Fenofibrate, a Peroxisome Proliferator-Activated Receptor α, Improves Myocardial Capillary Density in Diabetic Rats

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Abstract

Introduction: An early manifestation during the development of diabetes is cardiovascular damage. Studies illustrated the potential effects of peroxisome proliferator-activated receptor (PPAR) agonists on reduction of cardiovascular disease. In this study, we tried to delineate a possible effect of fenofibrate, a PPARα agonist, on coronary angiogenesis in diabetic rats.

Methods: Eighteen male Wistar rats were randomly divided into three groups of control, diabetic and diabetic + fenofibrate (100 mg/kg/day) (n = 6 for all groups). Diabetes was induced by a single dose of intraperitoneal streptozotocin (55 mg/kg). After 21 days, capillary density in the myocardial tissue was evaluated by immunohistochemical staining. The results were reported as capillaries per mm^2. Blood samples were taken before and after the induction of diabetes.

Results: Diabetes was associated with reduced myocardial capillary density compared to the control group (121.71 ± 13.32 vs. 153.78 ± 11.08/mm2; $p < 0.05$). Administration of fenofibrate significantly restored angiogenesis in myocardial tissue of diabetic animals (199.98 ± 20.54 vs. 121.71 ± 13.32 /mm^2).

Conclusion: Our results supported the hypothesis regarding a possible beneficial effect of fenofibrate on coronary artery diseases in diabetic subjects.

Keywords: Diabetes, Fenofibrate, Capillary Density (JPMA 62: S-9; 2012).

Introduction

Diabetes mellitus is a complex metabolic disease with adverse multiple clinical effects such as glucose intolerance, insulin resistance, cardiovascular pathologies and endothelial dysfunction.[1] Diabetes global incidence has been estimated to reach 366 million by 2030.[2] There has been increasing evidences that many of clinical effects of diabetes may relate to abnormalities of angiogenesis as compared with euglycemic individuals.[3,4] On one hand, increased angiogenesis has been seen in the retina or kidneys of diabetic subjects, and on the other hand, impaired coronary collateral vessel development has been implicated.[3]

Peroxisome proliferator-activated receptors (PPARs) are ligand-activated transcription factors of steroid receptor superfamily. Three isotypes of PPARs have been identified as PPARα, PPARγ and PPARβ/δ. PPARα is highly expressed in liver, heart, kidney, vascular endothelial cells and skeletal muscles.[5-7] PPARα agonists are known to ameliorate hyperlipidemia, hyperglycemia and vascular endothelial dysfunction in diabetic patients,[9] and improve cardiac function in subjects with diabetes in long term.[10,11] Recently, in vivo and in vitro studies have examined the angiogenic properties of PPARα.[12,13] This study attempted to investigate the role of fenofibrate, a known PPARα agonist, on coronary angiogenesis in a diabetic animal model.

Materials and Methods

Animals:

Eighteen male Wistar rats, weighing 180 ± 50 g, were purchased from Pasteur Institute of Iran. They were housed in an environmentally controlled room in a 12 h/12 h light/dark cycle. The animals were fed a standard rat chow and had free access to water ad libitum. All experimental procedures were approved by the Ethics Committee of Isfahan University of Medical Sciences, Isfahan, Iran.

Experimental protocol:

The animals were randomly divided into three groups of control, diabetic and diabetic + fenofibrate. Diabetes was induced by single intraperitoneal injection of streptozotocin (Sigma Co.) at a dose of 55 mg/kg.[14] After 48 hours, blood glucose levels were measured. The animals with blood glucose concentrations higher than 16.7 mmol/l were considered as diabetic.[15] The control diabetic groups received vehicle while diabetic + fenofibrate received fenofibrate (100 mg/kg/day) by gavage every day. The treatments lasted for 21days.[16]

Blood samples:

Blood samples were taken before and after the experiment. Blood samples were centrifuged at 3000 rpm for 20 minutes and serums were poured in separate eppendorf

tubes. Blood glucose levels were measured by a glucometer. Serum total cholesterol (TC), triglyceride (TG) and high-density lipoprotein cholesterol (HDL-C) levels were determined using calorimetric assay. Low-density lipoprotein cholesterol (LDL-C) was calculated with the Friedewald equation. Serum nitric oxide (NO) concentration was measured by Griess reagent method.

Measurement of capillary density:

After 21 days, the animals were sacrified by cervical dislocation and the left ventricular muscles were immediately dissected and put in 10% formalin overnight. After preparation of 5 µm histological sections, they were deparaffinized and incubated with a rat monoclonal antibody against murine CD31 (Abcam, Cambridge, UK). Then, the counted in ten random microscopic fields (×400) from each tissue preparation were reported as the number of capillaries per square millimeter.[17]

Statistical analysis:

Data was represented as mean ± SE. Statistical comparisons between groups were evaluated by one way analysis of variance (ANOVA). Paired t-test was used for data analysis before and after drug therapy. Bivariate correlations were calculated using Pearson's correlation coefficient. P values less than 0.05 were considered as statistically significant.

