Original Article

Effects of fenofibrate on plasma and hepatic transaminase activities and hepatic transaminase gene expression in rats

Akio Kobayashi1,2, Yusuke Suzuki1, Hideyuki Kuno1, Shoichiro Sugai1, Hiroyuki Sakakibara2,3 and Kayoko Shimo2,3,4

1Toxicology Research Lab., Central Pharmaceutical Research Institute, JAPAN TOBACCO INC., 23 Nakogi Hadano, Kanagawa 257-0024, Japan
2Graduate School of Nutritional and Environmental Sciences; 3Institute for Environmental Sciences and 4Global COE Program, University of Shizuoka, 52-1 Yada, Suruga-ku, Shizuoka 422-8526, Japan

(RECEIVED April 10, 2009; ACCEPTED May 11, 2009)

ABSTRACT — Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels are widely used as sensitive markers of possible tissue damage, particularly liver toxicity. Lipid-lowering drugs, such as fibrates, slightly increase serum transaminase levels in humans, but there is little evidence that the phenomenon is related to drug-induced liver injury (DILI). Some in vitro studies have indicated that the elevations of serum transaminase activities after treatment of humans with fenofibrate, one of the fibrates, are related to increased transaminase synthesis in the hepatocytes rather than to transaminase leakage from the hepatocytes associated with cell lysis. In this study, male F344/DuCrjCrj (Fischer) rats were treated once with fenofibrate at a dose level of 400 mg/kg and the relationships between the pharmacological effects, blood and hepatic transaminase activities and the gene expression of the transaminases in the liver were investigated. Fenofibrate treatment slightly increased plasma transaminase activities in rats with the findings directly related to the pharmacological action of the drug. The increases were in parallel with increases in hepatic transaminase activities associated with increases in the transaminase genes in the liver and were not considered to be a consequence of hepatotoxicity from the drug. The modification in transaminase gene expression is likely to be secondary to the pharmacological action of fenofibrate. The evidence obtained in our study underlines the importance of gene regulation as a possible alternative mechanism for increased blood transaminase activities.

Key words: Alanine aminotransferase, Aspartate aminotransferase, Induction, Fenofibrate, Rats

INTRODUCTION

Both in clinical and non-clinical studies, serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels are widely used as sensitive markers of possible tissue damage, particularly liver toxicity (Ramaiah, 2007). In the case of drug-induced liver injury (DILI), serum transaminase levels increase up to 100-fold, indicative of marked hepatocyte cell lysis. On the other hand, we sometimes encounter mild and transient increases in serum transaminase levels without any obvious finding indicative of DILI, including alteration of other liver function parameters and degenerative histopathological findings in the liver both in clinical and non-clinical studies for pharmaceuticals.

