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Interleukin-1 receptor antagonist improves normoglycemia and insulin sensitivity in diabetic Goto-Kakizaki-rats

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ABSTRACT

Type 2 diabetes mellitus has been considered as an auto-inflammatory syndrome. Interleukin-1 receptor antagonist (IL-1Ra) has attained considerable attention due to its broad spectrum anti-inflammatory therapeutic effects against various auto-immune diseases. The purpose of our study was to investigate its therapeutic effects of IL-1Ra on none-obese diabetic Goto-Kakizaki (GK) rats. We administered IL-1Ra subcutaneously for one month into GK rats. Insulin sensitivity and β-cell function was calculated by homeostatic model assessment (HOMA) and quantitative insulin sensitivity check index (QUICKI) models. IL-1Ra decreased the onset of hyperglycemia and did not impact the body weight and/or food intake. Insulin tolerance test (ITT) and intraperitoneal glucose tolerance test (IPGTT) results showed that IL-1Ra improved insulin sensitivity and glucose tolerance. The results of HOMA and QUICKI models revealed that IL-1Ra improved insulin sensitivity and β-cell function. Moreover, significant reduction of pro-insulin/insulin ratio and lipid profiles also exhibited significant therapeutic effects of IL-1Ra. Immunohistochemical analysis showed minimal macrophage infiltration in pancreatic islets demonstrating decreased intra-islet inflammation in IL-1Ra treated GK rats. The results of our present study revealed that IL-1Ra has broad spectrum therapeutic potentials but due to its short biological half-life (6–8 h) high doses with frequent dosing intervals are required. Therefore, there is a need for the development of such dosage form that may prolong its half-life via extended release.

1. Introduction

Type 2 diabetes mellitus is characterized as metabolic disorder that is manifested by chronically elevated levels of circulating glucose and lipid profiles, impaired insulin secretion from β-cells of pancreatic islets and increased insulin resistance in peripheral tissues (Maedler et al., 2004; Argoud et al., 2006; Donath et al., 2008; Akash et al., 2011) and last but not least the activation of various pro-inflammatory mediators (Dinarello, 2011; Donath and Shoelson, 2011; Akash et al., 2012a). Chronically elevated levels of glucose may lead to glucotoxicity, which is mostly associated with abnormalities in carbohydrates, lipids and protein metabolism. Alterations of blood lipid profiles may also lead to lipotoxicity, which is characterized by increased levels of triglycerides (TGs), free fatty acids, low density lipoproteins (LDLs), very low density lipoproteins (VLDL) and decreased levels of high density lipoproteins (HDLs) (Akash et al., 2012a). Lipotoxicity usually provokes the dysfunctioning of pancreatic islets (Briaud et al., 2002) whereas, both glucotoxicity and lipotoxicity that are collectively known as glucolipotoxicity are decisively involved to provoke insulin resistance in peripheral tissues and impairs insulin secretion from β-cells of pancreatic islets (Akash et al., 2012a).

Insulin resistance and impaired insulin secretion are the two main causative features that may propagate the pathogenesis of type 2 diabetes mellitus (Song et al., 2007). Pro-inflammatory mediators that are commonly known as pro-inflammatory cytokines and/or chemokines cause inflammation in their respective tissues where they are generated. Various factors are involved to activate the generation of these pro-inflammatory mediators (Dinarello, 2011; Donath and Shoelson, 2011; Akash et al., 2012a, 2013), among them, the most important role is played by hyperglycemia and dyslipidemia. Insulin resistance further increases the circulating levels of glucose and lipids at alarmingly elevated levels and thereafter these nutrients enter into pancreatic islets via systemic circulation and impart their potential cytotoxic effects through the activation of various pro-inflammatory mediators (Dinarello, 2011; Akash et al., 2012a) including IL-1β, IL-6, TNF-α, IL-1-dependent cytokines and chemokines (Ehses et al., 2007, 2010).

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IL-1β is a main causative factor among all pro-inflammatory mediators that induces the secretion of other pro-inflammatory cytokines and chemokines. It potentiates the inflammatory responses in various tissues (Homo-Delarche et al., 2006; Donath et al., 2009; Akash et al., 2013). In pancreatic islets, it causes β-cell dysfunctioning that ultimately leads to impaired insulin secretion (Akash et al., 2012a). In peripheral tissues, it induces inflammation due to which insulin resistance is increased. Hyperglycemia, dyslipidemia and insulin resistance further induce the secretion of IL-1β (Maedler et al., 2004; Boni-Schnetzler et al., 2009) via feedback mechanism. As evident from these inflammatory responses, type 2 diabetes mellitus has been recently considered as an auto-inflammatory syndrome (Donath et al., 2008; Dinarello, 2011) in which various inflammatory mechanisms are determinedly involved to generate inflammation in β-cells of pancreatic islets to induce impaired insulin secretion and insulin resistance (Donath and Shoelson, 2011, Akash et al., 2012a, 2013). Therefore, such therapeutic modalities should be adopted to treat type 2 diabetes mellitus that must possess anti-inflammatory effects against these inflammatory responses. IL-1Ra is the only naturally occurring anti-inflammatory antagonist of IL-1β that have the ability to sufficiently block the binding of IL-1β with interleukin-1 receptor-I (Dinarello, 1996, 2000) without causing any agonistic effects. Recent studies have also proved that IL-1Ra not only blocks the synthesis of IL-1β but it may also has a potential to prevent the synthesis of IL-6 (Lavi et al., 2007; Akash et al., 2012b), lipten, TNF-α, and numerous other IL-1-dependent cytokines and chemokines (Boni-Schnetzler et al., 2009; Lacraz et al., 2009; Ehses et al., 2009).

