Fenofibrate Reduces Age-related Hypercholesterolemia in Normal Rats on a Standard Diet

Ying Han¹, Mi-Hyang Do¹, Mi Sun Kim¹, Eunhui Seo¹, Mi-Kyoung Park², Duk Kyu Kim², Hye-Jeong Lee^{1,†}, and Su-Yeong Seo^{3,*}

Departments of ¹Pharmacology, ²Internal Medicine, ³Microbiology, Dong-A University College of Medicine, Medical Science Research Center, Busan 602-714, Korea

Plasma cholesterol is increased in normal aging in both rodents and humans. This is associated with reduced elimination of cholesterol and decreased receptor-mediated clearance of plasma low-density lipoprotein (LDL) cholesterol. The aims of this study were: (1) to determine age-related changes in plasma lipid profiles, and (2) to determine the effect of fenofibrate, an activator of peroxisome proliferator activated receptor alpha (PPAR α), on plasma lipid profiles in normal rats on a standard diet. Male Sprague-Dawley (SD) rats (n=15) were fed standard chow and water from 10 to 25 weeks of age. During that period, we measured daily food intake, body weight, fasting and random blood glucose levels, plasma total cholesterol (TC), triglycerides (TG), and free fatty acid (FFA) levels. At 20 weeks of age, all rats were randomly divided into two groups: a fenofibrate group (in which rats were gavaged with 300 mg/kg/day of fenofibrate) and a control group (gavaged with water). Fenofibrate treatment lasted 5 weeks. There were no significant changes in daily food intake, blood glucose, and plasma TG level with age. Body weight, plasma TC, and FFA levels were significantly increased with age. Fenofibrate significantly decreased plasma concentrations of TC and FFA, which had been increased with age. However, fenofibrate did not influence the plasma concentration of TG, which had not increased with age. These results suggest that fenofibrate might have a novel role in preventing age-related hypercholesterolemia in SD rats on a normal diet.

Key Words: Fenofibrate, PPAR α , Sprague-Dawley rats, Hypercholesterolemia, Aging

INTRODUCTION

A number of metabolic changes occur with normal aging in both animals and humans. Phenomena such as reduced physical activity, decreased oxygen consumption, redistribution of body tissues with a relative increase in adipose over muscle mass, decreased insulin sensitivity, and increased blood pressure may all contribute to the acceleration of atherosclerosis known to occur with age. Of particular interest is the fact that plasma levels of total and LDL-cholesterol are well known to increase with normal aging [1,2]. Elevated plasma LDL-cholesterol levels represent one of the key causal factors for the development of atherosclerosis and subsequent coronary heart disease (CHD) with its clinical manifestations of angina, heart failure, arrhythmias, myocardial infarction, transient ischemic attack, and ischemic stroke [3,4].

It has been demonstrated that hypercholesterolemia is

related to aging. Almost 25% of men and 42% of women older than 65 years have increased plasma TC levels [5]. Another study showed that 12-month-old Wistar rats have an increased TC level compared with a 3-month-old group [6]. The mechanisms behind this age-related increase in plasma cholesterol are still incompletely understood.

Peroxisome proliferator-activated receptor (PPAR) α is a member of the family of nuclear transcription factors that act as lipid sensors and regulate lipid metabolism [7-9]. A PPAR α activator, fenofibrate, is known to promote fatty acid oxidation and to lower circulating lipids, and has been used as a hypolipidemic drugs [10,11]. Several studies have examined the effects of fenofibrate on daily food intake, body weight, and lipid profile in rodent models of obesity. As they reported, fenofibrate reduced weight gain and plasma TG levels of fatty Zucker rats [12], and reduced weight gain and adiposity in Wistar rats in which a high-fat diet had induced obesity [13]. Fenofibrate also decreased food intake, body weight, plasma TG and FFA levels in obese Otsuka Long-Evans Tokushima Fatty rats [14]. Although these studies indicate that fenofibrate reduces food intake, body weight gain, and plasma lipid levels in obese rodent

