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The Influence of Gemfibrozil on Malondialdehyde Level and Paraoxonase 1 Activity in Wistar and Fisher Rats

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Abstract: There are diverse experimental data about the influence of gemfibrozil (GEM) on the production of hydrogen peroxide (H_2O_2) and antioxidant enzymes. We investigated the influence of GEM treatment on the production of malondialdehyde (MDA) level in tissues of normolipidaemic Wistar and Fisher rats which is an index of lipid peroxidation. Because serum paraoxonase 1 (PON1) is an important enzyme with specific protective function on metabolism of lipid peroxides, we examined the influence of GEM on PON1 activity in liver and serum. MDA level and enzyme activities were also determined 10 days after withdrawal of GEM treatment. The significantly increased levels of MDA in liver, kidney and heart of both rat strains were obtained after 3 weeks of GEM treatment. We propose two possibilities for the increase of MDA levels caused by GEM, induction of peroxisome proliferation and activities of enzymes that participated in occurrence of H_2O_2 and possible reduction of enzyme activities including in H_2O_2 metabolism. Ten days after withdrawal of GEM treatment, MDA levels in all tissue levels of both rat strains were less in comparison with GEM treatment. GEM caused a significant drop of PON1 activity in serum and liver of Fisher rats, and in liver of Wistar rats. We suggest that GEM, through induction of lipid peroxidation, caused the damage of hepatocytes with consequent reduction of PON1 synthesis. The increase in PON1 activity in serum and tissues of both rat strains 10 days after withdrawal of GEM treatment shows the fast recovery of enzyme synthesis.

Gemfibrozil (GEM) and other fibric acid derivatives (fenofibrate, clofibrate, bezafibrate, etc.) are the drugs of choice for the treatment of hypertriglyceridaemia because they reduce hepatic triglyceride production, decrease the levels of total, very low-density lipoprotein (VLDL) and low-density lipoprotein (LDL) cholesterol and raise high-density lipoprotein (HDL) cholesterol levels [1,2]. The efficiency of GEM therapy was confirmed by the conclusive results of many clinical trials that clearly demonstrated impressive results in reducing cardiovascular events during its administration [3–6].

Although the mechanism responsible for the effects of GEM and other fibrates on lipoprotein metabolism is not known in detail, it is considered that many of the above-mentioned effects are mediated by their interaction with peroxisome proliferator-activated receptor α (PPARα) [7]. PPARα are ligand-dependent transcription factors belonging to the nuclear hormone receptor superfamily [8,9] and are involved in the regulation of lipid metabolism in liver, heart, kidney and muscles [10–12]. PPARsα are primarily expressed in the liver of rodents and to a lesser extent in kidney, heart and skeletal muscles. Human beings are less susceptible to peroxisome proliferators than rodents. PPARsα are activated by natural ligands like polyunsaturated fatty acids, oxidized phospholipids and synthetic agents, including widely prescribed fibrates for the treatment of hyperlipoproteinaemia and hypertriglyceridaemia [9].

The fact is that fibrates induce proliferation of peroxisomes in liver cells with associated coordinated transcriptional activation of peroxisomal fatty acid β-oxidation system and production of reactive oxygen species (ROS) as are superoxide anion radical, hydroxyl radical, nitric oxide or hydrogen peroxide (H_2O_2) [10,11,13–17]. Large amounts of H_2O_2 stimulate lipid peroxidation and cause a significant increase of malonyldialdehyde (MDA) which is one of several low-molecular-weight end products formed via the decomposition of certain primary and secondary lipid peroxidation products. MDA is very often used as an index of oxidative status [18,19]. In healthy aerobic organisms, MDA is formed only in small amounts during the peroxidation of lipids, and its production is balanced by antioxidant defence systems. It must be mentioned that the increase in H_2O_2 production does not always stimulate lipid peroxidation probably due to the presence of high levels of some endogenous antioxidants [16]. In the literature data mentioned above, fenofibrate and clofibrate were the most frequent fibrates that were used for the investigation of their oxidant actions [13,15,17,18].

