Life Sciences 153 (2016) 180–187

Contents lists available at ScienceDirect

Life Sciences

journal homepage: www.elsevier.com/locate/lifescie

Suppression of connective tissue growth factor mediates the renoprotective effect of Sitagliptin rather than Pioglitazone in type 2 diabetes mellitus

Samia M. Ali [a], Hoda Khalifa [b], Dalia K. Mostafa [a,*], Amal El sharkawy [a]

[a] Department of Clinical Pharmacology, Faculty of Medicine, Alexandria University, Alexandria, Egypt
[b] Department of Histology and cell Biology, Faculty of Medicine, Alexandria University, Alexandria, Egypt

ARTICLE INFO

Article history:
Received 26 January 2016
Received in revised form 17 March 2016
Accepted 23 March 2016
Available online 3 April 2016

Keywords:
Diabetic nephropathy
Sitagliptin
Pioglitazone
CTGF
Metformin
Enalapril

ABSTRACT

Aim: Diabetic nephropathy (DN) is a leading cause of end-stage renal disease, and thus, the ability of antidiabetic drugs to ameliorate renal microvascular disease may be as important as their ability to control blood glucose. Therefore, we investigated the reno-protective effect of the antidiabetic drugs, Sitagliptin and Pioglitazone, versus combined Metformin/Enalapril in a rat model of type 2 diabetes.

Method: Male Wistar rats were randomly assigned to be either normal control or diabetic. Induction of type 2 diabetes was done by intraperitoneal injection of\ low dose streptozotocin (35 mg/kg) on top of 2 weeks of high fat diet. Hyperglycemic animals were divided into 4 groups: untreated diabetic, Sitagliptin (10 mg/kg), Pioglitazone (10 mg/kg) and Metformin/Enalapril (500, 10 mg/kg, respectively) treated. After 6 weeks, fasting blood glucose, plasma insulin, β-cell function, insulin resistance, serum lipids, urea & creatinine, albuminurea, kidney weight, renal oxidative stress, plasma connective tissue growth factor (CTGF) and renal histopathology were assessed.

Key findings: Sitagliptin decreased microalbuminurea, urea & creatinine, renal tropism, oxidative stress and CTGF to levels similar to Metformin/Enalapril group. It also preserved near normal renal histology. Although Pioglitazone treatment reduced urea, creatinine, renal tropism and oxidative stress, it did not improve renal pathological changes or significantly alter CTCF.

Significance: Early Sitagliptin treatment in type 2 diabetes can equally ameliorate renal functions and structural changes as combined Metformin/Enalapril. Moreover Sitagliptin is a better renoprotective than Pioglitazone, probably due to its suppressor effect on CTGF, a key factor mediating diabetic renal injury.

1. Introduction

Diabetic nephropathy (DN) has emerged as the leading cause of end-stage renal disease, and thus, preventing or delaying it, has been a major goal in biomedical research. Early detection of this chronic diabetes mellitus (DM) complication along with the treatment of its main risk factors, and the use of renoprotective drugs may decrease the progression of this kidney disease. In the management of Type 2 DM (occurring in up to 90% of patients with diabetes), the ability of antidiabetic drugs to ameliorate renal microvascular disease might be as important as their ability to control glucose [1,2]. Therefore, the reno-protective effects of antidiabetic drugs in common use constitute a dynamic area of extensive research. Pioglitazone, a high-affinity ligand for peroxisome proliferator-activated-receptors-gamma (PPARs-γ), improves glucose homeostasis by increasing insulin sensitivity and, until recently, it has been widely used in anti-diabetic therapy. While PPARs-γ are predominantly expressed in adipocytes, they are also present in vascular and inflammatory cells, as well as renal glomerular and tubular cells. Now there is a growing body of evidence supporting the role of inflammation, immune activation and lipotoxicity in DN [3].Therefore, PPAR-γ agonists could serve as promising therapeutic agents for controlling the progression of renal disease in patients with diabetes [4].

