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The effects of PPARα and PPARγ agonists on proteinuria and oxidative stress in patients with type 2 diabetes mellitus.

Zafer Ufuk Cinkara[1], Saime Paydas[2*], Mustafa Balal[2], Ertan Kara[3], Özlem G Öztürk[4], Tamer Inal[4]

[1]Department of Internal Medicine, Faculty of Medicine, Cukurova University, Adana, Turkey

[2]Department of Nephrology, Faculty of Medicine, Cukurova University, Adana, Turkey

[3]Department of Public Health, Faculty of Medicine, Cukurova University, Adana, Turkey

[4]Department of Biochemistry, Faculty of Medicine, Cukurova University, Adana, Turkey

Abstract

Objectives: In diabetes mellitus, renal-cardiovascular complications are important public health problems. We aimed to evaluate the effect of pioglitazone and fenofibrate, as PPAR agonists, on proteinuria and oxidative stress in diabetic patients.

Patients and methods: 60 type 2 diabetic patients were included in this study. 15 patients with HbA1c<7 and triglyceride<350 mg/dl comprised GA. Pioglitazone was added to the therapy in 15 patients with HbA1c>7 and triglyceride<350 mg/dl (GB). In GC, patients with triglyceride>350 mg/dl and HbA1c<7, fenofibrate was added to their therapy. Pioglitazone and fenofibrate were added to the therapy for 15 patients with HbA1c>7 and triglyceride>350 mg/dl (GD). Biochemical tests, serum total oxidative status, paraoxonase-1 enzyme activity, C-reactive protein (CRP), brain natriuretic peptide (BNP), and spot urine protein/creatinine were measured at baseline (1), the 6th week (2) and the 12th week (3). The glomerular filtration rate (GFR) was also calculated.

Results: In GB and GD, glucose (1) and HbA1c (1) were higher than glucose (2,3) and HbA1c (2,3) ($p<0.05$ for all). In GC and GD, triglycerides (1) were higher than triglycerides (2,3) ($p<0.05$ for all). Proteinuria, blood pressure, GFR, BNP, CRP, total oxidant status and paraoxonase-1 enzyme activity were not changed with pioglitazone and/or fenofibrate treatment.

Conclusion: In contrast to blood glucose/triglyceride levels, pioglitazone and fenofibrate alone or in combination did not alter proteinuria, BNP, GFR, CRP, TOS or PON-1 enzyme activity during a period of 12 weeks in diabetic patients with different glucose or triglyceride levels. This could be related to the short study period and limited patient number.

Keywords: Diabetes mellitus, Paraxonase-1, Total oxidant status, Pioglitazone, Fenofibrate.

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Introduction

Thiazolidinedione's and fibrate derivatives used for diabetes mellitus act on the family of peroxisome proliferator receptors. This family of receptors have beneficial effects on the anti-inflammatory and antioxidant systems, in addition to effects on lipid and carbohydrate metabolism.

Intracellular antioxidant enzymes include superoxide dismutase, catalase, glutathione peroxidase, paraoxonase, glutathione-S transferase and aldehyde dehydrogenase [1]. Pathogens are removed by the immune system via the total effects of free oxygen radicals. Some of these radicals are superoxide dismutase, nitric oxide and peroxynitrite, which are reactive products of these radicals [2]. Total oxidant status represents the effect of total oxidative stress in plasma and body fluid composition [3,4]. It has been found that paraoxonase enzyme activity (PON-1) is low in type 1 and 2 diabetes mellitus (DM) and also in haemodialysis patients. Low PON-1activity is related to insulin resistance and to high levels of serum cholesterol and inflammation [5-8].

In this study, we evaluated the effect of pioglitazone and/or fenofibrate on serum glucose control and/or triglycerides, blood pressure, serum glucose, serum lipids; renal functions, proteinuria, brain natriuretic peptide and oxidative stress in patients with type 2 diabetes mellitus.

