J Invest Biochem. 2013;2(1):65-70 *ISSN: 2146-8338*

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Journal of Investigational Biochemistry

available at www.scopemed.org

Original Research

Biochemical alterations and endothelial dysfunction in hypercholesterolemic rats: Effects of Bezafibrate

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Received: January 31, 2013

Accepted: March 05, 2013

Published Online: March 23, 2013

DOI: 10.5455/jib.20130305012038

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Key words: Hyperlipidemia, bezafibrate, lipid profiles, endothelial dysfunction

Abstract

Background: Hypercholesterolemia is a serious problem cause biohazards for human health and endothelial dysfunction.

Objective: The current study was conducted to test effects of hyperlipidemia alone or in combination with bezafibrate at various doses. Total cholesterol, triacylglecerol, high density lipoprotein (HDL), low density lipoprotein (LDL), apolipoprotein B, endothelin-1, histamine, nitric oxide (NO), IL-6, creatinine and alanine amino transferase (ALT) were assayed

Methodology: Rats were randomly divided into four groups (10 rats each). The first group fed a normal diet and represents the control group. The second group fed normal diet enriched with 1% cholesterol and 5% coconut oil (cholesterol–fed group). The third group fed on normal diet and bezafibrate at a dose of 100 mg/kg/day. The fourth group is cholesterol fed and subdivided into A, B, C and supplemented with bezafibrate at various concentration 50, 100, 200 mg/kg/day respectively. Feeding was continued daily for 6 weeks after incidence of hypercholesterolemia.

Results: The current study revealed that total cholesterol, triacylglycerols, apolipoprotein B and LDL concentration were significantly elevated in the cholesterol–fed rats compared to control alone and control plus bezafibrate. In addition, plasma endothelin-1, histamine and IL-6 concentration were significantly elevated in the cholesterol–fed rats compared to bezafibrate and control group rats. Bezafibrate administration to rats fed high cholesterol diet normalized significantly the changes in all parameters except NO levels compared to hyperlipidemic group. Meanwhile, the plasma nitric oxide in all groups was not significantly different from those of control. However, plasma HDL concentration in the cholesterol fed rats was significantly lowered compared to bezafibrate treated rats.

Conclusion: Bezafibrate may acts as a mixed blessing drug to normalize lipid profiles and endothelial dysfunction during hyperlipidemia.

INTRODUCTION

The problem of hypercholesterolemia is being of much interest because an elevated concentration of lipoproteins can develop chest pain, heart attack, and accelerate the development of atherosclerosis with its dual sequel of thrombosis and infraction [1]. Moreover, hyperlipidemia, particularly hypercholesterolemia is a major cause of atherosclerosis and atherosclerosis associated conditions including coronary heart disease, ischemic cerebrovascular disease and peripheral vascular disease [2]. In general, monotherapy with a pharmacologic agent should be attempted first, together with dietary adjustments. Combination treatment may be required for refractory severe hypertriglyceridemia, but should be attempted only with caution and frequent monitoring of serum concentrations of creatine kinase, transaminases and creatinine [3]. Hypercholesterolemia, defined as excessively high plasma cholesterol levels, has emerged as a strong risk factor for cardiovascular disease (CVD). As high total cholesterol levels are considered to be a major independent risk factor for development of coronary artery disease, considerable attention has been directed

toward evaluating the impact and mechanisms of cholesterol lowering therapies and interventions for cardiovascular outcomes [4-5].

As known, Fibric acid derivatives such as bezafibrate are the mainstay in hypertriglyceridemia treatment [6]. These fibrates can reduce plasma triglyceride levels by up to 50% and raise plasma HDL-C concentrations as much as 20% [7]. The complex mechanism of action of fibrates includes modulation of the activity of peroxisome proliferator–activated receptor-α in the liver, with reduced hepatic secretion of VLDL and increased lipolysis of plasma triglycerides [7]. Fibrates reduce the quantity of small, dense LDL particles and increase HDL-C [8]. So, it is better to treat hyperlipidemia with safe and less side effect drugs. Bezafibrate is widely used as anti-hyperlipidemic drug in human therapy. Based on these facts our study was designed to evaluate the hypolipidemic effect of bezafibrate at various doses on endothelium derived substances and hyperlipidemia related changes as total cholesterol, triacylglecerol, high density lipoprotein-cholesterol (HDL-C), low density lipoprotein-cholesterol (LDL-C), apolipoprotein B , endothelin -1 , histamine, nitric oxide (NO), interleukin-6 (IL-6), creatinine and alanine amino transferase (ALT).