There are small diverse experimental data about the influence of GEM on production of H_2O_2 activity of antioxidant enzymes [e.g. superoxide dismutase (SOD), glutathione peroxidase, glutathione reductase, catalase] and lipid peroxidation, while there are no data about the effect of

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GEM on MDA level. The investigations of GEM action on glutathione and glutathione-related enzymes in rats and hamsters showed that in rats, GEM decreased glutathione reductase, glutathione S-transferase and selenium-dependent glutathione peroxidase activities [20]. These results have suggested that there is a possible interdependent relation between GEM, H_2O_2 and MDA during GEM treatment. On the other hand, a significant recovery of plasma lipids and lipid peroxides without significant changes in vessel antioxidant enzymes was shown in diabetic rats treated with GEM [21].

Serum paraoxonase (aryldialkilphosphatase, EC 3.1.8.1, PON1) is an esterase enzyme, synthesized by the liver and secreted into the plasma where it is associated with the HDL and apolipoprotein (apo) AI, the structural peptide of HDL [22]. PON1 prevents the formation of oxidized LDL, protects phospholipids in HDL from oxidation, metabolizes lipid peroxides and because of these actions, it is considered that PON1 is also a part of the endogenous antioxidant defence system [23,24] There are few conflicting results about the influence of GEM and other fibrates on PON1 activity. So, Durrington *et al.* [25] found that the treatment of hyperlipidaemic patients with bezafibrate or GEM had no effect on plasma PON1 activity, while Balogh *et al.* [26] showed that administration of GEM in diabetic patients with hypertriglyceridaemia caused the significant increase in PON1 level. The results of our experiments in rats on normal diet and in rats with hypertriglyceridaemia showed that the addition of GEM significantly decreased plasma PON1 activity [27].

According to the facts that GEM treatment influences the activity of PON1 and antioxidant enzymes [23–27], the aim of this study was to investigate the influence of GEM on the production of MDA level in normolipidaemic rats of two different strains. Because PON1 is an important enzyme with specific protective function on the metabolism of lipid peroxides, we wanted to examine the influence of GEM on PON1 activity in liver and serum as well and to determine the MDA level and enzyme activity 10 days after withdrawal of GEM treatment. It was also of interest to examine whether there is the correlation between MDA level and PON1 activity. GEM was administered in a dosage that has often been used for the investigation of GEM effects on plasma lipid profile [28,29]. The same dosage and schedule of GEM treatment were also used in our recent report [27].

Material and Methods

Test substances. Gemfibrozil (CAS–25812-30-0) was obtained from Lek, Pharmaceutical and Chemical Company Ltd., Ljubljana (Slovenia) and administered daily (9.00–10.00 a.m.) into the stomach by oral gavage (50 mg/kg body weight/day) and suspended in saline. GEM was given during 3 weeks.

Treatment of animals. Male Wistar (Department of Pharmacology, School of Medicine, University of Zagreb) weighing 240–345 g and Fisher rats (Departments of Biology, School of Medicine, University of Zagreb) weighing 270–340 g were used in these studies. The animals were maintained under controlled laboratory conditions. Standard diet in pellet form was available *ad libitum*. Handling and treatment of the animals were conducted on basis of the international guidelines regarding the use of laboratory animals. The experiments were approved by the local ethics committee.

Study design. The Wistar and Fisher rats were divided into four control groups (n = 7 each) and four experimental groups (n = 7 each). The control groups were treated with saline, and the experimental groups were given GEM treatment for 3 weeks (treatment period, TP). Following an overnight fast of 12 hr, one control group of Wistar and one control group of Fisher rats together with one experimental group of Wistar and one experimental group of Fisher rats were killed under ether anaesthesia on the 22nd day of the treatment. The rats were killed between 9.00 and 10.00 a.m. under diethyl ether anaesthesia. The last two control groups of Wistar and Fisher rats and the last two experimental groups of both rat strains were given saline during the next 10 days (recovery period, RP), and following an overnight fast of 12 hr, all groups were killed the next day between 9.00 and 10.00 a.m. under diethyl ether anaesthesia. Blood samples for the measuring of serum PON1 activity and lipid levels were obtained by cardiac punction. Serum samples for measuring PON1 activity were frozen immediately after sampling at −20°C until further processing. Plasma lipids were measured 2–4 hr after sampling. Before collecting liver tissue, the liver had been washed out of blood with saline *in situ* via the vena cava superior. Liver, kidney and heart tissues for determining PON1 activity and MDA level were frozen immediately after the killing of the animals at −70°C until further processing.

