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Fenofibrate and dipyridamole treatments in low-doses either alone or in combination blunted the development of nephropathy in diabetic rats

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ABSTRACT

Low-doses of fenofibrate and dipyridamole have pleiotropic renoprotective actions in diabetic rats. This study investigated their combined effect relative to their individual treatments and lisinopril in rats with diabetic nephropathy. Streptozotocin (55 mg/kg, *i.p.*, once)-administered diabetic rats were allowed for 10 weeks to develop nephropathy. Diabetic rats after 10 weeks developed nephropathy with discernible renal structural and functional changes as assessed in terms of increase in kidney weight to body weight ratio (KW/BW), and elevations of serum creatinine, urea and uric acid, which accompanied with elevated serum triglycerides and decreased high-density lipoproteins. Hematoxylin–eosin, periodic acid Schiff and Masson trichrome staining confirmed renal pathological changes in diabetic rats that included glomerular capsular wall distortion, mesangial cell expansion, glomerular microvascular condensation, tubular damage and degeneration and fibrosis. Low-dose fenofibrate (30 mg/kg, *p.o.*, 4 weeks) and low-dose dipyridamole (20 mg/kg, *p.o.*, 4 weeks) treatment either alone or in combination considerably reduced renal structural and functional abnormalities in diabetic rats, but without affecting the elevated glucose level. Fenofibrate, but not dipyridamole, significantly prevented the lipid alteration and importantly the uric acid elevation in diabetic rats. Lisinopril (5 mg/kg, *p.o.*, 4 weeks, reference compound), prevented the hyperglycemia, lipid alteration and development of diabetic nephropathy. Lipid alteration and uric acid elevation, besides hyperglycemia, could play key roles in the development of nephropathy. Low-doses of fenofibrate and dipyridamole treatment either alone or in combination markedly prevented the diabetes-induced nephropathy. Their combination was as effective as to their individual treatment, but not superior in preventing the development of diabetic nephropathy.

Introduction

Diabetes mellitus is a greatly challenging disease of the 21 century while the prevalence and mortality rate due to this insidious disease continuously upsurge worldwide. Diabetes mellitus is a group of metabolic disorders, resulting in various microvascular and macrovascular complications [1,2]. The chronic and uncontrolled diabetes mellitus may affront with several undesirable complications, including diabetic nephropathy, which is associated with reduced glomerular filtration rate, and elevated serum levels of creatinine, and urea [3–5]. In addition, recent evidences implicate uric acid as a mediator of diabetic nephropathy [6].

Diabetic nephropathy is one of leading causes of morbidity and mortality [7]. Angiotensin-converting enzyme (ACE) inhibitors and angiotensin II-type 1 (AT_1) receptor blockers are employed for the clinical management of diabetic nephropathy [8–10]. However, we still have a dearth of promising pharmacological interventions that could satisfactorily improve the clinical outcomes of patients afflicted with diabetic nephropathy. Current treatment protocol for the management of diabetic nephropathy targets to resist glucose and blood pressure elevation. In addition, dyslipidemia plays an indispensable role in the induction and progression

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of nephropathy in diabetic condition [11,12]. A significant correlation was suggested to be existed between glomerular filtration rate, inflammation and lipid metabolism genes, supporting a possible role of abnormal lipid metabolism in the pathogenesis of diabetic nephropathy [13]. A recent study by Herman-Edelstein et al. [13] suggested that renal lipid metabolism might serve as a target for specific therapies aiming to slow the progression of glomerulosclerosis [13]. Activation of peroxisome proliferator-activated receptor alpha (PPARα) regulates the lipid metabolism. The PPARα activation by fibrates reduces triglycerides, and consequently elevates high-density lipoprotein [14,15]. Experimental and clinical studies evidenced that fenofibrate, a PPARα agonist, has a potential to reduce diabetic renal abnormalities [5,16–20]. We reported that the low-dose fenofibrate therapy in the diabetic rat significantly prevented the development of nephropathy [21]. We have recently shown that the pretreatment with low-dose fenofibrate prevented the onset of nephropathy in diabetic rats [22]. However, the low-dose fenofibrate treatment against the established diabetic nephropathy might not be effective [22].

Dipyridamole is a platelet inhibitor employed clinically for the secondary prevention of transient ischemic attack [23]. It has additional pleiotropic beneficial effects, beyond its role in platelet inhibition, through its anti-inflammatory, anti-proliferative and anti-oxidant properties [24–26]. We have recently reviewed the pleiotropic potentials of dipyridamole, including its renoprotective action [27]. The combination of dipyridamole and aspirin was shown to reduce the incidence of proteinuria in patients with diabetic nephropathy [28]. Likewise, treatment with dipyridamole and aspirin either alone or in combination significantly reduced the proteinuria in patients with diabetic nephropathy [29]. In addition, dipyridamole has been reported to reduce urinary albumin excretion in diabetic patients with microalbuminuria [30]. Dipyridamole at high-dose is considered proischemic, and it causes a marked '*coronary steal*' effect [31,32]. However, dipyridamole at low-dose orally might have a minimal hemodynamic effect [33]. We have recently shown that the low-dose dipyridamole treatment has a therapeutic potential in partially preventing the diabetes mellitus-induced experimental vascular endothelial and renal abnormalities by enhancing endothelial nitric oxide signals and inducing renovascular reduction of oxidative stress [34]. The present study has been designed to investigate the combined effect of low-doses of fenofibrate and dipyridamole in comparison to their individual treatment effects in diabetic rats with nephropathy. In addition, this study compared their combined and individual treatment effects with lisinopril, an ACE inhibitor.