MATERIALS AND METHODS

Animals and chemicals

Male white albino rats, 6 weeks age and weighting (140 – 150g) were used in the experiment. Rats were housed in separate metal cage with free access to water. Rats were kept under constant and nutritional environmental condition throughout the experiment. Rats were left for a week before beginning of experiment for acclimatization. Cholesterol and coconut oil were purchased from El-Goumhouria Co. for Trading Chemicals, Egypt. Hyperlipidemia induced by continuous supplementation of high fat (coconut oil 2% wt/wt) and high cholesterol (1% wt/wt) diet. Bezafibrate was obtained from EIPICO Pharmaceutical Co., Egypt. Bezafibrate was dissolved in 20% ethanol to be used at doses 50, 100 and 100 mg/kg.bw/day orally. All measured kits were purchased from Sigma Aldrich Co. USA.

Experimental design and sampling

Rats were divided into 3 groups (10 per each). Group 1 was fed on normal diet and served as control group. Group 2 was fed on high fat diet for 14 weeks. Group 3 was fed on normal diet and bezafibrate at a dose of 100 mg/kg/day. Another 4^{th} group (30 rats) were hyperlipidemic and were subdivided into 3 groups (10 rats each). Group 3A is hyperlipidemic and bezafibrate 50 (fed high fat diet plus bezafibrate at a dose 50 mg/kg/day). Group3B is hyperlipidemic and bezafibrate 100 (fed high fat diet plus bezafibrate at a dose 100 mg/kg/day). Group3C is hyperlipidemic and bezafibrate 200 (fed high fat diet plus bezafibrate at a dose 200 mg/kg/day). At the end of experimental procedures heparinized blood samples were collected from animals after 14 weeks (time of hypercholesterolemia) and at 2, 4 and 6 weeks from the beginning of bezafibrate administration. The samples were collected in the morning after overnight fasting. Plasma was separated by centrifugation at 3000 r.p.m for 10 minutes. The clear obtained plasma was kept in deep freezer at -20 °C until assayed.

Biochemical Assays

Kits for total cholesterol, triacylglecerol (TG), high-density lipoprotein-cholesterol (HDL-C), low-density lipoprotein-cholesterol (LDL-C), apolipoprotein B, endothelin-1, histamine, nitric oxide (NO), IL-6, creatinine and ALT were assayed spectrophotmetrically.

Statistical analysis

Statistical analysis was done using SPSS software version 15. The inter-group variation was measured by one way analysis of variance (ANOVA) followed by Post Hoc LSD test. Results were expressed as means ± SEM. The mean difference is significant at the $P<0.05$ level.

RESULTS

Effect of high cholesterol diet on lipid profiles in rats

The results in table 1, 2 and 3 revealed that rats fed hyperlipidemic diet showed significant increase in plasma lipid profiles as total cholesterol, triglyceride, LDL and apolipoprotein B (APOB), and significant decrease in HDL compared with control group at 2, 4, and 6 weeks after feeding. Moreover, no significant difference between control fed rats and control fed rats plus bezafibrate (100 mg/kg.bw) was observed.

Effect of bezafibrate on hyperlipidemic fed rats

To examine the effect of bezafibrate on changes induced by feeding rats high cholesterol diet, bezafibrate was given in 3 different doses 50, 100 and 200 mg/kg.bw/day. As seen in table 1, 2 and 3, bezafibrate administration significantly decreased plasma levels of total cholesterol, triglycerides, LDL and apolipoprotein B with significant increase in high density lipoprotein after 2, 4 and 6 weeks compared to hyperlipidemic non treated group.

Effect of bezafibrate on endothelial derived substances in hyperlipidemic rats.