PON1 activity in serum. PON1 activity in serum was measured using synthetic diethyl-*p*-nitrophenyl phosphate (paraoxon, *o*,*o*-diethyl-*p*-nitrophenylphosphate; Sigma Chemical Co., London, UK) and $CaCl_2$ (1 mM in 0.1 M TRIS buffer pH 7.4) as moderator. The activity towards paraoxon was determined by measuring the initial rate of substrate hydrolysis to *p*-nitrophenol. In brief, reaction (final volume 1100 µl) for the hydrolysis of paraoxon contained 200 µl of 0.1 M TRIS buffer (pH 7.4)–$CaCl_2$ and 800 µl of 1 mM paraoxon solution to which 100 µl undiluted serum was added to start the reaction. Increase in absorbance at 405 nm was monitored for 3–5 min. The blank sample containing incubation mixture without serum was run in parallel to correct for spontaneous substrate breakdown. The enzyme activity was calculated from E_{405} of *p*-nitrophenol (16000 l/mol/cm) and was expressed in µmol min/ml [30].

PON1 activity in liver. PON1 activity in liver was measured by using synthetic diethyl-*p*-nitrophenyl phosphate (paraoxon, *o*,*o*-diethyl-*p*-nitrophenylphosphate; Sigma Chemical Co.) and moderator $CaCl_2$ (1 mM in TRIS buffer pH 7.4). Slices of liver tissue (0.5 g) were homogenized in four volumes of saline 3500 g for 15 min. Then, supernatant was collected and stored at −20°C until further processing. The activity towards paraoxon was determined by measuring the initial rate of substrate hydrolysis to *p*-nitrophenol. The medium for the hydrolysis of paraoxon consisted of 200 µl 0.1 M TRIS buffer (pH 7.4)–$CaCl_2$ and 800 µl 1 mM paraoxon solution to which 100 µl supernatant was added to start the reaction (final volume 1100 µl). The increase in absorbance at 405 nm was monitored for 3 min. The blank sample containing incubation mixture without supernatant was run simultaneously to correct the simultaneous substrate breakdown. The enzyme activity was calculated from E_{405} of *p*-nitrophenol (16000 l/mol/cm) and was expressed in µmol min/g ($v = \Delta A/\text{min} \times 9.4$) [30].

Lipids and lipoproteins. Lipids were determined on the Olympus automatic analyser AU 2700 using original reagents produced by Olympus Diagnostics GmbH (Irish Branch), Lismeehan, Ireland. The plasma concentrations of total cholesterol (TC) and triglycerides (TGs) were determined by enzymatic colorimetric methods [31,32]. HDL level was estimated using compound method of immuno-inhibition with β-lipoprotein antibody in the first step, and enzymatic colorimetric measuring of accessible cholesterol in the second step [33]. LDL was calculated mathematically from the total cholesterol,

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the TGs and the HDL concentrations using Friedwald's formula LDLchol = total cholesterol - TGs/2.2 - HDLchol [34]. The approximate formula applies only for plasma without chylomicrons and TGs concentration <4.7 mM. All lipid concentrations were expressed as mM.

Spectrophotometric determination of MDA-TBA. Chemicals and reagents. Chemicals and reagents were of the highest analytical grade available as follows: BHT [2,6 di-ter-butyl-4-methylphenol, ≥99.0% (GC); (Sigma-Aldrich Inc., St. Louis, USA)], TCA (trichloracetic acid, ≥98%; Sigma-Aldrich Inc.), TBA (thiobarbituric acid, ≥98%; Sigma-Aldrich Inc.) and ethanol 98% (Merck, Darmstadt, Germany), HCl (37%; puriss. p.a. Sigma-Aldrich Inc.), KCl (≥99%; Sigma-Aldrich Inc.).

