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Original article

Cardioprotective effect of concomitant administration of trigonelline and sitagliptin on cardiac biomarkers, lipid levels, electrocardiographic and heamodynamic modulation on cardiomyopathy in diabetic Wistar rats

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ABSTRACT

Aim of the study: The present study was designed to investigate the possible association of concomitant administration of trigonelline (TRIG) and sitagliptin (SITA) on cardiomyopathy in diabetic rats.
Methods: Diabetes was induced in Wistar rats by administration of nicotinamide (NICO; 110 mg/kg) and streptozotocin (STZ; 65 mg/kg) intraperitonelly (i.p.). After confirmation of diabetes, rats were divided into following groups i.e. group 1: non-diabetic rats (ND); Group 2: diabetic rats (DC); Group 3: TRIG (50 mg/kg, p.o.); Group 4: SITA (5 mg/kg, p.o.); Group 5: TRIG + SITA (50 + 5 mg/kg, p.o.). Treatment with selected dose of TRIG and SITA was started from 3rd week after NICO–STZ injection and was continued up to 11th week. Cardiomyopathy was assessed by measuring serum glucose level, enzymatic cardiac markers, electrographic abnormalities, heamodynamic changes, lipid levels and histological examination of isolated heart tissue of treated animals.
Results: NICO–STZ diabetic rats showed extensive hyperglycemia, hyperlipidemia, and elevated levels of enzymatic cardiac markers compared to non-diabetic rats. Concomitant and monotherapy treatment of TRIG and SITA exhibited significant decrease in hyperglycemia as compared to diabetic rats. Whereas TRIG + SITA considerably reduced hyperlipidemia, alteration in levels enzymatic cardiac markers and improvement in cardiac function by improved electrographic abnormalities and heamodynamic changes. Overall, the findings revealed in this investigation demonstrated that concomitant administration of TRIG + SITA can be strong pharmacological therapy as compared to monotherapy used for the treatment of hyperglycemia, hyperlipidemia and cardiac dysfunctions in NICO–STZ-induced cardiomyopathy in Wistar rats.
Conclusion: We conclude that concomitant administration of TROG and SITA showed additive cardioprotective effect compared to monotherapy.

1. Introduction

Type 2 diabetes is a consequence of chronic sustained hyperglycemia, which leads to provoke certain macrovascular complications [1,2]. It has been reported that diabetes affects the heart in 3 ways: coronary artery disease (CAD) due to accelerated atherosclerosis; cardiac autonomic neuropathy (CAN); and diabetic cardiomyopathy (DbCM) [3]. Diabetic cardiomyopathy was first observed around four decades ago [4]. In 1972 Rubler et al. framed the term "diabetic cardiomyopathy" to describe this form of disease of heart muscle in diabetic patients [5,6]. It has been ascribed that diabetic cardiomyopathy is a distinct primary disease characterized by the presence of abnormal myocardial performance or structure in the absence of coronary artery disease, hypertension and significant vascular disease in patients with diabetes [7]. Incidence and prevalence of diabetic cardiomyopathy are growing worldwide, about 65–70% of diabetic people have been died due to cardiac dysfunction, so diabetes emerges as one of the leading consequences of cardiovascular disease. Therefore, cardiac protection is a very perceptive issue in diabetes [8].

Pathphysiology of diabetic cardiomyopathy is still contradictory [9]. Moreover, different mechanistic approaches have been

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proposed to understand mechanism, such as activation of peroxisome proliferator-activated receptor (PPAR) enzyme, formation of advanced glycated end products, activation of protein kinase-A pathway, which are correlated to oxidative stress [10,11]. Additionally it is acknowledged that utilization of fatty acid causes hyperlipidemia, activation of renin–angiotensin–aldosterone system (RAAS) that alters the function of cardiomyocytes, dysregulation of calcium homeostasis, which increases Ca^{2+} concentration in myofilament that may be responsible for cardiac contraction [12,13]. However, it is reported that specific strategy to prevent or treat cardiomyopathy associated with diabetes has not been fully established [14].

Sitagliptin is a dipeptidyl peptidase inhibitor (DPP-4), which alters incretin deficiency [15]. Incretin hormones, glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic peptide (GIP) secreted from ileum, colon and duodenum of intestine in the presence of food [16]. Sitagliptin showed cardioprotective effect in mice through increasing cardiovascular event [17,18]. In preclinical study, it has been reported that sitagliptin improved endothelial dysfunction and lipids levels, reduced oxidative stress, decreased inflammation and platelet aggregation [19]. Trigonelline is a plant alkaloid, which is commonly used to treat diabetes in China [20]. It showed antihyperglycemic effect through regeneration of pancreatic β-cells [21]. Up to date, very few evidences are available on cardioprotective effect of trigonelline in diabetes [22,23].