No sufficient data till now regarding the pharmacokinetic and pharmacodynamic effects of IL-1Ra against type 2 diabetes mellitus is available. The purpose of our present research was to investigate the therapeutic potentials of IL-1Ra on insulin sensitivity and β-cell secretory function using various HOMA and QUICKI models. We also investigated its effects on various lipid profiles and intra-islet inflammatory responses using CD68 as an inflammatory response marker. In our present study, we used Goto-Kakizaki (GK) rats as non-obese spontaneous model for type 2 diabetes mellitus which are characterized by hyperglycemia, tissue-specific insulin resistance, glucose intolerance, hyperinsulinaemia, impaired insulin secretion, β-cell dysfunction and decrease β-cell mass (Portha, 2005). These GK rats also exhibit elevated levels of circulating lipid profiles (Gronholm et al., 2005). As a control, we also used age-matched wistar rats.

2. Materials and methods

2.1. Materials

IL-1Ra was kindly donated by Zhejiang Hisun Pharmaceuticals Co., Ltd. Taizhou China. Glucose was purchased from Sinopharm Chemical reagent Co., China. Glucose kit (Shanghai Rongsheng Biotech Co., China), microplate reader (Model 680, Bio-Rad, Japan), glucometer (AccuChek Active, Roche Diagnostics, USA) were used. All other materials and reagents were at least analytical grade and used without further purification.

2.2. Animals

All experiments were conducted on age-matched male GK and wistar rats in accordance with approved protocols for animal care designed by laboratory of animal centre, Zhejiang University Hangzhou, China. Rats were purchased from Academy of Medical Science Zhejiang Province, China. All animals were kept in cages one week before the start of experiments and fed with water *ad libitum* with standard food in temperature, humidity and light (12 h cycles) controlled rooms. Animal were divided into three groups i.e., Wistar-Saline (treated with Normal saline, $n=5$), GK-Saline (treated with Normal saline, $n=7$) and GK-IL-1Ra (treated with 10 mg/kg IL-1Ra, $n=7$).

2.3. In vivo treatment of IL-1Ra

GK rats exhibit mild hyperglycemia following weaning (4 weeks old). Treatment was started 3 days later after their weaning at 12 h intervals and continued till one month. Blood glucose levels for all groups were measured with glucometer 2–3 times a week at 9–10 am. At the end of treatment, rats were anesthetized with pentobarbital for merciful killing. Whole blood was collected from abdominal vein in a sterile syringe and kept on ice till centrifugation. Serum was separated by centrifugation at $3000 \times g$ for 20 min at 4 °C and stored at −20 °C until further analysis. Pancreatic tissue was picked and preserved in 10% formalin solution till further analysis to perform immunohistochemistry.

2.4. Insulin sensitivity test (ITT)

ITT was performed on day 25 of treatment. Insulin (0.35 U/kg) was administered intraperitoneally after 8 h starvation. Blood was collected from tail before administration of insulin (0 time) to calculate the baseline reading of glucose and then after every 15 min, 30 min and 60 min of injection, blood was also collected to measure the serum levels of glucose.

2.5. Intraperitoneal glucose tolerance test (IPGTT)

IPGTT was carried out at day 28 of treatment. Glucose solution (2 mg/kg) was injected intraperitoneally into animals after an overnight starvation. Blood was collected from tail before administration of glucose solution (0 time) to calculate the baseline reading of glucose and insulin. Then after every 15 min, 30 min, 60 min, and 120 min of injection, blood was collected to measure the serum levels of glucose and insulin.

2.6. Insulin sensitivity and β-cell secretory function determinations

Homeostasis model assessment for insulin resistance (HOMA-IR) [HOMA-IR = Insulin (μU/ml) × Glucose (mmol/L)/2.25] and β-cell function (HOMA-β) [HOMA-β] = 20 × Insulin (μU/ml)/Glucose−3.5] were calculated as described previously (Matthews et al., 1985). Quantitative insulin sensitivity check index (QUICKI) [QUICKI] = 1/[log insulin + log glucose] was calculated as described by Katz et al. (2000). To calculate these parameters, the serum levels of glucose and insulin were estimated under fasting conditions during IPGTT.

2.7. Biochemical analysis

Serum insulin and pro-insulin were analyzed using rat insulin and pro-insulin ELISA kits (Mercodia AB, Sweden) while serum levels of triglycerides, total cholesterol urea nitrogen and uric acid were measured by enzymatic assay (Beckman coulter, USA). Serum creatinine was also measured by enzymatic assay (Autec Diagnostica, Germany).

2.8. Immunohistochemistry

Immunohistochemistry was performed for mouse monoclonal antibody CD68 (Abcam) with isolated pancreatic islets from sacrificed rats. Staining was visualized using peroxidase coupled secondary antibody with subsequent incubation. Antibody-stained surface area