Analytical procedure. The TBA assay was adapted from Angulo *et al.* [35] and Botsoglou *et al.* [36] and adjusted for our purposes by Lovrić *et al.* [37]. The 0.2-g tissues (heart, kidney or liver) were used to obtain 10% homogenates in 0.15 M KCl that were further treated with 25 µl 0.2% BHT (in 98% ethanol) as antioxidant. The homogenates with BHT were transferred into Eppendorf tubes and centrifuged (Hettich Universal 32 R, Tuttlingen, Germany) at 18,890 *g* for 20 min., followed by transfer of the supernatants into Nunc CryotubeE, successive additions of 5% aqueous TCA in proportion 1:4 and recentrifuged (Hettich Universal 32 R) at 1780 *g* for 15 min. [38,39]. TCA was used for protein precipitation because of its low toxicity. The aliquots of 500 µl of deproteinized supernatants were transferred into the microepruvetes Cartell, and 500 µl TBA 0.375% in 0.25 M HCl was added. It was heated at 100°C for 15 min., followed by cooling the samples to room temperature and measuring MDA level by UV–VIS spectrophotometer (HPV-220, Iskra, Slovenia) using 1-cm absorption cell. The concentration of MDA was calculated by reading the absorbance at 532 nm using a molar extinction coefficient of $\varepsilon = 1.56 \times 10^5$ M/cm [40,41]. Concentrations of MDA in all tissue samples are expressed in µM. During the whole procedure (until heating), samples were kept on ice.

Statistical analysis. Data are shown as the mean ± standard deviation (S.D.). Descriptive statistics, 95% confidence intervals for the difference between means and hypothesis testing were made using GraphPad Prism version 5.0 [42,43]. The statistical significance was determined by two-sample *t*-test. All applied procedures were two-tailed. The differences discussed in this paper were considered significant at the $p \leq 0.05$ level.

Results

Effects of GEM on MDA levels in plasma, liver, kidney and heart of Wistar rats.

Malondialdehyde values in heart, kidney and liver of Wistar rats treated by GEM were increased significantly over the basal levels by 47% ($p < 0.026$), 30% ($p < 0.002$) and 42% ($p < 0.026$) (fig. 1A). After withdrawal of GEM treatment and a RP of 10 days, the values of MDA in heart, kidney and liver were still higher (10–25%) (fig. 1C) in comparison with their control values.

Effects of GEM on MDA levels in liver, kidney and heart of Fisher rats.

Gemfibrozil caused a significant increase in MDA levels in heart, kidney and liver by 59% ($p < 0.031$), 38% ($p < 0.001$) and 34% ($p < 0.026$) (fig. 1B). After the withdrawal of GEM treatment and a RP of 10 days, MDA levels in heart, kidney and liver were a little higher (5–8%) than their control values (fig. 1D).

Effects of GEM on serum and liver PON1 activity and plasma lipids in Wistar rats.

Gemfibrozil treatment did not change the PON1 activity in serum while the enzyme activity in the liver was significantly decreased by 56% in comparison with their control groups ($p < 0.001$) (table 1). After the withdrawal of GEM treatment and a RP of 10 days, enzyme activity of liver PON1 was completely restored and increased by 29% above the control values established (table 1).

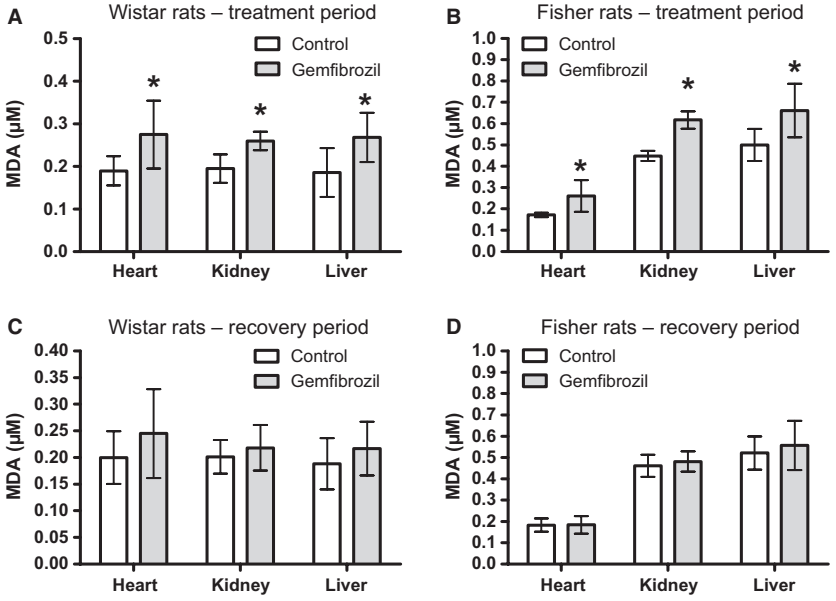

Fig. 1. Malondialdehyde levels in Wistar (A) and Fisher (B) rat tissue after 21 days of treatment with gemfibrozil (GEM), as well as 10 days after gemfibrozil discontinuation (C and D). Results are expressed as means ± 95% confidence intervals. *$p \leq 0.05$ compared to the control group.