As seen in table 1, 2 and 3, hyperlipidemic diet showed

a significant decrease of nitric oxide (NO) and a significant increase endothelin-1 when compared with control group. Bezafibrate administration caused significant decrease in endothelin-1after 2, 4 and 6 weeks of administration, and partial normalization in nitric oxide concentration after when compared to hyperlipidemic non treated group.

Effect of bezafibrate on IL-6, histamine, alanine transaminases and creatinine in hyperlipidemic rats

Table 1, 2 and 3 revealed that rats fed on hyperlipidemic diet showed a significant increase in IL-6, histamine, ALT and creatinine when compared to control group. Moreover, bezafibrate administration at different doses (table 1, 2 and 3) induced, a significant normalization in IL-6, histamine, ALT and creatinine levels at 2, 4 and 6 weeks when compared to hyperlipidemic non treated group in dose dependent manner.

Table 1. Effect of 2 weeks administration of bezafibrate on some biochemical blood parameters of hyperlipidemic rats

	Chol. (mg/dl)	TG (mg/dl)	HDL-C (mg/dl)	LDL-C .(mg/dl)	Apo(B) (mg/dl)	NO (nmol/l)	Endothelin-1 (ng/dl)	Histamine (ng/dl)	IL-6 (pg/mL)	ALT (IU/mL)	Creatinine (mg/dl)
Control	147.8^{c} ± 0.012	82.7^{a} ± 0.002	40.1^{a} ± 0.0009	133.3^{c} ± 0.003	105.5^{d} ± 0.003	0.2^{a} ± 0.008	1.86^{d} ± 0.006	0.6^{d} ± 0.001	9.33^{c} ± 0.005	11.66^{b} ± 0.001	0.85^{d} ± 0.02
Hyperlipidemic	218.4^{a} ± 0.006	198.5^{a} ± 0.003	23.5^{a} 23.5 ± 0. 009	207.4^{a} ±0.003	204.3^{a} 0.004±	0.17^{a} 0.008±	2.3^{d} 0.0001±	2.2^{a} 0.004	18.64^{a} 0.002±	20.00^{a} 0.005±	2.06^{a} 0.08±
Control + Beza100	150.7^{c} ± 0.009	100.75.3^{a} ± 0.002	35.2^{b} ± 0.0006	124.6^{c} ± 0.005	108.3^{d} ± 0.004	0.19^{a} ± 0.005	1.38^{a} ± 0.001	0.58^{d} ± 0.001	9.37^{c} ± 0.003	10.0^{b} ± 0.005	0.85^{d} ± 0.02
Hyperlipidemic + Beza 50 (mg/kg/day)	194.8^{b} ± 0.005	182.4^{a} ± 0.002	23.1^{d} ± 0.0008	155.2^{c} ± 0.005	167.2^{b} ± 0.001	0.17^{a} ± 0.005	1.01^{b} ± 0.002	2.0^{b} ± 0.003	18.2^{a} ± 0.005	19.66^{a} ± 0.002	1.93^{a} ± 0.02
Hyperlipidemic + Beza 100 (mg/kg/day)	196.2^{b} ± 0.005	135.8^{a} ± 0.003	26.1^{c} ± 0.0005	158.2^{c,b} ± 0.002	149.5^{c} ± 0.002	0.18^{a} ± 0.002	1.01^{b} ± 0.006	2.0^{b} ± 0.001	15.30^{b,a} ± 0.005	18.33^{a} ± 0.001	1.7^{b} ± 0.05
Hyperlipidemic + Beza 200 (mg/kg/day)	182.4$^{a;b}$ ± 0.002	157.3^{a} ± 0.002	33.1^{b} ± 0.0007	144^{a,c} ± 0.003	111^{d} ± 0.002	0.19^{a} ± 0.002	1.01^{c} ± 0.009	1.7^{c} ± 0.005	14.25^{b} ± 0.001	16.00^{a} ± 0.002	1.4^{c} ± 0.09

Data are presented as (mean ± S.E.). S.E = Standard error for 10 rats per each group. Mean values with different superscript letters in the same column are significantly different at (P<0.05).