Materials and Methods

Sixty patients with type 2 diabetes mellitus participated in the study and were divided into four groups. Group A: control group of type 2 diabetes mellitus patients with good serum glucose and lipid levels. Group B: 15 patients with type 2

diabetes mellitus with HbA1c>7% and non-dyslipidaemia and using pioglitazone for the first time. Group C: 15 patients with type 2 diabetes mellitus that had HbA1c<7% and serum triglyceride >350 mg/dl and were using fenofibrate for the first time. Group D: 15 patients with type 2 diabetes mellitus that had HbA1c>7% and serum triglyceride>350 mg/dl and were using fenofibrate and pioglitazone for the first time. History and physical examination findings of all patients were recorded. Body mass index was calculated with the weight (kg)/height (meter)^2 formula and glomerular filtration rate was calculated with Cocroft Gault, MDRD and CKD-EPI formulas. Proteinuria was calculated as the protein/creatinine ratio in morning spot urine. Total blood count, serum glucose, HDL, LDL, triglyceride, CRP, BNP, PON-1 and total oxidant status were measured at the beginning of the study, at the 6th week and at the 12th week for all patients. Biochemical tests were measured with a Roche modular DPP device. HbA1c was evaluated with a COBAS INEGRA 800 device. BNP was evaluated with an electrochemiluminescence assay using a COBAS 400-1 device. Urinary protein/ creatinine ratio was detected by immunoturbidimetric assay using a Beckman DXC-800 device. Total oxidant status and paraoxonase enzyme activity were detected from serum using a flow metric method. This study was funded as a Cukurova University Scientific Research Project (Project number=TF2013LTP14) and approved by the local ethics committee.

Statistical analysis

SPSS 20.0 package program was used for analysis of data. To evaluate differences between groups, the significance level was considered as 0.05.

Results

Mean age of the patients was 49.58 ± 9.5 years. Body mass index, age and sex are shown in Table 1.

There were statistically significant differences between groups A and B for baseline glucose (p=0.008) and baseline and 6th week HbA1c (p=0.005 and p=0.015). Baseline values of HbA1c, triglyceride and total cholesterol were significantly higher in group C than in group A (p<0.05 for all).

Compared to Group A, in group D baseline values of glucose (p=0.02), HbA1c (p=0.000), triglyceride (p=0.00), and total cholesterol (p=0.006) were higher.

At the 6th week, HbA1c and triglycerides were lower in group A than in groups C and D. The 6th week levels of PON-1 and total cholesterol were lower in group A than in group D (p=0.05 and p=0.05).

At the 12th week, there were statistically significant differences between groups A and D for values of HDL (p=0.005) and HbA1c (p=0.025) and between groups A and C for triglycerides (p=0.004).

Table 1. *Demographic characteristics of the patients.*

	A	B	C	D
Groups	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Body Mass Index	28.77 ± 6.33	30.58 ± 6.82	31.17 ± 5.32	31.15 ± 6.26
Age	53.60 ± 2.75	49.07 ± 2.17	47.87 ± 2.31	47.80 ± 2.54
Male/female	4-Nov	8-Jul	7-Aug	8-Jul

For all groups, blood pressure, serum lipids, serum glucose, HbA1c, BNP, total oxidant status, PON-1 and GFR at the beginning, 6th and 12th weeks of the study are shown in Tables 2 and 3.