Received March 9, 2010, Revised April 9, 2010,
Accepted April 16, 2010

*Corresponding to: Su-Yeong Seo, Department of Microbiology, Dong-A University College of Medicine, Dongdaeshin-dong, Seo-gu, Busan 602-714, Korea. (Tel) 82-51-240-2931, (Fax) 82-51-242-9750, (E-mail) syseo@dau.ac.kr

[†]Co-corresponding author: Hye-Jeong Lee, (Tel) 82-51-240-2859, (Fax) 82-51-241-0778, (E-mail) hjlee@dau.ac.kr

ABBREVIATIONS: TC, total cholesterol; TG, triglyceride; FFA, free fatty acid; SD, Sprague-Dawley; PPAR, peroxisome proliferator-activated receptor; OLETF, Otsuka Long-Evans Tokushima Fatty rats; LETO, Long-Evans Tokushima rats.

models, few studies have examined the effect of fenofibrate on age-related hyperlipidemia in rodent models on a standard diet.

In this study, we investigated age-related changes in plasma lipid profiles and determined whether fenofibrate improves the lipid profile and reduces age-related hyperlipidemia in normal rats on a standard diet.

METHODS

Animals and treatment

Eight-week-old male Sprague-Dawley rats (n=15) (Central Lab Animal Inc., Korea) were kept in individual cages and fed freely with standard rat chow and water. All rats were cared for during the entire period of experimentation in ac-

cordance with the Guidelines of Animal Experiments recommended by the Korean Academy of Medical Sciences.

When they were 20 weeks of age, all rats were randomly divided into two groups. One group (n=8) was gavaged with 300 mg/kg/day of fenofibrate (Green Cross, Korea) for 5 weeks. The other group (n=7) was gavaged with water for the same period.

Measurement of daily food intake, body weight, and blood glucose

Daily food intake and body weight were measured twice a week during the experimental period. Fasting blood sugar (FBS) levels were measured every other week using blood drawn from the tail vein after overnight fasting. Random blood sugar levels were also measured every other week using blood drawn from the tail vein.

Fig. 1. (A) Effect of fenofibrate on daily food intake. There was no significant change in daily food intake from 10 to 25 weeks of age. There was no significant difference in daily food intake between the fenofibrate and control groups at 25 weeks of age. (B) Effect of fenofibrate on body weight. Body weight of SD rats increased with age. There was no significant difference in body weight between fenofibrate and control groups at 25 weeks of age. Values represent means $\pm$ SEM of the control group (n=7) and the fenofibrate group (n=8). Feno, fenofibrate; Tx, treatment.

Fig. 2. Analysis of blood glucose levels. (A) Effect of fenofibrate on fasting blood glucose. There was no significant change in fasting blood glucose from 10 to 25 weeks of age. There was no significant difference in fasting blood glucose between the fenofibrate and control groups at 25 weeks of age. (B) Effect of fenofibrate on random blood glucose levels. There was no significant change in random blood glucose from 10 to 25 weeks of age. There was no significant difference in random blood glucose between the fenofibrate and control groups at 25 weeks of age. Values represent means $\pm$ SEM of the control group (n=7) and the fenofibrate group (n=8). Feno, fenofibrate; Tx, treatment.

Measurement of plasma lipid profiles

Plasma was collected by centrifugation of heparinized blood at $2,000\times g$ for 15 minutes. Plasma total cholesterol (TC) and triglyceride (TG) levels were analyzed with rat TC and TG kits (Asan Pharmaceutical, Korea). The plasma free fatty acid (FFA) level was analyzed with rat FFA kits (Shinyang Diagnostics, Korea).

Statistics

The significance of differences between groups was analyzed by an unpaired two-tailed Student's *t* test. All results are expressed as mean values $\pm$ SEM. Differences were considered significant when their *p* values were less than 0.05.

