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Short-term gemfibrozil treatment reverses lipid profile and peroxidation but does not alter blood glucose and tissue antioxidant enzymes in chronically diabetic rats

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Abstract

In this study, we investigated the efficiency of short-term treatment with gemfibrozil in the reversal of diabetes-induced changes on carbohydrate and lipid metabolism, and antioxidant status of aorta. Diabetes was induced by a single injection of streptozotocin (45 mg/kg, i.p.). After 12 weeks of induction of diabetes, the control and diabetic rats were orally gavaged daily with a dosing vehicle alone or with 100 mg/kg of gemfibrozil for 2 weeks. At 14 weeks, there was a significant increase in blood glucose, plasma cholesterol and triglyceride levels of untreated-diabetic animals. Diabetes was associated with a significant increase in thiobarbituric acid reactive substances (TBARS) in both plasma and aortic homogenates, indicating increased lipid peroxidation. Diabetes caused an increase in vascular antioxidant enzyme activity, catalase, indicating existence of excess hydrogen peroxide (H_2O_2). However, superoxide dismutase (SOD) and glutathione peroxidase (GSHPx) activities in aortas did not significantly change in untreated-diabetic rats. In diabetic plus gemfibrozil group both plasma lipids and lipid peroxides showed a significant recovery. Gemfibrozil treatment had no effect on blood glucose, plasma insulin and vessel antioxidant enzyme activity of diabetic animals. Our findings suggest that the beneficial effect of short-term gemfibrozil treatment in reducing lipid peroxidation in diabetic animals does not depend on a change of glucose metabolism and antioxidant status of aorta, but this may be attributed to its decreasing effect on circulating lipids. The ability of short-term gemfibrozil treatment to recovery of metabolism and peroxidation of lipids may be an effective strategy to minimize increased oxidative stress in diabetic plasma and vasculature. (Mol Cell Biochem **216**: 59–63, 2001)

Key words: gemfibrozil, streptozotocin-diabetes, aorta, superoxide dismutase, catalase, glutathione peroxidase, lipid peroxidation, hyperlipidemia

Introduction

Lipid peroxidation has been implicated in the pathogenesis of many degenerative disorders including naturally occurring and chemically induced diabetes [1, 2]. Oxidation of lipids in plasma lipoproteins and in cellular membranes is associated with increased oxidative stress leading to the development of cardiovascular disease in diabetes [1, 3]. Accordingly, we have previously observed that increased free radical production is responsible for abnormalities in both vasomotor activity and ultrastructural organisation of aorta in experimental diabetes [4–6]. Under normal conditions, antioxidant enzymes, catalase, glutathione peroxidase (GSHPx) and superoxide dismutase (SOD), offer protection to cell and tissues against oxidative injury [7]. If the balance between the formation of reactive oxygen species and their elimination

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by endogenous antioxidant defense systems is impaired, oxidative damage of the membrane and changes in the structural and functional integrity of subcellular organelles can occur. Gemfibrozil is most widely used fibric acid, an effective cholesterol- and triglyceride-lowering agent, which has been shown to be beneficial in the treatment of atherosclerosis [8]. It has been reported that gemfibrozil renders low-density lipoprotein (LDL) less susceptible to oxidative modification [9], decreases the rate of LDL oxidation *in vivo* and *in vitro* [10, 11], potentiates fibrinolysis [12], and increases the levels of high-density lipoprotein cholesterol (HDL-C) [13]. These mechanisms have been suggested to mediate its beneficial effects in reducing or preventing of atherogenic risk and cardiovascular disease [9, 11, 13]. The aim of the present study was to investigate whether the changes in lipid metabolism and aorta antioxidant enzyme profile in chronically diabetic rats could be reversed by acute gemfibrozil treatment.