Materials and methods

The experimental protocol used in the present study was approved by the 'Research & Ethics committee', AIMST University (Reference number: AUHAEC 13/FOP/2013). Male Sprague Dawley rats of weighing about 140–185 g were employed in the present study. Rats were acclimatized in the 'AIMST Central Animal House' and maintained on standard rat pellets and tap water. Rats were given *ad libitum* access to food and water. They were exposed to normal day and night cycles.

Induction of experimental diabetes mellitus

Streptozotocin (STZ, 55 mg/kg, *i.p.*, once) dissolved in freshly prepared cold 0.1 M citrate buffer (for 100 mL, added 1.05 g citric acid monohydrate and 1.48 g trisodium citrate dihydrate in distilled water and brought up to 100 mL with distilled water, pH 4.5) was administered to rats to induce experimental diabetes mellitus. The blood sugar level was monitored before and after 72 h of STZ administration. Rats showing blood glucose level of greater than 180 mg/dL were selected and named as diabetic rats. At the end of the experimental protocol, the serum glucose level was again estimated.

Estimation of serum glucose

The serum glucose concentration was estimated using commercially available Reflotron strips employing Reflotron Plus Apparatus (Roche Diagnostics, Germany). After application to the test strip, the serum sample flows into the reaction zone. D-Glucose is oxidized to δ-D-gluconolactone by atmospheric oxygen in the presence of glucose oxidase (GOD). The resulting hydrogen peroxide oxidizes an indicator in presence of peroxidise (POD). The dye formed is directly proportional to the glucose concentration present in the sample.

$$\text{Glucose} + O_2 \xrightarrow{\text{GOD}} \delta\text{-D-gluconolactone} + H_2O_2$$

$$H_2O_2 + \text{Indicator} \xrightarrow{\text{POD}} \text{Dye} + H_2O$$

At a temperature of 37 °C, the dye formed was measured at 642 nm, and the serum glucose concentration was expressed in mg/dL.

Experimental protocol

Seven groups were employed in the present study. Fenofibrate and dipyridamole were suspended in 0.5% carboxymethylcellulose. Lisinopril was dissolved in distilled water. Thirty five out of 42 rats administered STZ (55 mg/kg, *i.p.*, once) developed diabetes mellitus (Normal *vs.* Diabetic rats; 102.28 ± 1.808 *vs.* 330.15 ± 25.308) after 72 h. The diabetic rats were randomly divided into 5 groups (Group 2, Groups 4–7) as shown below.

Group I (*Normal Control*, $n = 6$), rats were maintained on standard food and water, and no treatment was given. Group II (*Diabetic Control*, $n = 6$), rats were administered STZ (55 mg/kg, *i.p.*, once) dissolved in freshly prepared ice cold citrate buffer of pH 4.5, and were allowed for 10 weeks to develop experimental diabetic nephropathy. Group III (*Fenofibrate + Dipyridamole per se*, $n = 6$), normal rats were maintained on standard food and water, and no treatment was given for first 6 weeks. Thereafter, these rats were administered low-dose combination of fenofibrate (30 mg/kg/day, *per os*) and dipyridamole (20 mg/kg/day, *per os*) for 4 weeks. Group IV (*Fenofibrate Treated*, $n = 6$), the diabetic rats after 6 weeks of STZ-administration were treated with low-dose fenofibrate (30 mg/kg/day, *per os*) for the last 4 weeks of the 10 weeks protocol. Group V (*Dipyridamole Treated*, $n = 6$), the diabetic rats after 6 weeks of STZ-administration were treated with low-dose dipyridamole (20 mg/kg/day, *per os*) for the last 4 weeks of the 10 weeks protocol. Group VI (*Fenofibrate and Dipyridamole Treated*, $n = 6$), the diabetic rats after 6 weeks of STZ-administration were treated with low-dose combination of fenofibrate (30 mg/kg/day, *per os*) and dipyridamole (20 mg/kg/day, *per os*) for the last 4 weeks of the 10 weeks protocol. Group VII (*Lisinopril Treated*, $n = 6$), the diabetic rats after 6 weeks of STZ-administration were treated with lisinopril (5 mg/kg/day, *per os*, a standard drug) for the last 4 weeks of the 10 weeks protocol.

Induction of experimental diabetic nephropathy

Rats administered STZ (55 mg/kg *i.p.*, once) were allowed for 10 weeks to develop experimental nephropathy.

Assessment of diabetic nephropathy

The development of diabetic nephropathy, 10 weeks after STZ administration, was assessed in rats by estimating serum creatinine, urea and uric acid using commercially available Reflotron