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Table 1.

Effect of GEM (50 mg/kg/daily for 3 weeks) on PON1 activity and lipids in Wistar rats. The study was divided into a GEM treatment period (TP) and a recovery period (RP).

Parameter	Study period	Control[1]	Gemfibrozila[1] (50 mg/kg/day)	Difference[1] (95% CI)[2]	*p*-value[3]
Paraoxonase	TP	0.19 ± 0.030 (N = 6) (100)	0.20 ± 0.007 (N = 7) (105)	−0.01 ± 0.024 (−0.06 to 0.05)	0.775
serum μmol/min/ml	RP	0.26 ± 0.013 (N = 6) (100)	0.27 ± 0.025 (N = 6) (104)	−0.01 ± 0.028 (−0.07 to 0.05)	0.707
Paraoxonase	TP	0.27 ± 0.018 (N = 6) (100)	0.12 ± 0.024 (N = 7) (44)	0.15 ± 0.031 (0.07 to 0.21)	<0.001
liver μmol/min/g	RP	0.34 ± 0.060 (N = 6) (100)	0.44 ± 0.046 (N = 7) (129)	−0.10 ± 0.077 (−0.27 to 0.07)	0.219
Total cholesterol mM	TP	1.64 ± 0.100 (N = 6) (100)	2.14 ± 0.079 (N = 7) (139)	−0.50 ± 0.123 (−0.77 to −0.23)	0.002
	RP	1.44 ± 0.100 (N = 6) (100)	1.84 ± 0.131 (N = 7) (128)	−0.40 ± 0.168 (−0.77 to −0.030)	0.037
HDL cholesterol mM	TP	1.14 ± 0.083 (N = 6) (100)	1.59 ± 0.051 (N = 7) (139)	−0.45 ± 0.094 (−0.66 to −0.24)	<0.001
	RP	1.00 ± 0.075 (N = 6) (100)	1.35 ± 0.096 (N = 7) (135)	−0.35 ± 0.125 (−0.62 to −0.07)	0.019
LDL cholesterol mM	TP	0.48 ± 0.050 (N = 6) (100)	0.66 ± 0.054 (N = 7) (137)	−0.18 ± 0.074 (−0.34 to −0.02)	0.035
	RP	0.45 ± 0.030 (N = 6) (100)	0.52 ± 0.053 (N = 7) (115)	−0.07 ± 0.064 (−0.21 to 0.07)	0.304
Triglycerides mM	TP	1.06 ± 0.061 (N = 6) (100)	0.72 ± 0.055 (N = 7) (68)	0.34 ± 0.084 (0.15 to 0.52)	0.002
	RP	1.16 ± 0.200 (N = 6) (100)	1.03 ± 0.084 (N = 7) (89)	0.13 ± 0.201 (−0.31 to 0.58)	0.521

Relative changes (%) are given in brackets.

GEM, gemfibrozil; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

[1]The values are mean ± S.D.

[2]Ninety-five per cent confidence interval between means.

[3]*p* Value of independent two-sample *t*-test.

Gemfibrozil-treated rats had significantly higher plasma levels of TC (30%, $p < 0.002$), HDL (39%, $p < 0.001$) and LDL cholesterol (37%, $p < 0.035$), while the level of TGs fell significantly by 32% ($p < 0.002$). After withdrawal of GEM treatment and a RP of 10 days, the values of TC, HDL and LDL were still higher (significantly in the case of TC and HDL) than their control values and level of TGs tended to decrease by 11% in comparison with the control (table 1).

Effect of GEM on serum and liver PON1 activity and plasma lipids in Fisher rats.

The level of PON1 activity in serum indicated a decrease by 15% while the decrease was significant in liver by 38% ($p < 0.001$) in comparison with the control groups (table 2). After the withdrawal of GEM treatment and a RP of 10 days, serum PON1 activity was practically identical with control values. The liver PON1 activity was lower (12%) in comparison with the control (table 2).

Gemfibrozil treatment caused a significant increase in TC, HDL and LDL cholesterol, 43% ($p < 0.002$), 48% ($p < 0.001$) and 75% ($p < 0.001$), respectively, while TG was significantly lower (42%) ($p < 0.001$). After the withdrawal of GEM treatment and a RP of 10 days, the values of TC, HDL and LDL were higher by 9–23% in comparison with their control values (table 2). The level of TGs was still lower (23%) in comparison with the control (table 2).