Materials and methods

Animals, induction of diabetes and gemfibrozil treatment

The 'Animal Care Ethics Committee' of Ankara University has approved the study. Male Wistar rats weighing 200–250 g were divided into the following groups: (1) Untreated-control rats (n = 8); (2) gemfibrozil treated-control rats (n = 9); untreated-diabetic rats (n = 9); gemfibrozil treated-diabetic rats (n = 10). Diabetes was induced by streptozotocin (45 mg/kg, i.p.) injection. Control animals were injected with the vehicle alone. Two days after streptozotocin injection, development of diabetes was confirmed from tail vein blood glucose, and rats with blood glucose levels of 250 mg/dl or above were considered to be diabetic. Twelve weeks after diabetes induction, gemfibrozil was given for 2 weeks (100 mg/kg/day in carboxymethylcellulose 10%, per oral). The treatment dose of gemfibrozil was chosen according to a previous study [14]. Rats were maintained under standard housing conditions for 14 weeks before experiments were conducted.

Blood and tissue analysis

Blood glucose concentrations were measured by an Ames glucometer (Glucometer III, Bayer Diagnostics, France). Plasma immunoreactive insulin concentrations were determined by standard radioimmunoassay technique using Coat-A-Count (DPC) insulin kit available from Diagnostic Products Corporation, USA. Plasma triglyceride and cholesterol concentrations were measured using a commercially available enzyme kit (Wako, Osaka, Japan).

Thiobarbituric acid reactive substances (TBARS), as an index of malondialdehyde production and hence lipid peroxidation, were measured by the fluorimetric technique of Yagi [15].

Superoxide dismutase (SOD) activity in aortic homogenates was measured spectrophotometrically at 560 nm using the method of Spitz [16]. The reaction mixture in 50 mM phosphate buffer (pH 7.8) consisted of SOD-induced inhibition of the reduction of nitro blue tetrazolium, using a free radical-generating system of 0.1 nM xanthine and an amount of xanthine oxidase to produce a rate of absorbance change of 0.025/min.

Catalase was measured spectrophotometrically by the method of Aebi [17]. Cleaned and minced aortas were homogenized in 3 volumes of 50 mM phosphate buffer (pH 7) with Tirton X-100. Twenty µM of homogenate was added to a cuvette containing the same phosphate buffer to make final volume of 2.0 ml. The reaction was started by the addition of 1.0 mL of 30 mM H_2O_2. The rate of decomposition of H_2O_2 was read at 20°C against a blank containing prepared enzyme solution but no substrate in absorbancy at 240 nm. The activity of tissues is expressed as k/sec/mg protein, where *k* is the first order rate constant.

The method of Lawrence was used to measure glutathione peroxidase (GSH-Px) activity [18]. In this method, GSHPx activity is coupled to NADPH utilization, and the production of $NADP^+$ was measured spectrophotometrically at 340 nm. The assay mixture consisted of 76 mM phosphate buffer with EDTA and NaN_3 (pH 7.0), 0.150 mg 10,000 × g supernatant protein of tissue, 0.1 mM NADPH, 4.0 mM GSH and 1.5 U glutathion reductase in a final volume of 500 µL. The reaction was started by addition of 3.0 mM H_2O_2. GSHPx activity was expressed as µmol of NADPH oxidized to $NADP^+$/min/mg protein for tissues.

Drugs and statistical analysis

All chemicals were purchased from Sigma Chemical (St. Louis, MO, USA). Results are expressed as the mean ± S.E.M. Statistical analysis was carried out using one-way analysis of variance followed by Neuman-Keul's test. Results were considered significantly different if $p < 0.05$.

Results

Body weights and blood glucose levels

Initial and final body weights and blood glucose levels of animals are presented in Table 1. The baseline weight at the beginning of the study was similar in all groups. At the end of the treatment period, untreated-diabetic rats exhibited loss of body weight. There was no significant difference between initial and final body weight levels in gemfibrozil treated group.