Table 2. Effect of 4 weeks administration of bezafibrate on some biochemical blood parameters of hyperlipidemic rats

	Chol. (mg/dl)	TG (mg/dl)	HDL-C .(mg/dl)	LDL-C .(mg/dl)	Apo(B) (mg/dl)	NO (nmol/l)	Endothelin-1 (ng/dl)	Histamine (ng/dl)	IL-6 (pg/mL)	ALT (IU/ML)	Creatinine (mg/dl)
Control	152.4^{c} ± 0.013	89.8^{a} ± 0.003	43.3^{a} ±SE 0.001	133.2^{d,c} ± 0.002	102.2^{c} ± 0.001	0.2^{a} ± 0.006	1.3^{d} ± 0.006	0.6^{e} ± 0.02	9.85^{d} ± 0.003	11.6^{d} ± 0.001	0.85^{d} ± 0.02
Hyperlipidemic	246.8^{a} ± 0.06	207.8^{a} ± 0..005	19.8^{e} ±0.006	21.6^{a} ± 0..006	207.4^{a} ± 0..004	0.17^{d} ± 0.009	2.9^{a} ± 0..008	2.3^{a} ± 0..07	20.61^{a} ± 0..002	25.66^{b,a} ± 0..001	2.27^{a} ± 0.006
Control + Beza100	148^{c} ±.07	71.3^{a} ± 0.003	40.5^{b} ± 0.0006	126.9^{d} ± 0.005	106.1^{c} ± 0.002	^{a} 0.24 ± 0.01	1.2^{d} ± 0.008	0.5^{e} ± 0.01	08.65^{d} ± 0.002	19.00^{b,c} ± 0.005	0.84^{d} ± 0.06
Hyperlipidemic + Beza 50 mg/kg/day	186.8^{b} ± 0.005	168.2^{a} ± 0.003	28.1^{d} ± 0.001	153^{b} ± 0.007	139.5^{b} ± 0.003	0.18^{d,c} ± 0.004	1.0^{b} ± 0.003	2.0^{b} ± 0.01	15.3^{b} ± 0.005	20.00^{c} ± 0.001	1.8^{b} ± 0.06
Hyperlipidemic + Beza 100 mg/kg/day	183^{b} ± 0.007	168.2^{a} ± 0.003	30.2^{d} ± 0.002	143.4^{d,c} ± 0.001	134.3^{b} ± 0.004	0.19^{c} ± 0.0008	1.8^{c} ± 0.001	1.6^{c} ± 0.07	12.25^{b,c} ± 0.001	17.66^{b,c} ± 0.001	1.5^{c} ± 0.009
Hyperlipidemic + Beza 200 mg/kg/day	167^{c,b} ± 0.003	137.5^{a} ± 0.002	34.9^{c} ± 0.0006	133.5^{d,c} ± 0.004	113.6^{c} ± 0.006	0.23^{b} ± 0.003	1.2^{c} ± 0.009`	1.5^{d} ± 0.01	11.96^{c} ± 0.006	15.33^{a} ± 0.008	1.3^{c} ± 0.09

Data are presented as (mean ± S.E.). S.E = Standard error for 10 rats per each group. Mean values with different superscript letters in the same column are significantly different at (P<0.05).

Table 3. Effect of 6 weeks administration of bezafibrate on some biochemical blood parameters of hyperlipidemic rats