Lipid-lowering drugs, such as fibrates and statins, slightly increase serum transaminase levels (less than three times the upper limit of normal) in humans, but there is little evidence that the phenomenon is related to DILI (Tolman, 2000). Treatment with some drugs that modify glucose metabolism as their pharmacological action, such as α-glucosidase inhibitors, is sometimes associated with slight and asymptomatic increases in serum transaminase levels in humans (Bomhard, 1996). Toxicology studies on lipid-lowering drugs and α-glucosidase inhibitors have also demonstrated transient increases in blood transaminase levels but no histopathological evidence of hepatotoxicity (Tolman, 2000; Bomhard, 1996), although some non-clinical toxicology studies have revealed that very high doses of statins caused hepatocellular necro-
sis in rabbits (Kornbrust et al., 1989). These clinical and non-clinical findings indicate that mild and asymptomatic transaminase elevations do not necessarily reflect hepatotoxicity. Possible mechanisms for the transaminase elevation include increased transaminase synthesis, decreased transaminase clearance and transaminase leakage from hepatocytes whose membranes may have been altered by changes in their lipid content. Transaminases, both ALT and AST, are some of the key enzymes involved in amino acid/glucose metabolism pathways and play an important role in gluconeogenesis in the liver and kidney (DeRosa and Swick, 1975). Taking the functions of transaminases into account, transaminase synthesis must be altered as well as for other enzymes involved in the amino acid/glucose metabolism pathways if hormonal or nutritional fluctuation, which leads to acceleration or deceleration of gluconeogenesis, occurs. In fact, the expression of the rat cytosolic AST gene in the hepatoma cell line Fao is modified by glucocorticoids, insulin and cAMP (Aggerbeck et al., 1993; Barouki et al., 1989) and by a protein-rich diet or during prolonged fasting (Horio et al., 1988). Further, ALT and AST levels in the serum and organs are increased by these hormonal or nutritional modifications (Katchman and Zipf, 1970; Katsunuma et al., 1966; Ramesh and Pugalendi, 2005; Rosen et al., 1959; Hoffman et al., 1989). Elevation of transaminase activities in the serum or organs associated with hormonal or nutritional modifications are slight in magnitude and are not accompanied by any finding suggestive of tissue damage including hepatocellular necrosis. These nutritional or hormonal aspects for the elevation of transaminase activities have led some researchers to in vitro studies for the investigations of the relationship between transaminase activities and their gene expression in human and animal hepatocytes treated with drugs which modify lipid metabolism as their pharmacological action. Edgar et al. (1998) have reported that fenofibrate increased ALT and AST activities in human HepG2 cells as well as both mRNA levels. Another in vitro study conducted by Tomkiewicz et al. (2004) showed that fenofibrate increased the expression of the cytosolic AST gene in human liver cells. The results of these in vitro studies strongly support the suggestion that the elevation of serum transaminase activities after fenofibrate treatment in humans is related to increased transaminase synthesis, which may be a consequence of the alteration of lipid metabolism in the hepatocytes rather than to transaminase leakage from the hepatocytes associated with cell lysis.

Despite several in vitro approaches for the non-hepatotoxic elevations of transaminase activities with the peroxisome proliferator-activator receptor alpha (PPARα) agonism, in vivo trials have not been reported for the study of the mechanism of the elevation in the transaminase activities in animals or humans treated with PPARα activators. In the present study, we further investigated the relationships between the pharmacological effects, the blood and hepatic transaminase activities and the gene expression of transaminases in the liver after treatment in vivo with fenofibrate in order to verify the pharmacological aspects of the fenofibrate-induced transaminase elevations.

**MATERIALS AND METHODS**

**Animals**

Five-week-old male F344/DuCrjCrlj (Fischer) rats were purchased from Charles River Japan Inc. (Kanagawa, Japan). The animals were housed individually in wire-mesh cages kept in an air-conditioned room with a 12-hr light-dark cycle at a temperature of 23 ± 1°C, a relative humidity of 55 ± 5% and a ventilation rate of about 15 times per hour. The rats were allowed free access to a commercial diet (CRF-1, Oriental Yeast Co., Ltd., Tokyo, Japan) and tap water ad libitum. The animals were quarantined and acclimated for 1 week. All animal experiment procedures were approved by the Institutional Animal Care and Use Committee of the Toxicology Research Laboratories, Central Pharmaceutical Research Institute, Japan Tobacco Inc. This study was conducted in accordance with the Japanese Law for the Humane Treatment and Management of Animals (Law No. 105, as revised in 2006, issued in October 1, 1973).

**Treatment**

Fenofibrate was purchased from Sigma Chemical Co. (St. Louis, MO, USA) and was suspended in 0.5% methylcellulose (MC; Shin-Etsu Chemical Co., Ltd., Tokyo, Japan) aqueous solution. The dose level selected in this study was based on our preliminary results, which indicated that the pharmacological effects of fenofibrate, decreases in plasma lipid levels, reached uppermost limit at a single dosage of 400 mg/kg (data not shown). The animals were 6 weeks of age at the time of treatment.

**Necropsy and sampling of blood**

Animals were euthanized under ether anesthesia at 24, 48 and 72 hr after dosing under non-fasted conditions. Blood samples were collected into heparinized tubes at necropsy and the samples were centrifuged for separation of the plasma. The plasma was stored at −80°C until use.