Table 2. *Blood pressure (BP), serum lipids, serum glucose, HbA1c, BNP, total oxidant status, PON-1 and glomerular filtration rate (GFR) at the baseline of the study (1), 6th (2) and 12th (3) weeks.*

Groups	A	B	C	D
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Systolic BP mmHg	120 ± 17	122 ± 6.76 **II**	113.67 ± 8.55	115.33 ± 10.6
	116.67 ± 12.91	117.33 ± 8.83	116.67 ± 9.75	117.33 ± 5.93
	120 ± 8.43	114 ± 8,28 II	114.67 ± 11.25	14.67 ± 9.90
Diastolic BP mmHg	78 ± 8.61	77.33 ± 4.57	73.33 ± 8.16 **M**	70.33 ± 8.16
	77.67 ± 4.16	79.33 ± 7.03	78.56 ± 5.6 **M**	75.33 ± 5.16
	78 ± 5.60	76.67 ± 7.23	75.33 ± 6.39	74.67 ± 6.39
Glucose mg/dl	131.07 ± 38.68	171.40 ± 39.02 **I,II**	172.93 ± 72.75	182.80 ± 75.17 **E F**
	131.33 ± 33.16	141.47 ± 34.59 **I**	154.93 ± 54.58	130.60 ± 28.19 **E**
	137.20 ± 47.243	134.47 ± 25.80 **II**	164.07 ± 58.61	133.13 ± 25.02 **F**

HbA1c	6.39 ± 0.84 **X**	8.07 ± 0.69 **I, II**	7.35 ± 1.59 **M**	8.76 ± 1.39 **E G**
	6.05 ± 0.69 **X**	6.83 ± 0.93 **I**	6.87 ± 1.44 **M**	7.65 ± 1.17 **G E F**
	6.3 ± 0.83	6.49 ± 0.55 **II**	6.87 ± 1.2	6.9 ± 0.6 F **G**
PON-1 U/lL	30.57 ± 31.07	34.27 ± 26.61	41.6 ± 37.09	62.27 ± 55.75
	30.53 ± 25.06	38.53 ± 30.87	45 ± 38.5	60.13 ± 51.96
	35.67 ± 29.28	41.27 ± 35	43.07 ± 34.59	60.27 ± 51.91
TOS µmol/l	3.97 ± 2.23	3.17 ± 1.89	2.07 ± 2.2	1.34 ± 1.64 **E**
	3.81 ± 1.9	3.74 ± 1.84	2.99 ± 1.91	2.59 ± 2.31 **E**
	3.4 ± 2.04	3.7 ± 2.42	3.37 ± 2	2.55 ± 2.78
Proteinuria mg/day	665.07 ± 88.88	78.41 ± 73	248.20 ± 91	118.78 ± 82
	428.07 ± 138	83.49 ± 79	279.07 ± 142	102.75 ± 105
	492.96 ± 105	90.08 ± 76	229.67 ± 100	114.86 ± 112
BNP pg/ml	71.99 ± 104.72	39.04 ± 35.67	65.28 ± 72.76	36.48 ± 43.79
	68.43 ± 87.61	48.,85 ± 35.38	77.82 ± 74.88	50.32 ± 36,67
	66.1 ± 75.7	61 ± 53.69	59.74 ± 64.25	43.61 ± 47.81
CRP mg/dl	0.85 ± 1.40	0.50 ± 0.33	0.68 ± 0.29	0.7 ± 0.43
	0.95 ± 1.65	0.46 ± 0.46	0.68 ± 0.33	0.59 ± 0.44
	0.53 ± 0.29	0.52 ± 0.46	0.86 ± 0.81	0.71 ± 0.68
LDL mg/dl	104.67 ± 19.01	113.07 ± 32.57	95.67 ± 32.72	102.67 ± 89.06
	109.87 ± 38.05	118 ± 27.78	116.53 ± 35.35	110.67 ± 418
	125.07 ± 45.75	125.80 ± 41.7 **1**	114 ± 38.91	99.67 ± 29.85
HDL mg/dl	39.18 ± 6.47	43.8 ± 10.4	39.23 ± 7.84	38.71 ± 7.33
	38.12 ± 6.82	42.90 ± 10.79	37.15 ± 8.07	38.41 ± 8.18
	35.47 ± 7.04	44.59 ± 9.09	37.36 ± 6.89	36.93 ± 4.89
Triglyceride mg/dl	158.93 ± 57.65	155.93 ± 70.22 **II**	445.07 ± 130.57 **M N**	700.67 ± 482.42 **E**
	155.53 ± 78.67	129.47 ± 44.39	262.47 ± 99.96 **M**	313.27 ± 162.27 **E G**
	153.33 ± 83.94	117.73 ± 48.14 **II**	264.33 ± 108.67 **N**	300.27 ± 145.86 **G**