Discussion

The significantly increased levels of MDA in liver, kidney and heart of Wistar and Fisher rats were obtained in our experiments after 3 weeks of GEM treatment (fig. 1A,B), and we suggest that this increased level of MDA is the consequence of peroxisome proliferation and lipid peroxidation caused by GEM.

Considering the H_2O_2 level as the central point in the beginning of lipid peroxidation, we believe that a brief description of the results on the effects of fibrates on the H_2O_2 and activity of enzymes involved in its level is essential for discussion of our results. We were interested only in pharmacological action of fibrates, regardless of their dosages and duration of treatments used in the experiments. Thus, during fibrate administration, there are three possible modalities of the occurrence of high H_2O_2 level that could lead to an increased lipid peroxidation. The higher H_2O_2 level may be a consequence of the increased activities of enzymes that are included in the occurrence of H_2O_2 during peroxisome proliferation. In liver homogenates of rats and mice, fenofibrate (0.23% w/w in the diet) administered for 2–3 weeks caused hepatic peroxisome proliferation, induction of peroxisome hydrogen peroxide oxidases [13,14] and an increase in H_2O_2 production [15,16]. In the study by Arnaiz *et al.* [15], treatment with fenofibrate for 2 weeks induced a 10–12 times the increase in acyl CoA-oxidase activity of peroxisomal fractions when compared to the control. Because acyl CoA-oxidase catalyses the first oxidation step of peroxisomal β-oxidation with reduction of molecular oxygen to H_2O_2, the increase in H_2O_2 concentration is the logical consequence of peroxisome proliferation caused by fenofibrate. The higher production of H_2O_2 and other activated ROS was found in peroxisomes from plants treated by clofibrate. The same experiment demonstrated stimulation of the lipid peroxidation of peroxisomal membranes [13].

The higher level of H_2O_2 caused by fibrates can also be the consequence of the decreased activity of antioxidant enzymes that are included in metabolism of H_2O_2. Fenofibrate (25 and 100 mg/kg daily for 30 days) and clofibrate (300 mg/kg daily for 30 days) caused a marked decrease in liver SOD activity and glutathione peroxidase and a significant increase of MDA [17,44]. The decrease in catalase and SOD activities was also found in intact plant peroxisomes treated with clofibrate [13]. The investigations of GEM action on glutathione and glutathione-related enzymes in rats and hamsters showed that GEM administered in two different doses (1000 and

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Table 2.

Effect of GEM (50 mg/kg/daily for 3 weeks) on PON1 activity and lipids in Fisher rats. The study was divided into a GEM treatment period (TP) and a recovery period (RP).

Parameter	Study period	Control[1]	Gemfibrozil[1]	Difference[2] (95% CI)	*p*-value[3]
Paraoxonase	TP	0.27 ± 0.020 (N = 5) (100)	0.23 ± 0.008 (N = 6) (85)	0.04 ± 0.020 (0.00 to 0.090)	0.050
serum μmol/min/ml	RP	0.33 ± 0.020 (N = 6) (100)	0.32 ± 0.016 (N = 7) (97)	0.01 ± 0.025 (−0.04 to 0.07)	0.480
Paraoxonase	TP	0.21 ± 0.014 N = 7) (100)	0.13 ± 0.009 (N = 7) (62)	0.08 ± 0.016 (0.04 to 0.11)	<0.001
liver μmol/min/ml	RP	0.33 ± 0.019 (N = 6) (100)	0.29 ± 0.021 (N = 7) (88)	0.04 ± 0.029 (−0.02 to 0.10)	0.198
Total cholesterol mM	TP	1.11 ± 0.042 (N = 7) (100)	1.59 ± 0.107 (N = 8) (143)	−0.49 ± 0.122 (−0.74 to −0.22)	0.002
	RP	1.20 ± 0.069 (N = 6) (100)	1.33 ± 0.074 (N = 7) (111)	−0.13 ± 0.102 (−0.35 to 0.096)	0.232
HDL cholesterol mM	TP	0.88 ± 0.031 (N = 7) (100)	1.30 ± 0.078 (N = 8) (148)	−0.42 ± 0.089 (−0.61 to −0.22)	<0.001
	RP	0.97 ± 0.055 (N = 6) (100)	1.06 ± 0.052 (N = 7) (109)	−0.09 ± 0.076 (−0.23 to 0.07)	0.264
LDL cholesterol mM	TP	0.20 ± 0.013 (N = 7) (100)	0.35 ± 0.034 (N = 7) (175)	−0.15 ± 0.036 (−0.27 to −0.08)	0.001
	RP	0.30 ± 0.025 (N = 6) (100)	0.37 ± 0.046 (N = 7) (123)	−0.07 ± 0.056 (−0.19 to 0.05)	0.242
Triglycerides mM	TP	0.62 ± 0.076 (N = 7) (100)	0.36 ± 0.037 (N = 8) (58)	0.26 ± 0.081 (0.08 to 0.43)	0.007
	RP	0.70 ± 0.069 (N = 6) (100)	0.54 ± 0.067 (N = 7) (77)	0.16 ± 0.096 (−0.05 to 0.38)	0.112