RESULTS

Effect of fenofibrate on food intake and body weight

SD rats consumed about 24 ± 1 g of standard rat chow every day from 10 to 25 weeks of age, and there was no significant change in daily food intake. Five weeks of fenofibrate treatment did not alter daily food intake (Fig. 1A). Body weights of the SD rats increased from 363 ± 4 g at 10 weeks of age to 503 ± 14 g at 25 weeks of age. Five weeks of fenofibrate treatment did not alter body weight (Fig. 1B).

Effect of fenofibrate on blood glucose

There was no difference between fasting and random blood glucose from 10 to 20 weeks of age. After 5 weeks of fenofibrate treatment, there was no difference in fasting or random blood glucose between the fenofibrate and control groups (Fig. 2).

Change in lipid profile with age

The plasma TC level was significantly increased with age. It was 75 ± 5 mg/dl at 10 weeks of age, 100 ± 10 mg/dl at 15 weeks of age, 116 ± 7 mg/dl at 20 weeks of age, and 125 ± 4 mg/dl at 25 weeks of age (Fig. 3A). The plasma TG level was not altered with age. It was 50 ± 5 mg/dl at 10 weeks of age, 61 ± 5 mg/dl at 15 weeks of age, 58 ± 3 mg/dl at 20 weeks of age, and 51 ± 5 mg/dl at 25 weeks of age (Fig. 3B) the plasma FFA level was significantly increased with age. It was 392 ± 13 μ Eq/l at 10 weeks of age, 456 ± 16 μ Eq/l at 15 weeks of age, 484 ± 14 μ Eq/l at 20 weeks of age, and 475 ± 21 μ Eq/l at 25 weeks of age (Fig. 3C).

Effect of fenofibrate on the lipid profile

There was no difference in plasma TC level between the two groups before fenofibrate treatment. After five weeks of fenofibrate treatment, the plasma TC level of the fenofibrate group was significantly decreased compared with that of the control group (81 ± 10 mg/dl vs. 125 ± 4 mg/dl, $p < 0.05$) (Fig. 4A). There was no difference in the plasma TG level between the two groups before fenofibrate treatment. Five weeks of fenofibrate treatment did not change plasma TG levels compared with the control group (58 ± 6 mg/dl vs. 51 ± 5 mg/dl, $p=0.21$) (Fig. 4B). There was no difference in plasma FFA level between the two groups before fenofi-

brate treatment. After five weeks of fenofibrate treatment, the plasma FFA level of the fenofibrate group was significantly decreased compared with that of the control group (311 ± 12 μ Eq/l vs. 475 ± 21 μ Eq/l, $p < 0.05$) (Fig. 4C).

Fig. 3. Changes in plasma TC, TG, and FFA levels with age. (A) Plasma TC level was significantly increased from 10 to 25 weeks of age. (B) The plasma TG level was not changed from 10 to 25 weeks of age. (C) Plasma FFA levels were significantly increased from 10 to 25 weeks of age. Values represent means $\pm$ SEM of the SD rats ($n=15$). * $p < 0.05$ vs. 10 weeks of age; ** $p < 0.01$ vs. 10 weeks of age.

DISCUSSION

Fig. 4. Effects of fenofibrate on plasma TC, TG, and FFA levels. There was no difference in plasma TC, TG, and FFA levels between the fenofibrate and control groups before fenofibrate treatment. (A) After 5 weeks of fenofibrate treatment, the plasma TC level of the fenofibrate group was significantly lower than that of the control group. (B) After 5 weeks of fenofibrate treatment, there was no significant difference in plasma TG level between the fenofibrate and control groups. (C) After 5 weeks of fenofibrate treatment, the plasma FFA level of the fenofibrate group was significantly lower than that of the control group. Values represent means $\pm$ SEM of the control (n=7) and fenofibrate (n=8) groups. *p<0.05 vs. control group.