Nowadays, there is increased use of bioactive constituents isolated from plants in communities. Biologically active constituent, such as alkaloid when administered with prescription medications may show positive health outcome [24]. Currently researchers and physicians are much inclined towards the concomitant drug therapy for treatment of diabetic complications [25].

Previously we have reported that concomitant administration of trigonelline + sitagliptin was effective in diabetic nephropathy in Wistar rats [26]. Furthermore, to date, there is paucity of reports relating to the effect of concomitant administration of trigonelline + sitagliptin on hemodynamic parameters, cardiac biochemical marker and lipid profile in diabetic cardiomyopathy in animals. The objective of present study was to investigate the effect of concomitant administration of trigonelline + sitagliptin on cardiomyopathy in diabetic Wistar rats.

2. Materials and methods

2.1. Drugs and chemicals

Trigonelline (Sigma chemical Co. USA), Sitagliptin (Streptozotocin) [STZ], (Sigma chemical Co. USA) and GOD/POD kit (Acuurex, India) were purchased from respective vendors. All chemicals used were of analytical grade.

2.2. Animals and research protocol approval

Male Wistar rats (200–250 g) were procured from National Toxicological Center, Pune. Animals were housed in room at 24 °C, with relative humidity of 45–55% and 12:12 h dark light cycle. The animals had free access to standard pallets chow (Chakan Oil Mills, Pune, India) throughout experimental protocol, with the exception of overnight fasting before induction of the diabetes, Water was provided *ad libitum*. The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC) constituent under CPCSEA guidelines at Poona College of Pharmacy, Pune, India.

2.3. Preparation of drug solution

Trigonelline and sitagliptin were dissolved in distilled water. Streptozotocin was dissolved in citrate buffer (pH 4.5) and nicotinamide was dissolved in normal physiological saline.

2.4. Experimental design

For the induction of diabetic cardiomyopathy in Wistar rats, we followed previously mentioned protocol [27]. Overnight fasted rats of all groups, except (group1), which was non-diabetic group, were injected with NICO (110 mg/kg, i.p.) then after 15 min, rats were injected with STZ (65 mg/kg, i. p). The rats were received 5% glucose for the next 24 h to prevent hypoglycemia. Hyperglycemia was confirmed after 3 days. Steady state of hyperglycemia was reached after 15 days. Serum glucose level was determined by the glucose oxidase–peroxidase method (GOD/POD). Rats having serum glucose between 350–450 mg/dL were selected for the study. Water and feed were provided *ad libitum*. The body weight and serum glucose levels were measured every week. Diabetic rats weighing 200–250 g body weight were divided into five groups, each group consisting six rats ($n = 6$) as follows:

- group 1: non-diabetic;
- group 2: diabetic;
- group 3: TRIG (50 mg/kg);
- group 4: SITA (5 mg/kg);
- group 5: TRIG (50 mg/kg) + SITA (5 mg/kg).

The rats were allowed to develop diabetic cardiomyopathy for three weeks. The treatment was started from beginning of 4th week and continued until the end of 11th week. The animals from normal and diabetic group received only vehicle (distilled water). Blood withdrawal and biochemical estimation were carried out first then histological examination of isolated hearts were carried out.

2.5. Cardiac markers

Cardiac damage markers viz. creatine kinase (CK-MB), lactate dehydrogenase (LDH), aspartate transaminase (AST) were estimated by the commercially available kits. (Accurex Pvt Ltd, Mumbai, India).

2.6. Effect on lipid profile level

The serum was analyzed for the lipid profile viz. for serum cholesterol, triglycerides, high-density lipoprotein (HDL) and very low-density lipoproteins (VLDL) using kits procured from Accurex Pvt Ltd, Mumbai, India.

2.7. Electrocardiographic and heamodynamic parameters

On last day of study, animals were anaesthetized by urethane (1.25 g/kg, i.p.). Electrocardiographic (ECG) was recorded using 8 channel recording Power Lab System (AD Instruments, LABCHART 7.3 software, Australia). Heamodynamic changes were measured by means of a polyethylene cannula (PE 50) filled with heparinised saline (100 IU/mL) inserted into the right carotid artery. The cannula was connected to a transducer and the signal was amplified. Left ventricular systolic pressure was measured by means of a Millar mikro-tip transducer catheter (Model SRP-320, Millar instrument, INC 320-7051, Houston, Texas 77023-5417) inserted into the left ventricle via the right carotid artery. The left ventricular functions like dP/dt max, dP/dt min and left ventricular end diastolic pressure signals were obtained from primary signals (left ventricular systolic pressure and blood pressure) by means of an acquisition