Relative changes (%) are given in brackets.
GEM, gemfibrozil; HDL, high-density lipoprotein; LDL, low-density lipoprotein.
[1]The values are mean ± S.D.
[2]Ninety-five per cent confidence interval between means.
[3]*p* Value of independent two-sample *t*-test.

16,000 p.p.m daily) for 6, 34 and 90 days decreased the rat glutathione reductase, glutathione S-transferase and selenium-dependent glutathione peroxidase activities [45].

Fibrates can increase the activity of some antioxidant enzymes that are included in H_2O_2 metabolism. Lalwani *et al.* [46] established *in vitro* that GEM together with other NINE hypolipidaemic compounds (e.g. fenofibrate, clofibrate) produced a marked but variable increase in the activities of peroxisomal enzymes catalase, carnitine acetyltransferase, heat-labile enoyl-Coa hydratase and the fatty acid beta-oxidation system. The results of Arnaiz *et al.* [16] showed that the administration of fenofibrate during 2 weeks in mice increased the production of H_2O_2, but H_2O_2 did not stimulate lipid peroxidation, probably due to the presence of high levels of some endogenous antioxidants (ubiquinols and glutathione). An increase of 55% was found in ubiquinol levels, and an increase of 67% and 58% in total and oxidized glutathione content was also obtained in treated mice after 22 days of treatment with fenofibrate when compared with the controls.

According to the results of the studies mentioned above as well as our results, we propose two possibilities for increasing MDA-level tissues of both rat strains caused by GEM. First, GEM induced both peroxisome proliferation and activities of enzymes that participated in the occurrence of H_2O_2. Second, it is possible that GEM decreased the activities of some enzymes including in H_2O_2 metabolism, and in this way increased lipid peroxidation and high H_2O_2 level. Although the data of Lalwani *et al.* [46] have shown that GEM *in vitro* increased the activity of catalase, we are of the opinion that an *in vivo* condition of GEM exerts the inhibitory action on antioxidative enzymes. We suggest that our hypothesis is confirmed by results of O'Brien *et al.* [20] who showed that GEM in rats decreased the activity of some antioxidant enzymes. The low dose of GEM used in this study was 1000 ppm daily. Inhibitory action of GEM on CYP2C8 and CYP3A4 in human liver microsomes [47,48] has shown the possibility that GEM has an influence on other enzyme systems as well. After the RP, MDA levels in heart, kidney and liver of both previously GEM-treated Wistar and Fisher rats were less in comparison with TP, although they remained moderately higher *versus* controls (fig. 1C,D). We suggest that this decrease in MDA level after the RP has been shown on the recovery of the activity of the antioxidant enzymes that are included in H_2O_2 metabolism.