In this study, we found that age-related lipid profiles showed increased plasma TC and FFA levels with age, while plasma TG levels were not changed with age. Fenofibrate treatment decreased plasma TC and FFA levels but did not alter plasma TG levels. Fenofibrate, an activator of PPAR α , is known to mainly decrease plasma TG levels. Although there are many studies demonstrating that fenofibrate decreases plasma TG levels [12,14,15], there was no difference in plasma concentrations of TG between the fenofibrate and control groups in this study. Most of the studies mentioned above studied obese rodents or rodents on a high-fat diet, subjects who have already developed dyslipidemia. In contrast, the SD rats we used in our study were fed with standard chow and there was no significant change in plasma TG level during aging. Thus, we cautiously suggest that fenofibrate does not alter plasma TG levels, which are not changed with age, while it does decrease plasma TG levels, which are already increased over the normal range. On the other hand, plasma TC and FFA levels did increase with aging, and fenofibrate treatment significantly decreased plasma TC and FFA levels. Although fenofibrate mainly decreases plasma TG level, it also reduces plasma TC levels in humans and rodents that have increased TC and FFA levels [10,16,17]. Our study suggests that fenofibrate treatment decreases plasma concentrations of lipids such as TC and FFA because they were significantly increased with aging.

The molecular mechanisms for the effects of aging and fenofibrate on lipid are not established. During aging in rats, hypercholesterolemia occurs in concert with full activation, a lowered degradation rate and an unchanged level of the rate limiting cholesterol biosynthesis enzyme, 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMGCR). The molecular base of the unchanged HMGCR level and the lowered degradation rate in aged rats is not clear. Pallottini et al reported that the proteins involved in cholesterol homeostasis—HMGCR, Insig-induced gene (Insig) protein, sterol regulatory element binding protein (SREBP), SREBP cleavage activating protein (SCAP), and low density lipoprotein receptor (LDLR)—are modified in age-related hypercholesterolemia [18]. Even though we did not examine the molecular mechanisms underlying changing cholesterol metabolism with aging, our group is now investigating the effects of fenofibrate on the factors involved.

The duration of drug treatment has varied according to the purpose of the experiment. Usually for experiments with rodents, the period of fenofibrate treatment is from 3 days [19] to 8 weeks [20]. In the present study, we determined that treatment duration of 5 weeks was optimum. However, further study will be needed to examine the effects of short-term and long-term treatments with fenofibrate.

We and others reported that fenofibrate decreases body weight and daily food intake in obese rats or in rats on a high-fat diet [12,14]. But, in the present study, fenofibrate did not affect body weight or daily food intake. Perhaps this lack of effect occurred because the duration of fenofibrate treatment in this study was not long enough. Nevertheless, it is convincing that fenofibrate did not affect body weight and daily food intake of normal rats on a standard diet. Another interesting finding in this study was that daily food intake did not change while body weight increased from 10 to 25 weeks of age. The same phenomenon was observed in our other study [15]. Although it may be attrib-

uted to decreased physical activity or decreased energy consumption with age, future studies are required to determine the reason why daily food intake does not increase while body weight increases during aging.

In conclusion, fenofibrate decreases age-related hypercholesterolemia in SD rats on a normal diet, while it does not alter the plasma TG level, which does not increase with age. We suggest that fenofibrate might be a preventive agent for age-related hypercholesterolemia.

ACKNOWLEDGEMENTS

This article was supported by Dong-A University Research Fund in 2007.