Group-A: X (comparison of 1with to 2), Y (comparison of 1with to 3), Z (comparison of 2 with to 3)
Group-B: I (comparison of 1with to 2), II (comparison of 1with to 3), III (comparison of 2 with to 3)
Group-C: M (comparison of 1with to 2), N (comparison of 1with to 3), P (comparison of 2 with to 3) Group-D: E (comparison of 1with to 2), F (comparison of 1with to 3), G (comparison of 2with to 3) X, Y, Z, I, II, III ,M ,N, P, E ,F, G p<0.05

Table 3. *Glomerular filtration rate (GFR) at the baseline (1) 6th, (2) and 12th (3) weeks of the study.*

Group	GFR	1	2	3
A	Cockroft gault	99.093 ± 40.75	106.087 ± 46.36	101.287 ± 34.19
	MDRD	98.907 ± 26.03	101.340 ± 28.29	99.987 ± 29.98
	CKD-EPI	94.713 ± 20.19	96.033 ± 20.05	92.880 ± 20.99
B	Cockroft gault	141.587 ± 56.20	133.873 ± 51.34	134.167 ± 57.72
	MDRD	130.427 ± 27.97	121.867 ± 17.55	121.087 ± 23.76
	CKD-EPİ	110.633 ± 11.25	109.847 ± 8.32	107.433 ± 11.99
C	Cockroft gault	110.020 ± 61.65	120.480 ± 88.19	100.180 ± 51.86
	MDRD	121.980 ± 35.24	107.700 ± 22.11	110.820 ± 28.64
	CKD-EPİ	104.987 ± 19.29	102.967 ± 17.25	102.660 ± 17.96
D	Cockroft gault	140.767 ± 63.85*	127.980 ± 49.31*	125.900 ± 37.50
	MDRD	110.167 ± 42.90*	99.347 ± 32.93*	98.060 ± 23.86
	CKD-EPİ	100.160 ± 19.62*,**	94.927 ± 19.70*	97.320 ± 17.82**

*Significant decreases at 6th week compared to baseline levels p<0.05,
**Significant decreases at 12th week compared to baseline levels p=0.043

Discussion

Diabetes mellitus and its complications are gradually increasing in the population. Studies that investigated the relationship of diabetes mellitus and its complications to reactive oxygen species have emphasized the role of non-enzymatic glycosylation, metabolic stress, and activation of the sorbitol pathway, ischemia-reperfusion and impairment of the anti-oxidant system [9]. Since oxidative stress is considered to be an important risk factor for developing diabetes mellitus and its complications, new strategies could be developed for the treatment of diabetes mellitus and its complications. Fenofibrate and pioglitazone may be preferred for treatment since they have anti-oxidant properties in cellular metabolism via PPAR receptors.

Most studies have shown that serum PON-1 enzyme activity in diabetic patients is significantly lower than in control groups [10,11]. Paragh et al. have determined that fenofibrate treatment increased PON-1 enzyme activity and anti-oxidant capacity in patients with coronary artery disease and type 2 diabetes mellitus [12]. In contrast to this study, Beltowski et al. established that dose dependent fenofibrate treatment caused decreased PON-1 enzyme activity by approximately 20-40 per cent in rats [13]. In a randomized clinical study, Hossein et al. did not find any change in oxidative stress and PON-1 enzyme activity with pioglitazone and metformin treatment, as in our study [14]. In our study, PON-1 enzyme activity increased in group B (receiving pioglitazone) but this change was not statistically significant when compared to baseline values. PON-1 enzyme activity also showed no significant change with fenofibrate (Group C) and fenofibrate and pioglitazone combination (Group D).

