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Differential effects of low-dose fenofibrate treatment in diabetic rats with early onset nephropathy and established nephropathy

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ABSTRACT

We have previously shown that low-dose fenofibrate treatment has an ability to prevent diabetes-induced nephropathy in rats. We investigated here the comparative pre- and post-treatment effects of low-dose fenofibrate (30 mg/kg/day p.o.) in diabetes-induced onset of nephropathy. Rats were made diabetics by single administration of streptozotocin (STZ, 55 mg/kg i.p.). The development of diabetic nephropathy was assessed biochemically and histologically. Moreover, lipid profile and renal oxidative stress were assessed. Diabetic rats after 8 weeks of STZ-administration developed apparent nephropathy by elevating serum creatinine, blood urea nitrogen and microproteinuria, and inducing glomerular-capsular wall distortion, mesangial expansion and tubular damage and renal oxidative stress. Fenofibrate (30 mg/kg/day p.o., 4 weeks) pretreatment (4 weeks after STZ-administration) markedly prevented diabetes-induced onset of diabetic nephropathy, while the fenofibrate (30 mg/kg/day p.o., 4 weeks) post-treatment (8 weeks after STZ-administration) was less-effective. However, both pre-and post fenofibrate treatments were effective in preventing diabetes-induced renal oxidative stress and lipid alteration in diabetic rats though the pretreatment was slightly more effective. Conversely, both pre-and post fenofibrate treatments did not alter elevated glucose levels in diabetic rats. It may be concluded that diabetes-induced oxidative stress and lipid alteration, in addition to a marked glucose elevation, play a detrimental role in the onset of nephropathy in diabetic rats. The pretreatment with low-dose fenofibrate might be a potential therapeutic approach in preventing the onset of nephropathy in diabetic subjects under the risk of renal disease induction. However, low-dose fenofibrate treatment might not be effective in treating the established nephropathy in diabetic subjects.

1. Introduction

The prevalence of diabetes mellitus is predicted to increase from 285 million at present to about 438 million in the year 2030, with increasing prevalence of this metabolic disorder particularly in Asia (Rossing and de Zeeuw, 2011). The persistent and uncontrolled diabetes mellitus is a major risk factor for the induction of nephropathy (Balakumar et al., 2009a; Luis-Rodríguez et al., 2012; Shelbaya et al., 2012). Diabetic nephropathy is associated with mesangial cell expansion, thickening of glomerular and tubular basement membrane, glomerulosclerosis and tubular necrosis. These structural changes could lead to the occurrence of albuminuria, elevation of serum creatinine and urea nitrogen levels, and reduction in glomerular filtration rate (Fioretto and Mauer, 2007; Kanwar et al., 2008; Balakumar et al., 2009b; Bonakdaran et al., 2011). In spite of effective interventions such as angiotensin converting enzyme inhibitors and angiotensin-II-type 1 (AT_1) receptor blockers available to treat diabetic nephropathy (Hunsicker, 2004; Balakumar et al., 2009c, 2012a), hitherto, no promising interventions are in the practice that could satisfactorily improve the clinical outcomes of diabetic nephropathy. Current treatment protocol for the management of diabetic nephropathy targets for tight glucose and blood pressure control as hyperglycemia and hypertension are major risk factors for the disease progression of nephropathy (Sowers and Epstein, 1995; Zelmanovitz et al., 2009; Long and Dagogo-Jack, 2011). In addition, dyslipidemia has been suggested to be strongly associated with an induction and progression of diabetic nephropathy (Leiter, 2005; Balakumar et al., 2012b). While renal lipid accumulation-induced lipotoxicity could develop diabetic nephropathy (Thompson et al., 2011), peroxisome proliferator-activated receptor α (PPARα) agonists could have a place in the treatment of diabetic nephropathy (Chung and Park, 2011). PPARα plays an essential role in regulating lipid metabolism. PPARα activation by

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fibrates could reduce triglycerides, total cholesterol and low-density lipoprotein levels and consequently elevate high-density lipoproteins (Park et al., 2006; Ansquer et al., 2009; Frazier-Wood et al., in press). Experimental and clinical studies suggested that fenofibrate, a fibrate class of hypolipidemic agent, acts as a PPARα agonist, ameliorated diabetic renal damage by preventing renal oxidative stress, inflammation and fibrosis (Ansquer et al., 2005; Sacks, 2008; Balakumar et al., 2009d; Li et al., 2010; Chung and Park, 2011; Bishnoi et al., 2012). Moreover, we have recently reported that the low-dose fenofibrate therapy in the diabetic rat partially but significantly prevented the development of diabetic nephropathy in rats (Arora et al., 2010). Since diabetes mellitus-induced dyslipidemia might play a key role in the renal disease induction (Chen et al., 2005; Rutledge et al., 2010; Balakumar et al., 2012b), the present study investigated the comparative pre- and post-treatment effects of low-dose fenofibrate in diabetes mellitus-induced nephropathy.