On the other hand, Sitagliptin is one of the best known incretin enhancers or gliptins, which increases incretin contents due to the inhibition of dipeptidyl peptidase-4 (DPP-4) activity. Previous reports have indicated that a high level of plasma DPP-4 is positively correlated with DM [5,6]. Such reports raise the interest of a possible renoprotective effect of incretin based therapy in diabetic patients.

Accordingly, investigating and comparing the effect of Pioglitazone and Sitagliptin on progress of DN is not only a basic interest, but also may have important clinical implication.

Due to the local activation of the renin angiotensin system (RAS) and/or increased intrarenal sensitivity to angiotensin II in DM, blockade of RAS with angiotensin converting enzyme inhibitors (ACEIs), or angiotensin receptor blockers (ARBs) combined with strict glycemic control is currently the most accepted strategy to combat DN [7]. Enalapril is

* Corresponding author at: Almoassat Medical Campus, Clinical Pharmacology Department, Faculty of Medicine, Alexandria, Egypt.
E-mail address: dalia.kashishy@alexmed.edu.eg (D.K. Mostafa).

http://dx.doi.org/10.1016/j.lfs.2016.03.043
0024-3205/

the most widely used ACEI in clinical trials, therefore it is used in our study in combination with Metformin, the most commonly prescribed oral antidiabetic [8], as a reference treatment to which the possible renoprotective effects of Pioglitazone and Sitagliptin were compared.

To date, albuminuria remains the only biomarker acceptable for diagnosis and evaluation of DN. However, some growth factors like transforming growth factor-β (TGF-β) and vascular endothelial growth factor (VEGF) are expected to replace it in the future. The contribution of TGF-β to the pathology of glomerulosclerosis has been established [9]. However, TGF-β is a pleiotropic cytokine with beneficial anti-inflammatory properties that are not related to fibrosis and scarring. Therefore, identification of causal factors downstream of TGF-β could serve as better therapeutic targets for disease intervention in DN. In recent years, Connective tissue growth factor (CTGF) has been identified as a prosclerotic cytokine induced by and acting downstream of TGF-β mediating its fibrogenic properties [10]. CTGF was shown to be involved in the regulation of cellular chemotaxis, migration, differentiation, and formation of extracellular matrix. Several lines of evidence have implicated CTGF as a possible contributor to the various fibrotic complications observed in diabetes [11]. CTGF was found to be a potent inducer of extracellular matrix accumulation in glomerular mesangial cells (MC). In addition, increased glucose concentration has been shown to induce CTGF mRNA and protein levels in human and rat MC *in-vitro*. Moreover, CTGF has been implicated in the development of glomerulosclerosis and tubulointerstitial injury observed in the renal cortex leading to disease progression in DN [11].

Given these encouraging data, we aimed to investigate the role of CTGF in DN, and to explore whether or not its level can be modified by the drugs under study.

Whereas several animal models have been described to induce DN, we utilized the high-fat diet (HFD)-in Streptozotocin (STZ)-injected rats as a model of type 2 DM and DN. This rat model exhibited both insulin resistance and concomitant ß-cell dysfunction which are the two typical features that discriminate type 2 diabetes [12]. Accordingly, this study evaluated and compared the effects of Pioglitazone and Sitagliptin on renal functional and structural alterations associated with DN and explored the involvement of CTGF in mediating the effects of either drugs.

2. Materials and methods

2.1. Experimental animals

This study was conducted on 60 adult male Wistar rats of body weight ranging from 220 to 250 g. Animals were purchased from the Medical Research Institute, Alexandria University. The rats were housed in animal cages (4 rats/cage) and were kept under standard conditions of light and temperature with free access to food and water ad libitum. The experiments were carried out in accordance with the Code of Ethics of EU Directive 2010/63/EU and are approved by the ethics committee of Alexandria faculty of Medicine.