Aslan at al. reported that oxidative stress was strongly increased in diabetic nephropathies and also that oxidative stress was related to micro albuminuria in comparison to diabetic patients who did not have diabetic nephropathies [15]. Interestingly, in our study total oxidant status was significantly lower at baseline and at the 6th week in patients with higher levels of glucose, HbA1c, and triglycerides (Group D) compared to patients with good glucose and lipid levels (group A). Contrary to expectations, treatment increased the total oxidant activity when we expected a reduction in total oxidant activity. There was no significant difference between baseline and 12th week values. This finding was similar to other studies. However, we think that this situation may be related to the short treatment period.

In our study, proteinuria was not altered at 12 weeks by fenofibrate treatment, in contrast to other reports [16,17]. It has been shown that hyperlipidaemia is a significant and independent risk factor for diabetic nephropathies, as well as other important risk factors [18]. PPARα agonists decrease serum lipids by inhibition of hepatic fatty acid synthesis. Decreased serum lipids have a protective effect on renal damage by the inhibition of renal lipid deposition, lipo-toxicity, renal inflammation and oxidative stress [19]. DAIS, FIELD and ACCORD studies showed that long term treatment with fenofibrate had a renal protective effect and caused decreased micro albuminuria [17,20,21]. We did not find significant differences in proteinuria for all patients. This finding may be related to the short treatment period or the low level of proteinuria at the beginning of the study for all patients. Also, there was no significant difference in GFR or serum creatinine in all groups. In patients with higher glucose and HbA1c levels (group B), pioglitazone decreased blood glucose and HbA1c levels significantly at the 6 and 12 weeks when compared to the beginning of the study. The beneficial effect achieved at the 6th week for blood glucose and HbA1c persisted to the 12th week but no additional improvement was observed. Fenofibrate caused a reduction in HbA1c in the first 6 weeks only in group C. But there was no significant effect on serum glucose levels. We think that this finding may be related to the use of other anti-hyperglycaemic drugs, diet or life style modification. In group D (receiving pioglitazone and fenofibrate) serum glucose and HbA1c decreased significantly at the 6th and 12th weeks. Also, serum glucose and HbA1c levels at the 12th week decreased significantly compared to the 6th week. This finding represents an expected effect of both drugs. Susumu et al. investigated the effect of pioglitazone on BNP and ANP. They found that BNP was a useful marker to follow up left ventricular dysfunction in patients taking pioglitazone [22]. In our study, BNP levels in group B were non-significantly lower at the beginning and at the 12th week compared to the control group (P=0.065). During the study period, BNP levels increased within the normal range. This situation may be related to the effect of pioglitazone of impairing water excretion. In our study there was no change in blood pressure. Total cholesterol and triglycerides in group B (receiving pioglitazone), group C (receiving fenofibrate) and group D (receiving pioglitazone and fenofibrate) decreased significantly at the 12th week compared to the values at the beginning of the study. This finding represents the beneficial effect of fenofibrate and pioglitazone on serum lipids. However, there were no significant differences for high density lipoprotein and low density lipoprotein during the study period. In conclusion, it is interesting that in patients with higher levels of glucose and triglyceride, PON-1 was higher at the 6th week than in patients with good levels of glucose and triglyceride. Fenofibrate and pioglitazone decreased HbA1c and triglyceride levels at the 6th and 12th weeks of treatment without adverse effects in diabetic patients. In contrast to some reports, we did not observe significant differences with/without PPARϒ and PPARα agonists for proteinuria, BNP, GFR, CRP, TOS and PON-1 enzyme activity during the 12 weeks, though this could be related to the short study period and the limited number of patients.

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*Correspondence to

Saime Paydas

Department of Internal Medicine Nephrology

Cukurova University

Adana

Turkey