Table 1. Body weights and blood glucose levels of animals

	Untreated-control (n = 8)	Untreated-diabetic (n = 9)	Gemfibrozil treated-control (n =- 9)	Gemfibrozil treated-diabetic (n = 10)
Initial body wt (g)	234 ± 9	247 ± 6	230 ± 11	238 ± 10
Final body wt (g)	401 ± 16	212 ± 11***	411 ± 5	239 ± 13***
Initial blood glucose (mg/dl)	110 ± 8	118 ± 5	98 ± 8	105 ± 4
Final blood glucose (mg/dl)	130 ± 3	385 ± 25***	128 ± 2	330 ± 30***

Data are means ± S.E.M. **p < 0.01; ***p < 0.001 vs. untreated-control.

The streptozotocin-induced diabetic animals showed consistent fasting hyperglycaemia throughout the study. Gemfibrozil treatment did not induce a significant fall in blood glucose levels of diabetic animals and had no effect on body weight and blood glucose levels of control rats.

Plasma insulin levels, lipid metabolism and oxidative stress markers

Plasma insulin levels of untreated- or gemfibrozil treated-diabetic rats were found to be significantly decreased when compared with control rats (Table 2).

Plasma cholesterol and triglyceride concentrations markedly increased in untreated-diabetic rats, as expected. As shown in Table 2, short-term gemfibrozil treatment completely reversed plasma triglyceride and cholesterol levels of diabetic animals. Gemfibrozil treatment of control rats did not induce significant changes in plasma insulin, cholesterol and triglyceride concentrations (Table 2).

Lipid peroxidation levels, measured as TBARS, was higher in the aorta and plasma of untreated-diabetic rats than control rats. Gemfibrozil treatment normalized plasma and aorta lipid peroxidation levels in diabetic animals (Table 2).

SOD and GSHPx activities were not significantly different in aorta of untreated- or gemfibrozil treated-diabetic versus control animals. In contrast, diabetes resulted in a significant increase in aorta catalase activity (Table 2). Short-term gemfibrozil treatment did not significantly change antioxidant enzyme activities in diabetic aorta. Control rats received two weeks treatment with gemfibrozil showed similar values in oxidative stress markers when compared with untreated-control rats (Table 2).

Discussion

Our experiments have demonstrated that TBARS levels, as an index of lipid peroxidation, are increased in aorta and plasma of untreated-diabetic rats. This is concomitant with augmented plasma cholesterol and triglycerides, and is consistent with the earlier reports [4, 5, 19]. The increased lipid peroxidation, firstly, implies the increased levels of reactive oxygen species (i.e. superoxide, hydrogen peroxide and hydroxyl radical) that could be due to their increased production or decreased destruction. Hyperglycemia may lead to an increase in the production of reactive oxygen species mainly through glucose autooxidation, non-enzymatic protein glycation, increased sorbitol pathway and depletion of some non-enzymatic or enzymatic scavengers [1, 3, 20]. Furthermore, hyperlipidemia alone may be unique reason for the enhanced levels of TBARS in diabetics since previous research has directly linked hyperlipidemia with increased serum and tissue concentrations of lipid peroxidation products [21, 22].

In order to evaluate antioxidant status, superoxide dismutase (SOD), catalase and glutathione peroxidase (GSHPx) activities in aortic homogenates were measured, and a significant increase in vascular catalase activity was observed without significant changes in either SOD or GSHPx in diabetic rats.

Table 2. Plasma insulin, cholesterol, triglyceride levels and oxidative stress markers of animals