The results of studies concerning the effect of fibrates on oxidative stress are controversial and have shown both prooxidative and antioxidant effects. On the basis of our results, we suggest that GEM exerts prooxidant action. This is supported by results obtained *in vitro* and *in vivo*. O'Brien *et al.* [45] found that both doses of GEM (1000 and 16,000 ppm) administered for 6, 34 and 90 days in rats did not affect the modifying SOD activity, but significantly decreased the content of α-tocopherol after 6 and 34 days of treatment. As well, only a low dose of GEM significantly increased the DT-diaphorase activity in rats at 6 and 43 days of study. On the basis of these results, the authors concluded that rats were compromised in antioxidant capabilities following GEM treatment. The single oral dose of GEM (1200 mg) administered to five male healthy volunteers significantly enhanced the induced ROS production by blood phagocytes, polymorphonuclear leucocytes and monocytes with respect to control values when these cells were stimulated by phorbol myristate acetate [49]. A 12-week course of GEM therapy (600 mg twice daily) in 11 hypertriglyceridaemic individuals caused the significant increase in polyunsaturated fatty acid proportion in cholesterol ester and phospholipid fractions of plasma lipids at the expense of saturated and monounsaturated fatty acids, while the lag time, the principle measure of lipoprotein susceptibility to oxidation, was decreased [50]. The results of the experiments in normal and beta-thalassaemic red blood cells have shown that GEM increased oxidative stress in the red blood cells [51].

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Antioxidant action of fibrates was established by Škrha *et al.* [52] who found that fenofibrate treatment (200 mg daily for 3 months) in patients with type 2 diabetes and dyslipidaemia caused a significant decrease in serum triglyceride concentration, associated with a decrease in plasma MDA and an increase in plasma plasminogen activator inhibitor. Aviram *et al.* [53] studied the effect of GEM and its metabolite on serum LDL, VLDL and HDL susceptibility to oxidation and found that that GEM metabolite I (*p*-hydroxy metabolite) possesses an antioxidative potential.

In our study, GEM treatment caused a significant drop of PON1 activity in the livers of Wistar and Fisher rats (tables 1 and 2). PON1 activity in GEM-treated Wistar rats was equal to the control, while the level of PON1 activity in serum of Fisher rats indicated a decrease by 15% (tables 1 and 2). Current results are similar with our recent results [27] obtained in plasma of normolipidaemic rats and rats with hypertriglyceridaemia which showed that the addition of GEM significantly decreased PON1 activity in plasma. We have suggested that GEM through induction of lipid peroxidation caused the damage of hepatocytes with consequent reduction of PON1 synthesis. Because PON1 is an important enzyme with specific protective function on the metabolism of lipid peroxides, we also suggest that the decrease in PON1 activity may induce lipid peroxidation as well. The increase in PON1 activity in liver and serum of Wistar and Fisher rats (tables 1 and 2) after the RP shows the recovery of enzyme synthesis and the reversible negative influence of GEM on PON1 synthesis. In our results, the significant negative correlation between PON1 activity and MDA level was not found because of the small number of animals.

In contrast to our results, Beltowski *et al.* [12] showed that fenofibrate treatment (30 and 300 mg/kg daily for 7 days) caused the significant decrease in PON1 activity of 81.5% and 69.2% and also the significant decrease in plasma MDA level that depended on the dosages. At present, we can not interpret these differences, but, according to our results and the results of all studies mentioned above, it is possible that GEM and other fibrate derivatives may indicate both an antioxidant and a prooxidant action and that this ambiguous action can not depend only on the dosages and duration of the treatments.

The actions of GEM on total cholesterol, HDL and triacylglycerol were typical for fibrates in both rat strains; thus, the comment is not necessary. The increase in LDL in rats is without any importance, because the plasma cholesterol in rats is essentially represented by HDL. Although rodents are more susceptible to peroxisome proliferators than human beings, the possible prooxidant effects of long-term GEM treatment in human beings are of importance under special pathophysiological conditions, such as diabetes mellitus, cardiovascular diseases or depression, where the increased production of ROS has been observed as well.

In conclusion, our results have shown that GEM administered for 3 weeks induced the significant increase in MDA levels in liver, kidney and heart of Wistar and Fisher rats. Two possibilities are responsible for the increase of MDA levels caused by GEM – the induction of both peroxisome proliferation and activities of enzymes that participated in occurrence of H_2O_2 and the inhibition of enzyme activities included in H_2O_2 metabolism. GEM treatment has also caused the significant decrease in liver PON1 activity in both rat strains, while the significant fall of serum PON1 was obtained only in Fisher rats. We suggest that because of lipid peroxidation, GEM has induced a damage of hepatocytes and lowered the synthesis of PON1. All actions of GEM on MDA level and PON1 activity were reversible. In the view of these results, further studies on the influence of GEM on oxidative status and antioxidative enzymes are needed.

Conflict of Interest

All authors declare that they have no conflicts of interest regarding this article.

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