2. Materials and methods

The experimental protocol used in the present study was approved by Institutional Animal Ethics Committee (IAEC) in accordance to the guidelines given by 'Committee for the Purpose of Control and Supervision of Experiments on Animals' (CPCSEA). Wistar albino rats of either sex weighing about 180–250 g were employed in the present study. The rats were acclimatized in the institutional animal house and maintained on rat chow (Ashirwad Industries, Mohali, India) and tap water. Rats were given ad libitum access to food and water. They were exposed to normal day and night cycles.

2.1. Induction of experimental diabetes mellitus

Experimental diabetes mellitus was induced in rats by single administration of streptozotocin (STZ) (55 mg/kg, i.p.) dissolved in freshly prepared citrate buffer (pH 4.5). The blood sugar level was monitored once after 72 h and serum glucose concentration was estimated using the commercially available kit (Transasia Bio-medicals Ltd., Solan, India). The rats showing blood glucose level of greater than 200 mg/dl were selected and named as diabetic rats.

2.2. Assessment of diabetes mellitus

The STZ-induced diabetes mellitus was assessed by estimating serum glucose concentration. The serum glucose level was estimated 72 h after STZ administration by glucose oxidase–peroxidase (GOD-POD) method using the commercially available kit (Transasia Bio-medicals Ltd., Solan, India). At the end of the experimental protocol, the serum glucose level was again estimated. This method is based on the principle that glucose is oxidized to gluconic acid and hydrogen peroxide catalyzed by glucose oxidase. Hydrogen peroxide, thus formed, reacts with 4-hydroxy benzoic acid (4-HBA) and 4-aminoantipyrine (4-AAP) in the presence of peroxidase to form a colored Quinoneimine dye complex whose absorbance was read.

The serum glucose level was calculated using the following formula:

$$\text{Glucose concentration (mg/dl)} = \frac{\text{absorbance of T}}{\text{absorbance of S}} \times 100$$

2.3. Experimental protocol

Five groups were employed in the present study and each group comprised six rats. Fenofibrate was suspended in 0.5% w/v of carboxy methyl cellulose. The pretreatment indicates low-dose fenofibrate treatment in rats with early stage of diabetic nephropathy (4 weeks after STZ administration) while the post-treatment indicates low-dose fenofibrate treatment in rats with established nephropathy (8 weeks after STZ administration). *Group* I, *Normal Control*: rats were maintained on standard food and water and no treatment was given. *Group* II, *Diabetic Control*: rats were administered streptozotocin (55 mg/kg, i.p., once) dissolved in citrate buffer (pH 4.5), and allowed for 8 weeks to develop diabetic nephropathy. *Group* III, *Fenofibrate* per se: the normal rats were administered fenofibrate (30 mg/kg p.o.) for 4 weeks. *Group* IV, *Fenofibrate Pre-treated*: the diabetic rats after 4 weeks of STZ administration were treated with low-dose fenofibrate (30 mg/kg p.o.) for 4 weeks. *Group* V, *Fenofibrate Post-treated*: the diabetic rats after 8 weeks of STZ administration were treated with low-dose fenofibrate (30 mg/kg p.o.) for another 4 weeks.

2.4. Assessment of diabetic nephropathy

The development of diabetic nephropathy, 8 weeks after the administration of STZ (Group II and Group IV) and 12 weeks after the administration of STZ (Group V), was assessed in rats by estimating serum creatinine, blood urea nitrogen and protein in urine using commercially available kits.

2.4.1. Estimation of serum creatinine

The serum creatinine concentration was estimated by alkaline picrate method using the commercially available kit (Angstrom Biotech Pvt. Ltd., Vadodara, India). Briefly, 100 μl serum sample and 100 μl standard creatinine solution (2 mg/dl) were taken separately in glass tubes, which were named as test (T) and standard (S), respectively. The working reagent (1000 μl) containing alkaline picrate solution was added in both tubes, mixed and the reaction temperature was kept at 30 °C. The absorbance of test and standard at 20 s (T_1, S_1) and again at 80 s (T_2, S_2) was noted against blank spectrophotometrically. The formation of a colored complex as a result of a reaction between creatinine present in serum sample and alkaline picrate present in working reagent was measured at 510 nm.

The serum creatinine concentration was calculated using the following formula:

$$\text{Serum creatinine concentration (mg/dl)} = \frac{(T_2 - T_1)}{(S_2 - S_1)} \times 2$$

2.4.2. Estimation of blood urea nitrogen

The blood urea nitrogen was estimated by modified Berthelot method using the commercially available kit (Reckon Diagnostics Pvt Ltd., Vadodara, India). Briefly, 10 μl distilled water, 10 μl standard solution (40 mg/dl) and 10 μl serum sample were taken separately in blank, standard (S) and test (T) glass tubes, respectively. The working enzyme reagent (1000 μl) (containing urease and a mixture of salicylate, hypochlorite and nitroprusside) was added in all glass tubes with thorough mixing. All glass tubes were incubated at 37 °C for 5-min. Then, the working color reagent (1000 μl) (containing alkaline buffer) was added to all glass tubes, and they were again incubated at 37 °C for 5-min. The absorbance of standard and test was noted against blank spectrophotometrically. The principle involved in this estimation follows. Urease breaks down urea into ammonia and carbon dioxide. In alkaline medium, ammonia reacts with hypochlorite and salicylate to form a colored compound, dicarboxyindophenol.