	Chol. (mg/dl)	TG (mg/dl)	HDL-C .(mg/dl)	LDL-C .(mg/dl)	Apo(B) (mg/dl)	NO (nmol/l)	Endothelin-1 (ng/dl)	Histamine (ng/dl)	IL-6 ((pg/mL)	ALT (IU/ML)	Creat .(mg/dl)
Control	145 ^{d,c} ± 0.01	84.7^{d} ± 0.003	41.3^{a} 0.0005	127.3 ^{c} ± 0.003	101.1^{d} ± 0.0005	0.2 ^{b} ± 0.006	1.4 ^{a} ± 0.004	0.6 ^{e} ± 0.02	9.85 ^{c,d} ± 0.3	11.66 ^{c} ± 0.001	0.85 ^{e} ± 0.02
Hyperlipidemic	260.8^{a} ± 0.008	230.3^{a} ± 0.006	18.2^{a} ± 0.0007	224.8 ^{a} ± 0.006	209.2^{a} ± 0.005	0.14 ^{c} ± 0.004	2.2 ^{c} ± 0.003	2.4 ^{a} ± 0.09	22.34 ^{a} ± 0.17	25.66 ^{b} ± 0.001	2.27 ^{a} ± 0.001
Control + Beza100	124^{d} ± 0.005	65.7^{e} ± 0.002	42.3^{a} ± 0.0007	125.9 ^{c} ± 0.003	105.1^{c} ± 0.004	0.24 ^{a} ± 0.01	.2.0 ^{c} ± 0.001	0.6 ^{e} ± 0.01	8.7 ^{d} ± 0.24	13.66 ^{c} ± 0.008	0.846 ^{e} ± 0.02
Hyperlipidemic + Beza 50 mg/kg/day	173.8^{b} ± 0.006	155.3^{b} ±0.003	33.2^{a} ± 0.0009	143.4 ^{b} ± 0.001	136.3^{b} ± 0.0006	0.19 ^{b} ± 0.005	1.4^{a} ± 0.01	1.5 ^{b} ± 0.04	14.25 ^{b} ± 0.11	16.66 ^{b} ± 0.001	1.4 ^{b} ± 0.09
Hyperlipidemic + Beza 100 mg/kg/day	161.6 ^{c,b} ± 0.005	148.1^{b} ±.002	35.6^{a} ± 0.001	133.5 ^{c,b} ± 0.004	128.6^{c} ± 0.006	0.2 ^{b} ± 0.0008	1.6 ^{c,b} ± 0.002	1.3 ^{c} ± 0.01	11.96 ^{b,c} ± 0.06	13.33 ^{a} ± 0.008	1.23 ^{c} ± 0.003
Hyperlipidemic + Beza 200 mg/kg/day	142.4 ^{d,c} ± 0.004	127^{c} ± 0.003	37.9^{a} ± 0.007	124.6 ^{c} ± 0.005	115.3^{d} ± 0.005	0.23 ± 0.004	1. 2 ^{c} ± 0.001	1.1 ^{d} ± 0.02	10.13 ^{c,d} ± 0.013	11.33 ^{c} ± 0.008	1. 1^{d} ± 0.008

Data are presented as (mean ± S.E.). S.E = Standard error for 10 rats per each group. Mean values with different superscript letters in the same column are significantly different at (P<0.05).

DISCUSSION

The findings of the present study showed that hypercholesterolemia induced alteration in lipid profiles (VLDL, LDL and TG). Many reviews like that of Selvin and Erlinger [5], Ros [7] and Huijgen [9] discussed the dangerous effects of hypercholesterolemia on cardiovascular system but no direction for the treatment. Unlike our study, we focused on the problem and gave attention for the treatment through the usage of bezafibrate at various doses. Saturated fats mostly increase the concentration of cholesterol, LDL, and to a less extent VLDL levels. The increase in LDL levels produced by saturated fats seems to be related mainly to the decrease in catabolic rate. Moreover, Csont [1] demonstrated that elevated levels of LDL, appearing in the circulation upon feeding the hyperlipidemic diet, is mainly derived from LDL is up taken by the receptor in liver and extra hepatic tissue. The production of LDL exceeds the capacity of LDL receptors i.e. efflux of cholesterol from the liver is more than influx [1]. This could be explaining the elevated serum LDL levels observed in our study. The lipid-lowering effects of bezafibrate and reduction of the risk of cardiovascular events in dyslipidemic patients with coronary artery disease is the target of many physicians. The molecular mechanism underlying the triglyceride-reducing effect of bezafibrate is due to in part to the induction of lipoprotein lipase activity mediated by the activation of peroxisome proliferators-activated receptor α (PPAR-α) [10]. Furthermore, Pennacchio *et al*., [12], demonstrated that, the activation of PPAR-□ by bezafibrate induces lipoprotein lipase in the liver, which plays a key role in triglyceride-rich lipoprotein catabolism. It also affects the binding and clearance in liver of remnant lipoprotein particles by LDL-related receptors. The fibrate-induced increased lipoprotein catabolism may also be related to a PPAR mediated lower hepatic Apo CIII synthesis [11]. It is well known that Apo CIII delays the catabolism of triglyceride rich lipoproteins, since it inhibits their binding to the endothelial surface and lipolysis by lipoprotein lipase and interferes with Apo E-mediated receptor clearance of remnant particles from plasma [12]. Finally, a new additional mechanism contributing to the fibrate-induced reduction in triglyceride rich-lipoproteins was also proposed is a recently discovered lipoprotein that influences plasma triglyceride levels [13]. Moreover, Fruchart *et al* [14] demonstrated that fibrates increase HDL-C may be related to accelerate triglyceride-rich lipoprotein catabolism leading to an increase in pre-HDL, which is the key acceptor of cholesterol for peripheral cells during reverse cholesterol transport.

