; low-density lipoprotein-cholesterol- LDL-C, and very low-density lipoprotein-cholesterol- VLDL-C), alanine transferase (ALT), and alanine aspartate (AST) levels
- Serum leptin and adiponectin
- Serum free fatty acids, uric acid, and GLP-1 level
- Hepatic lipid (TC and TG) and hepatic oxidative stress
- Histopathology of the liver, epididymal white adipose tissue (eWAT), and intercapsular brown adipose tissues (iBAT)
- Interleukin-6 (IL-6), tissue necrosis factor (TNF-α), and monocyte chemoattractant protein-1 (MCP-1) levels in eWAT and iBAT
- Oxidative stress in eWAT and iBAT
- Protein expression of phosphorylated AMPK and acetyl Co-A (ACC) in the liver
- Protein expression of P-AMPK in eWAT and iBAT
- mRNA expression of adiponectin (ADIPOQ) in liver and eWAT
- mRNA expression of carnitine palmitoyltransferase -1A (CPT-1A), fatty acid synthase (FASN), and peroxisome proliferator-activated receptor -α (PPARα) in the liver
- mRNA expression of peroxisome proliferator-activated receptor-gamma coactivator 1-alpha (PPARGC1-A), Nuclear respiratory factor-1 (NRF-1), transcription factor A in mitochondria (TFAM), and UCP-1 (Uncoupling protein-1) in eWAT and iBAT

Results

Objective I

Sixteen weeks of HFFW diet successfully developed the phenotype of obesity, fatty liver and metabolic syndrome in animal models of obesity. Significantly increased body weight was observed in addition to impaired glucose homeostasis at the end of eight weeks of HFFW. Hence the treatment protocol was initiated from the ninth week onwards. In the MS group, we observed significantly elevated levels of FBG, cumulative food intake, and organ weight index. Moreover, OGTT, ITT, FSI, and HOMA-IR were also found to be significantly impaired. However, sitagliptin significantly improved the insulin resistance at all the doses, as evident from the improved FBG, glucose and insulin tolerance tests, FSI, and HOMA-IR. However, we observed a significant decrease in body weight and liver and visceral fat index, with the higher doses of sitagliptin. But no effect of sitagliptin was observed on the cumulative food intake. In the MS group, a high-fat diet precipitated dyslipidemia in addition to increased serum free fatty acids, leptin, adiponectin, uric acid, and GLP-1 levels. Sitagliptin significantly improved TC, TG, HDL-C, LDL-C, and VLDL-C in the treatment groups. Serum free fatty acids, adipokines leptin and adiponectin, uric acid, and GLP-1 were also improved significantly at the higher doses of sitagliptin. There was a significant increase in the hepatic fat content in the MS group, as evident from the increased lipid deposition in the histopathology of the liver, along with the increased hepatic injury markers ALT and AST and also the hepatic oxidative stress. However, sitagliptin significantly decreased the hepatic TG and TC deposition, which was also

observed in the improved morphology of the liver in addition to the reduced ALT and AST levels and oxidative stress in the liver, at the higher doses.

Objective II

Sixteen weeks of HFFW significantly triggered the inflammation in white adipose tissue, followed by impaired adipocytokine secretion and hepatic fatty acid metabolism. The pro-inflammatory cytokines IL-6, TNFα and chemokine MCP-1 were increased significantly in the eWAT in the MS group. However, their levels were ameliorated significantly in the treatment groups at the higher doses of sitagliptin. Furthermore, the hepatic and eWAT mRNA expression of adiponectin in the MS group was significantly reduced, which was improved at the higher doses of sitagliptin. With the HFFW diet, hypertrophy of the adipocytes occurred in the eWAT in the MS group, as evidenced by the histopathology of eWAT. However, the relative number of the large hypertrophic adipocytes was found to be reduced by sitagliptin. There was significant reduction in the hepatic expression of P-AMPK and P-ACC in the MS group. However, higher doses of sitagliptin significantly improved the hepatic P-AMPK and P-ACC in the treatment groups. Followed by this, the hepatic mRNA expression of CPT-1A, FASN, and PPARα was dysregulated in the MS group, which were improved by sitagliptin at the higher doses.

Objective III

With the HFFW diet, a significant increase was observed in the weight of eWAT and iBAT in the MS group, which were reduced with the chronic administration of sitagliptin at the higher doses. Moreover, the HFFW diet precipitated oxidative stress in the MS group in eWAT and iBAT, which was significantly improved by sitagliptin.

Furthermore, the P-AMPK protein expression in eWAT and iBAT was decreased in the MS group, which was improved significantly at the higher dose of sitagliptin. The mRNA expression of thermogenesis and mitochondrial biogenesis markers, PPARα, PGC-1α, NRF-1, TFAM, and UCP-1 were significantly reduced in the eWAT and iBAT after sixteen weeks of the HFFW diet, in the MS group. With the exception of UCP-1, the expression of all other markers were improved significantly in both the eWAT and iBAT, in the sitagliptin treated groups, at the higher dose. While the UCP-1 mRNA expression was significantly improved by the higher dose of sitagliptin in iBAT only.

Conclusion

The findings of the present study suggest the potential effects of the anti-diabetic agent sitagliptin, in the treatment of obesity, fatty liver, and metabolic syndrome in an animal model of obesity. It provides the future roadmap for the treatment of this nutritional pandemic of the 21st century.