**Blood biochemistry tests**

ALT exists in the mitochondrial and cytosol frac-
Effects of fenofibrate on plasma and hepatic transaminases

tions of animal and human liver. However, the measurement methods of plasma mitochondria-type ALT or cytosol-type ALT have not been established (DeRosa and Swick, 1975). Differences in the physicochemical properties between mitochondrial and cytosolic AST have been reported and the measurement methods of plasma mitochondria-type AST activity have been established (Watazu et al., 1993). Plasma lipid parameters, activities of ALT, AST and mitochondria-type AST, and liver function parameters were measured at 37°C with a Toshiba TBA-120FR automated analyzer using standard reagents and the following principle: triglycerides (TGL), GPO-DAOS method (Wako Pure Chemical, Tokyo, Japan), total cholesterol (T-C), cholesterol oxidase-ǍDAOS method (Wako Pure Chemical), ALT, UV method (Wako Pure Chemical), AST, UV method (Wako Pure Chemical), mitochondria-type AST, protease method (Sysmex Corporation, Hyogo, Japan), alkaline phosphatase, p-nitrophenyl phosphoric acid method (Wako Pure Chemical), lactate dehydrogenase, Wróblewski-LaDue method (Wako Pure Chemical), total bilirubin, enzyme method (Wako Pure Chemical). Cytosol-type AST activity was calculated by subtracting mitochondria-type AST activity from the total AST activity.

Preparation of cytosol and mitochondria fractions of the liver

After the blood collection, the livers were removed and weighed. A liver sample of approximately 1 g was collected from each animal. All the samples were frozen in liquid nitrogen and stored at -80°C in an ultra-low temperature refrigerator. The frozen liver samples were thawed and 0.25 mol/l sucrose aqueous solution containing 0.05 mol/l tris(hydroxymethyl)aminomethane (pH 7.6, herein-after, 0.25 mol/l sucrose solution) was added to the samples at a volume of 5 ml per 1 g wet tissue weight. The liver samples were homogenized using a Potter-homogenizer (a Teflon homogenizer) in an ice-water bath. The mitochondria fraction was prepared by differential centrifugation of a 20% liver homogenate in 0.25 mol/l sucrose solution; the homogenate was centrifuged at 1,100 g for 3 min. The supernatant was saved, while the sediment of the nuclear fraction was resuspended in 0.25 mol/l sucrose solution and centrifuged at 1,100 g for 3 min. All of the combined supernatant fractions were centrifuged at 9,000 g for 15 min and the supernatant (cytosol fraction) was saved. The resulting mitochondria-enriched pellets (containing peroxisomes, mitochondria and lysosomes) were then resuspended in 0.25 mol/l sucrose solution and centrifuged again at 20,200 g for 15 min (Elliott et al., 1986; Esbenshade et al., 1990; Pedersen et al., 1978). The pellets obtained as the mitochondria fraction were suspended in 0.25 mol/l sucrose solution. Both the mitochondria and cytosol fractions were frozen at -80°C until assayed for ALT and AST activities.

Enzyme assay of cytosol and mitochondria fractions of the liver

All assays were conducted at 37°C and the units were expressed as international units (IU). ALT activity was determined by an interrupted assay wherein the amount of pyruvate formed during incubation was determined from the oxidation of NADH in the presence of excess lactate dehydrogenase. AST activity was determined in a coupled assay by measuring the formation of oxalacetate. The oxidation of NADH was determined in the presence of excess malate dehydrogenase. The protein concentration was determined by the Biuret method with human serum albumin as a standard. The total activity of ALT or AST in the whole liver was calculated using the following formula: activities in the whole liver (IU/liver) = (activities/g tissue in the mitochondria fraction + activities/g tissue in the cytosol fraction) × liver weights.

Measurements of mRNA in the liver

For RT-PCR analysis, total RNA was isolated from the liver using the Qiagen RNasy Kit according to the manufacturer's instruction. The RT-PCR primers and probes for cytosolic AST and mitochondrial AST were designed by using the Primer Express program from Applied BioSystems Japan Ltd. (Tokyo, Japan) from the insilico-derived cDNA sequences. The primer/probe sequences for above enzymes are shown in Table 1. The endogenous control for normalization was β-actine; the primer and probe were purchased from Applied BioSystems Japan Ltd. A two-step cDNA synthesis was performed using the High

Table 1. Primers sequences of aspartate aminotransferase used for real-time RT-PCR