Results

Body weight and blood samples:

Table illustrates serum lipid profile, blood glucose and body weight of the experimental groups. Body weight significantly decreased in diabetic rats over time ($p < 0.05$) while the reduction in body weight among diabetic rats that received fenofibrate was insignificant. On the other hand, body weight increased in the control group. Fasting blood glucose level in the diabetic rats was higher than the control group. In addition, administration of fenofibrate failed to reduce blood glucose. While plasma levels of TG significantly decreased, HDL-C levels significantly increased in the diabetic group treated with fenofibrate compared to the untreated group ($p < 0.05$). Serum NO level in the fenofibrate-treated diabetic group was significantly higher than the non-treated group ($p < 0.05$).

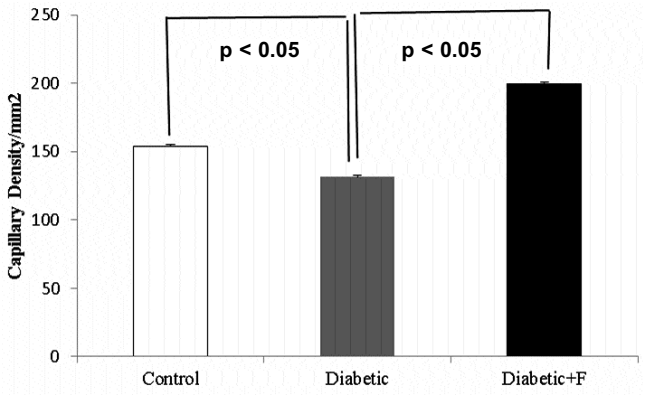

Figure-1: The effects of diabetes and administration of a PPARα agonist (fenofibrate) on myocardial capillary density (n = 6 in each group; F: Fenofibrate).

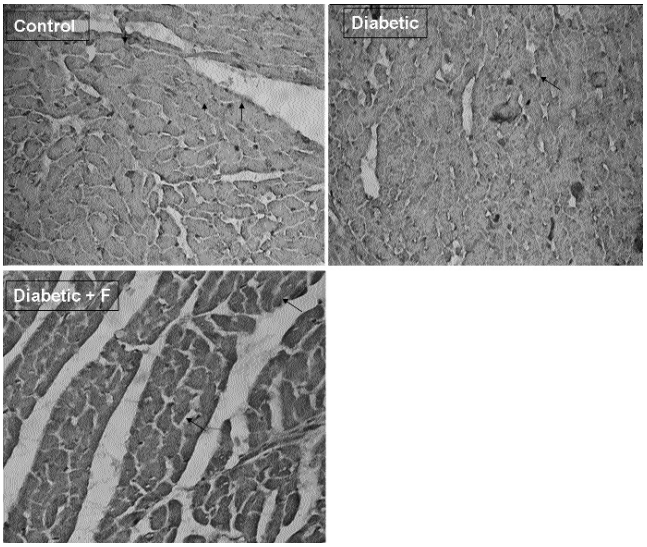

Figure-2: Representative photographs of immunohistochemical staining (×400) with anti-CD31 monoclonal antibody in the three experimental groups (Arrows indicate CD31 positive cells. F: Fenofibrate).

Myocardial capillary density:

Myocardial capillary density in the diabetic group was lower than the control group (121.71 ± 13.32 vs. 153.78 ± 11.08 /mm^2; $p < 0.05$). Administration of fenofibrate

Table: Body weight (g), fasting blood glucose level (mg/dl), serum lipid profile and NO concentrations before and after the experiment.

Group	Control		Diabetic		Diabetic + Fenofibrate	
Days	Day 0	Day 21	Day 0	Day 21	Day 0	Day 21
Body weight (g)	346.65 ± 10.91	345.3 ± 11.07	243.94 ± 3.21	212.42 ± 7.26*	247.83 ± 5.35	225.56 ± 16.03
Fasting Blood Glucose Level (mmol/l)	6.33 ± 0.03	6.53 ± 0.03	6.12 ± 0.03	25.35 ± 0.03	26.39 ± 1.48*	24.53 ± 1.15
Total Cholesterol (mg/dl)	66.00 ± 6.79	84.40 ± 8.74	70.33 ± 5.44	72.25 ± 7.06	69.18 ± 4.46	89.66 ± 7.82
Triglyceride (mg/dl)	93.16 ± 6.72	73.40 ± 5.88	81.80 ± 15.02	71.25 ± 12.37	93.45 ± 7.74	66.33 ± 3.56*
HDL-C (mg/dl)	28.33 ± 4.19	44.66 ± 5.01	26.00 ± 3.55	33.40 ± 2.76	28.00 ± 2.75	42.83 ± 4.51*
LDL-C (mg/dl)	25.56 ± 1.94	28.52 ± 3.66	19.63 ± 2.44	20.76 ± 2.56	22.49 ± 1.94	28.25 ± 1.89
NO (µmol/l)	-	5.8 ± 0.2	-	4.1 ± 0.2	-	9.1 ± 0.9

*: $p < 0.05$ compare to day 0.