2.2. Drug administration

STZ (Sigma-Aldrich, USA) was administered as a single intraperitoneal (*i.p*) injection in 0.1 M citrate buffer solution [12]. Drugs: [Sitagliptin (Januvia ®, Merck Sharp and Dohme, Egypt), Pioglitazone (Actos®, Abbott, Egypt), Metformin (Glucophage®, Bristol-Myers Squibb), and Enalapril (Vasotec®, Valeant Pharmaceutical, Inc)] or vehicle were administered by oral gavage, as a single daily dose in the morning for 6 weeks after induction of DM.

2.3. Experimental design

Induction of type 2 DM was done in all animals except for those used for normal control by feeding them with high-fat diet (HFD), 58% calories as fat (Table 1), followed after 2 weeks by *i.p.* injection of low dose STZ (35 mg/kg) [12]. Control rats were fed standard rat diet (SD) and received *i.p.* injection of citrate buffer instead. Induction of type 2 DM was confirmed by measuring blood glucose from retro-orbital blood samples one week after STZ injection. Only hyperglycemic animals with random blood glucose level above 180 mg/dl were further enrolled in the study.

Accordingly, rats were assigned into the following 5 groups of 10 rats each: normal control group, untreated–diabetic group receiving vehicle, Sitagliptin (SG)-treated diabetic group (10 mg/kg/d) [1], Pioglitazone (PIO)-treated diabetic group (10 mg/kg/d) [14]; and finally Metformin/Enalapril (MET/ENAL)-treated diabetic group (300 mg/kg/d and 10 mg/kg/d, respectively) [15,16].

2.4. Experimental procedures

At the end of the 6th week, animals were kept in metabolic cages and 24 hour urine was collected for estimation of albuminuria. Rats were then fasted for 12 hrs, anesthetized and blood samples were collected from the aorta into either EDTA or Becton Dickenson tubes, centrifuged at 2000 rpm. for 10 min. for separation of plasma or serum, respectively. Samples are then aliquoted and stored at −20 °C to be used for assay of the following parameters: Fasting plasma glucose (FPG), Glycosylated hemoglobin (HbA1c), plasma insulin (PI) level, plasma CTGF, serum urea & creatinine, total cholesterol (Total-C) and triglycerides (TGs).

Animal were then euthanized and both kidneys were excised and kidney weight was determined (tropism). One kidney was perfused with phosphate buffered saline (PBS) solution at pH 7.4, to remove any red blood cells and clots. Then, the kidney tissue was homogenized in 10 ml cold buffer (50 mM potassium phosphate)/g tissue. The homogenates were then centrifuged at 4000 rpm for 15 min at 4 °C. The supernatants were removed and frozen at −80 °C for further evaluation of the level of malondialdehyde (MDA) as a marker of oxidative stress. The other kidney was then fixed in 10% Formaldehyde and processed for routine paraffin block preparation. Sections of 5 μmm were stained with Haematoxylin and Eosin (H&E), and periodic acid Schiff (PAS) stains for histopathological examination.

2.5. Biochemical estimates

FPG was determined by the glucose oxidase method as described by Barham and Trinder [17]. HbA1c was determined using the turbidimetric inhibition immunoassay (TINIA) [18]. Quantitative determination of serum Total-C and TGs was done using enzymatic colorimetric methods (N.S. BIOTEC, Wellkang Ltd, UK). Serum urea and creatinine were estimated using diagnostic kits based on the methods of Tomas [19], while albuminuria was determined with fluorescein-labeled antibody with commercially available kit [20]. PI and CTGF were assessed using ELISA kits (Uscn, life science Inc) according to manufacturer instructions. Both FPG and PI were used by Homeostasis model assessment

Table 1
Component of high fat diet and standard diet [13]

Component	High fat diet (HDF) g%	Standard diet (SD) g%
Casein	19.61	17.32
Corn starch	36.24	56.39
Sucrose	10.75	10.34
Cellulose	7	6.16
Butter oil	19	3
Soybean	1	1
Vitamin mixture	1.27	1.27
L-cystine	0.25	0.25
Acetyl choline	0.27	0.27
Cholesterol	0.59	–
Mineral mixture	4	4