<table>
<thead>
<tr>
<th>Gene</th>
<th>Forward primer</th>
<th>Reverse primer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mitochondrial AST</td>
<td>5'-CAACCATCTTGACCTTCAGACTT-3'</td>
<td>5'-CTTCTTCTGAGATTCGAGAACCAC-3'</td>
</tr>
<tr>
<td>Cytosolic AST</td>
<td>5'-AACAGCCCACTCTCAGGGAAT-3'</td>
<td>5'-CCATTTGACAAACCTGACAGT-3'</td>
</tr>
<tr>
<td>β-Actin</td>
<td>5'-ACGCGTGAAGAGATACCCAGAT-3'</td>
<td>5'-AGCCCTTGAGTGGTACGTACATG-3'</td>
</tr>
</tbody>
</table>

Vol. 34 No. 4
Capacity Kit from Applied BioSystems Japan Ltd. as per the manufacturer’s instructions. Relative quantity values were determined following normalization with β-actine and using the mixed tissue pool as the calibrator sample. ALT mRNA levels were not measured due to the lack of a suitable primer.

Histopathology
The livers from the control and treatment groups were fixed in 10% neutral buffered formalin. Specimens were prepared for histopathological examination by embedding in paraffin wax, sectioning and staining with hematoxylin and eosin (HE). The liver slides were examined microscopically.

Statistical analysis
The mean values and standard deviations in each group were calculated for the liver weights and the biochemical parameters. Statistical analyses were performed using an unpaired Student’s t-test in Microsoft Excel (Microsoft Corporation) for the effects of fenofibrate. The levels of significance were set at 5% and 1% (two-tailed). Correlation analyses were conducted using the JMP (SAS Institute Japan, Tokyo, Japan).

RESULTS

Plasma lipid parameters
Plasma total cholesterol levels decreased at 24 and 48 hr after dosing in the fenofibrate-treated group but did not change at 72 hr after dosing (Fig. 1A). Plasma triglyceride levels decreased from 24 hr after dosing and the change was statistically significant at 48 and 72 hr after dosing in the fenofibrate-treated group (Fig. 1B).

Plasma ALT and AST activities and other liver function parameters
Plasma ALT activities in the fenofibrate-treated group were higher than those in the control group at all time points (with 1.2-, 1.3- and 1.3-fold increases at 24, 48 and 72 hr after dosing, respectively) (Fig. 2A). Plasma AST activities in the fenofibrate-treated group were higher than those in the control group at 48 and 72 hr after dosing (with 1.2-fold increases both at 48 and 72 hr after dosing) (Fig. 2B). There were no changes thought to be meaningful in the plasma cytosol-type AST activities at any time point in the fenofibrate-treated group, although some statistically significant differences were noted between the control and fenofibrate-treated groups (Fig 2C). Plasma mitochondria-type AST activities in the fenofibrate-treated group were higher than those in the control group at 48 and 72 hr after dosing (with 1.4- and 1.5-fold increases at 48 and 72 hr after dosing, respectively) (Fig. 2D). There were no changes in the other liver function parameters including lactate dehydrogenase (LDH), ALP or total bilirubin levels at any time point (Table 2).

Liver weights
Liver weights increased at all time points (with 1.2-, 1.3- and 1.3-fold increases at 24, 48 and 72 hr after dosing.

Fig. 1. Effects of fenofibrate on plasma lipid parameters in rats.
A: plasma total cholesterol, B: plasma triglyceride. Fenofibrate, at a dose level of 400 mg/kg, or vehicle was given once orally to rats. Plasma samples (n = 5/group/point) were used for the measurement. Plasma total cholesterol and triglyceride levels were measured at 37°C with an automated analyzer using standard reagents. Data are shown as mean ± S.D. ** p < 0.01 relative to the control group (Student’s t-test).
Effects of fenofibrate on plasma and hepatic transaminases

Fig. 2. Effects of fenofibrate on plasma transaminase activities in rats.
A: plasma ALT, B: plasma AST, C: plasma cytosol-type AST, D: plasma mitochondria-type AST. Fenofibrate, at a dose level of 400 mg/kg, or vehicle was given once orally to rats. Plasma samples (n = 5/group/point) were used for the measurement. Activities of plasma ALT, AST and mitochondria-type AST were measured at 37°C with an automated analyzer using standard reagents. Data are shown as mean ± S.D., * p < 0.05, ** p < 0.01 relative to the control group (Student’s t-test).