	Untreated-control (n = 8)	Untreated-diabetic (n = 9)	Gemfibrozil treated-control (n = 9)	Gemfibrozil treated-diabetic (n = 10)
Plasma insulin (μU/ml)	31.6 ± 4.3	12.1 ± 4.5**	30.7 ± 5.3	15.2 ± 3.4**
Plasma cholesterol (mg/dl)	67 ± 6	154 ± 8***	77 ± 7	79 ± 6
Plasma triglycerides (mg/dl)	84 ± 9	291 ± 33***	57 ± 11	102 ± 25
Plasma TBARS (mmol/l)	1.54 ± 0.10	2.49 ± 0.07***	1.66 ± 0.12	1.43 ± 0.12
Aorta TBARS (nmol/mg protein)	0.040 ± 0.002	0.061 ± 0.001**	0.042 ±0.002	0.041 ± 0.003
Aorta Superoxide dismutase (U/mg protein)	9.69 ± 1.81	9.20 ± 0.71	9.47 ± 0.62	10.99 ± 1.23
Aorta catalase (k/s/mg protein)	0.016 ± 0.002	0.034 ± 0.006**	0.014 ± 0.002	0.030 ± 0.004**
Aorta glutathione peroxidase (nmol/dk/mg protein)	0.076 ± 0.003	0.095 ± 0.008	0.075 ± 0.004	0.088 ± 0.005

Data are means ± S.E.M. **p < 0.01; ***p < 0.001 vs. untreated-control.

The selective increase in catalase but not SOD or GSHPx has previously been reported in diabetic blood vessels [23] and in other diabetic tissue [24]. Increased catalase activity could be a compensatory mechanism in response to increased oxidative stress, more specifically, peroxidative stress, due to elevated production of hydrogen peroxide (H_2O_2) in vessels. It has been reported that the preferential pathway for detoxification of H_2O_2 in blood vessels is dependent on the concentration of H_2O_2. Catalase has been implicated in the detoxification of high H_2O_2 concentrations whereas GSHPx is effective at lower concentrations of H_2O_2 [25]. Thus, in the present of a high H_2O_2 concentration, the induction of catalase in diabetic vessels is an expected result. In addition to this, the reason for an increase in catalase but not GSHPx may also be related to location of each enzyme, as reported previously [26]. The catalase is located mainly in the peroxisomal compartment as same as fatty acyl CoA oxidase, is an H_2O_2-generating enzyme in the presence of fatty acid and keton body metabolism increasing states such as diabetes. The observation of increased catalase without a significant change in SOD activity has been reported to be possible [23], since the rate constant for the degradation of H_2O_2 by catalase is lower than the rate constant for the dismutation of superoxide anion radicals to form H_2O_2 by SOD. Increased H_2O_2 is known to inactivate SOD, thus an increase in catalase activity due to oxyradicals may protect SOD inactivation.

In this study, we have shown for the first time that short-term gemfibrozil treatment initiated 12 weeks after induction of diabetes is able to reverse hyperlipidemia and the excess lipid peroxidation in plasma and vascular tissue without significant changes in glucose metabolism and vessel antioxidant enzymes in diabetic rats. It has been shown that gemfibrozil increases lipoprotein lipase activity, increases the clearance of very low-density lipoprotein (VLDL) triglycerides and some times enhances LDL clearance [8, 10, 11]. In addition, lipid-lowering effect of gemfibrozil was reported to be due to a decrease in the plasma triglyceride concentration by inhibiting the VLDL synthesis [27]. It seems that, the beneficial effect of gemfibrozil on the oxidation of lipids is mainly linked with its hypolipidemic effect. In accordance with our interpretation is a previous observation which showed that antioxidant effect of gemfibrozil was related to two independent phenomena: the reduction of the amount of substrate for the oxidation process due to its hypolipidemic activity, and the alteration in the type of fatty acids transported by the lipoproteins towards an enrichment in species resistant to the oxidation process [28]. Furthermore, gemfibrozil can affect lipoprotein oxidizability by inhibiting their susceptibility to oxidation, and *in vivo* metabolites of gemfibrozil has been reported to inhibit VLDL, LDL and HDL oxidation by free radical scavenging activity of themselves [29]. Gemfibrozil has recently been proposed to be suitable for the treatment of diabetic hypertriglyceridemia [26, 30]. We conclude that in addition to the management of diabetic dyslipidemia, gemfibrozil may provide a useful therapeutic option in the reversal of oxidative stress in diabetes mellitus.

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