REFERENCES

1. Ericsson S, Eriksson M, Vitols S, Einarsson K, Berglund L, Angelin B. Influence of age on the metabolism of plasma low density lipoproteins in healthy males. *J Clin Invest.* 1991;87:591-596.
2. Ericsson S, Berglund L, Frostegard J, Einarsson K, Angelin B. The influence of age on low density lipoprotein metabolism: effects of cholestyramine treatment in young and old healthy male subjects. *J Intern Med.* 1997;242:329-337.
3. Levine GN, Keaney JF Jr, Vita JA. Cholesterol reduction in cardiovascular disease: Clinical benefits and possible mechanisms. *N Engl J Med.* 1995;332:512-521.
4. Bays HR, Dujovne CA, Lansing AM. Drug treatment of dyslipidemias: Practical guidelines for the primary care physician. *Heart Dis Stroke.* 1992;1:357-365.
5. Grundy SM, Cleeman JI, Rifkind BM, Kuller LH. Cholesterol lowering in the elderly population. Coordinating Committee of the National Cholesterol Education Program. *Arch Intern Med.* 1999;159:1670-1678.
6. Trapani L, Violo F, Pallottini V. Hypercholesterolemia and 3-hydroxy-3-methylglutaryl coenzyme A reductase regulation in aged female rats. *Exp Gerontol.* 2010;45:119-128.
7. Willson TM, Wahli W. Peroxisome proliferator-activated receptor agonists. *Curr Opin Chem Biol.* 1997;1:235-241.
8. Issemann I, Green S. Activation of a member of the steroid hormone receptor superfamily by peroxisome proliferators. *Nature.* 1990;347:645-650.
9. Bays H, Mandarino L, DeFronzo RA. Role of the adipocyte, free fatty acids, and ectopic fat in pathogenesis of type 2 diabetes mellitus: peroxisomal proliferator-activated receptor agonists provide a rational therapeutic approach. *J Clin Endocrinol Metab.* 2004;89:463-478.
10. Najib J. Fenofibrate in the treatment of dyslipidemia: a review of the data as they relate to the new suprabioavailable tablet formulation. *Clin Ther.* 2002;24:2022-2205.
11. Francis GA, Annicotte JS, Auwerx J. PPAR-alpha effects on the heart and other vascular tissues. *Am J Physiol Heart Circ Physiol.* 2003;285:1-9.
12. Chaput E, Saladin R, Silvestre M, Edgar AD. Fenofibrate and rosiglitazone lower serum triglycerides with opposing effects on body weight. *Biochem Biophys Res Commun.* 2000;271:445-450.
13. Mancini FP, Lanni A, Sabatino L, Moreno M, Giannino A, Contaldo F, Colantuoni V, Goglia F. Fenofibrate prevents and reduces body weight gain and adiposity in diet-induced obese rats. *FEBS Lett.* 2001;491:154-158.
14. Lee HJ, Choi SS, Park MK, An YJ, Seo SY, Kim MC, Hong SH, Hwang TH, Kang DY, Garber AJ, Kim DK. Fenofibrate lowers abdominal and skeletal adiposity and improves insulin sensitivity in OLETF rats. *Biochem Biophys Res Commun.* 2002;296:293-299.
15. Han Y, Joe Y, Seo E, Lee SR, Park MK, Lee HJ, Kim DK. The hyperleptinemia and ObRb expression in hyperphagic obese rats. *Biochem Biophys Res Commun.* 2010;394:70-74.
16. Klosiewicz-Latoszek L, Szostak WB, Grzybowska B, Bialobrzaska-Paluszkievicz J, Wisniewska B, Stolarska I. Comparison of combined statin-fibrate treatment to monotherapy in patients with mixed hyperlipidemia. *Kardiol Pol.* 2004;60:567-577.
17. Krempf M, Rohmer V, Farnier M, Issa-Sayegh M, Corda C, Sirugue I, Gerlinger C, Masseyeff-Elbaz MF. Efficacy and safety of micronised fenofibrate in a randomised double-blind study comparing four doses from 200 mg to 400 mg daily with placebo in patients with hypercholesterolemia. *Diabetes Metab.* 2000;26:184-191.
18. Pallottini V, Martini C, Cavallini G, Donati A, Bergamini E, Notarnicola M, Caruso MG, Trentalance A. Modified HMG-CoA reductase and LDLr regulation is deeply involved in age-related hypercholesterolemia. *J Cell Biochem.* 2006;98:1044-1053.
19. Kobayashi A, Suzuki Y, Kuno H, Sugai S, Sakakibara H, Shimoi K. Effects of fenofibrate on plasma and hepatic transaminase activities and hepatic transaminase gene expression in rats. *J Toxicol Sci.* 2009;34:377-387.
20. Alvarez de Sotomayor M, Mingorance C, Andriantsitohaina R. Fenofibrate improves age-related endothelial dysfunction in rat resistance arteries. *Atherosclerosis.* 2007;193:112-120.