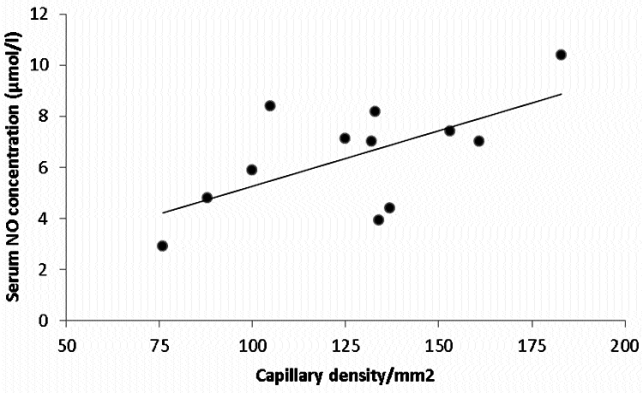

Figure-3: The correlations between capillary density in myocardial tissue and serum nitric oxide (NO) concentration in the fenofibrate-treated group (r = 0.63).

significantly improved myocardial capillary density in diabetic rats (Figure-1). Images of some histological sections of ventricular muscles in all experimental groups are presented in Figure-2.

Correlation analysis:

In the correlation analysis, there was a positive correlation between capillary density in myocardial tissue and serum NO concentrations (r = 0.63) (Figure-3).

Discussion

The present study aimed to determine the impact of diabetes on heart capillary density. It also assessed the efficacy of fenofibrate, a PPARα agonist, administration on glycemic control and myocardial capillary density improvement. It therefore evaluated the glycemic status and capillary density in ventricular muscles of diabetic rats. We found diabetes to be associated with impaired formation of heart collateral vessels. In addition, fenofibrate administration restored heart capillary density without a glucose lowering effect.

Cardiovascular diseases are the main cause of morbidity and mortality in diabetic subjects. One of the suggested mechanisms is defective neovascularization in myocardial tissue under hypoxic condition resulting in reduced myocardial blood flow and increased morbidity.[21] Therapeutic angiogenesis is a novel physiological approach for improving tissue perfusion and clinical consequences in diabetes. Imbalanced growth factors and cytokines and defected signal transduction of vascular endothelial growth factor (VEGF) may be two important mechanisms explaining inadequate angiogenesis.[3] For first time, Abaci et al. declared diabetic subjects to have reduced coronary artery collateral formation compared to non-diabetics.[18] Other studies also suggested the expression of some angiogenic factors such as VEGF and its receptors to decrease in the myocardium of diabetic individuals while production of anti-angiogenic factor (angiostatin) increased.[19,20]

In this study, we also found that fenofibrate could improve collateral vessel formation in heart muscles of a rat model of type 1 diabetes. In recent years, the angiogenic potentials of PPARα agonists have been studied.[1,16,22] Activation of PPARα by fenofibrate and WY14643 (a synthetic PPARα agonist) has been reported to inhibit endothelial cell migration mediating by VEGF via targeting Akt phosphorylation.[23] Furthermore, fenofibrate decreases plasma VEGF in patients with hyperlipidemia and atherosclerosis.[24] In contrast to these observations and in agreement with our results, Biscetti et al. found PPARα agonists to induce angiogenesis indirectly via upregulation of the angiogenic factor VEGF. However, they found that inhibition of VEGF did not completely inhibit the induced angiogenesis.[12] Thus, other angiogenic factors may involve in PPARα stimulated angiogenesis. Similarly, we found increased serum NO concentrations after fenofibrate treatment which suggests a possible mechanism for increased neovascularization. In another study, we found that fenofibrate resulted in ischemia-induced angiogenesis in hindlimb ischemia of diabetic rats via increasing serum NO concentrations (data not published, yet).

Conclusion

In conclusion, our data showed that diabetes is associated with impaired coronary artery collateral formation. We also found the administration of fenofibrate to be able to restore coronary angiogenesis. It therefore can be considered as an bneficial in treatment of diabetic patients with cardiovascular diseases.

Acknowledgment

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