The importance of the endothelium in vessel reactivity and subsequent abnormalities of arterial responses has fostered the use of the term "endothelial dysfunction". Importantly, endothelial dysfunction includes alterations in any of the functional roles. The endothelium plays role in maintenance of normal tone, limiting thrombosis, and protecting against leukocyte adhesion [15]. Reactivity of the arterial wall is controlled in part by biomechanical inputs through blood flow and blood pressure. Endothelin-1 (ET-1) promotes blood vessel constriction, as evident with the decrease in blood flow after endothelial ET-1 release [16]. ET-1 also induces smooth muscle cells proliferation. These ET-1 responses are countered by endothelial release of nitric oxide (NO), which stimulates vasodilatation, thus increasing blood flow [17-18]. Endothelial nitric oxide synthase (eNOS) is the key enzymatic step in producing NO, a secondary messenger produced by EC that can inhibit NF-κB activation and attenuate endothelial inflammatory responses, including adhesion molecule expression. The increase in ET-1 production and decrees in NO production, results in vasoconstriction, abnormal

vasomotor responses and promotion of atherosclerosis [19].

The results of this study are parallel with that of Stokes *et al.,* [20] where hypercholesterolemia leads to an inflammatory response within the microvasculature that reflected by endothelial cell activation, leukocyte recruitment, rolling and adherence, as well as platelet activation and adhesion. Moreover, monocyte chemo tactic protein-1 (MCP-1), IL-6 and histamine are important in hypercholesterolemic patients, acting to increase monocyte recruitment and adherence which leads to wall remodeling. Also, Stapleton et al. stated that macrophages derived from monocytes, begin to accumulate LDL and oxidized LDL (ox-LDL) which develop into foam cells between the basal lamina of the endothelium and the smooth muscle layer [21]. These foam cells lead to the production of numerous inflammatory and oxidative stress markers, cytokines, chemokines, and growth factors which aggravate the balance of endothelial equilibrium leading to vascular dysfunction. The increase in IL-6 and histamine is interesting point as Gervois and his colleagues [22] reported that bezafibrate reduced IL-6 and histamine possibly through the activation of the peroxisome proliferator-activated receptor (PPAR)-α with a consequent reduction of NF-κB activation. These discrepancies may be due to the differences in inflammatory status at baseline, duration of the study period, or the lack of control group reported in their study. The hyperlipidemic diet induced a significant increase ALT activity and creatinine compared with control group. Those findings may be attributed to the increase in LDL levels which is regulated by LDL receptors [23]. The elevation of aminotransferases levels has been frequently observed after the administration of bezafibrate and this phenomenon is considered to be non-pathological because bezafibrate activates the gene expression of the aminotransferases. Recently, fibrate has been used not only for hypercholesterolemia but also for primary biliary cirrhosis [24-25]. In conclusion, these results indicate that bezafibrate normalized the biochemical changes induced during hyperlipidemia and its accompanied endothelial dysfunction in rats and suggested that other studies are needed in order to directly translate bezafibrate effects in humans.

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