Specific activities of ALT and AST in liver
Mitochondrial ALT activities in livers from the fenofibrate-treated animals were higher than those in livers from the control animals only at 24 hr after dosing (Fig. 4A). There were no changes in cytosolic ALT activities in livers from the fenofibrate-treated animals at any time point when compared with the control animals (Fig. 4B). Mitochondrial AST activities in livers from the fenofibrate-treated animals were higher than those in the livers from the control animals at 48 and 72 hr after dosing (Fig. 4C). Cytosolic AST activities in the livers from the fenofibrate-treated animals were lower or tended to be lower than those in the livers from the control animals at all time points (Fig. 4D). ALT activities per whole liver in the fenofibrate-treated animals were higher than those in the control animals at all time points (Fig. 5A). AST activities per whole liver in the fenofibrate-treated animals were higher than those in the control animals at 48 and 72 hr after dosing (Fig. 5B).

Correlation between plasma and hepatic transaminase activities
Individual plasma transaminase activities significantly correlated with the total activities of transaminases per
A. Kobayashi et al.

Table 2. Effects of fenofibrate on plasma liver function parameters in rats

<table>
<thead>
<tr>
<th></th>
<th>24 hr after treatment</th>
<th>48 hr after treatment</th>
<th>72 hr after treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Fenofibrate</td>
<td>Control</td>
</tr>
<tr>
<td>Lactate dehydrogenase (IU/l)</td>
<td>59.4 ± 9.4</td>
<td>52.8 ± 19.2</td>
<td>51.6 ± 7.0</td>
</tr>
<tr>
<td>Alkaline phosphatase (IU/l)</td>
<td>1212.0 ± 123.2</td>
<td>1326.0 ± 81.4</td>
<td>1209.0 ± 78.2</td>
</tr>
<tr>
<td>Total bilirubin (mg/dl)</td>
<td>0.05 ± 0.01</td>
<td>0.04 ± 0.00</td>
<td>0.04 ± 0.00</td>
</tr>
</tbody>
</table>

Fenofibrate, at a dose level of 400 mg/kg, or vehicle was given once orally to rats. Plasma samples (n = 5/group/point) were used for the measurement. Plasma levels of liver function parameters were measured at 37°C with an automated analyzer using standard reagents. Data are shown as mean ± S.D.. There were no statistically significant differences for any parameter at any time point (Student's t-test).

Fig. 3. Effects of fenofibrate on liver weights in rats.
Fenofibrate, at a dose level of 400 mg/kg, or vehicle was given once orally to rats. Data are shown as mean ± S.D.. ** p < 0.01 relative to the control group (Student's t-test).

whole liver in the fenofibrate-treated animals (Figs. 6A and B, ALT: r = 0.839, p < 0.01, AST: r = 0.702, p < 0.01).

Correlation between the plasma transaminase activities and plasma lipid levels
Individual plasma ALT and plasma mitochondria-type AST activities correlated significantly with individual plasma TGL levels in fenofibrate-treated animals (Figs. 7A, B, plasma ALT activities versus TGL levels: r = -0.779, p < 0.01, plasma mitochondria-type AST activities versus TGL levels: r = -0.770, p < 0.01).

Expression of AST mRNA in liver
Mitochondrial AST mRNA levels in liver increased in the fenofibrate-treated animals both at 24 and 48 hr after dosing (with 1.5-fold increases both at 24 and 48 hr after dosing) (Fig. 8A). There were no changes in mRNA levels for cytosolic AST in livers from the fenofibrate-treated animals at either time point (Fig. 8B).

Histopathology
There were no histopathological findings suggestive of hepatotoxicity including necrosis or degeneration of the hepatocytes in the fenofibrate-treated groups at any time point. Granular eosinophilic cytoplasm was observed in the fenofibrate-treated groups at all time points, but the finding was slight in its severity.

DISCUSSION
It has been reported that serum ALT and AST levels were increased in some patients who received fibrates, a PPARα activator (Balfour et al., 1990; Blane, 1987). In general, the increase is mild (less than three times the upper limit of normal), sometimes transient and is not accompanied by any finding suggestive of hepatotoxicity (Balfour et al., 1990; Blane, 1987). From the results of in vitro studies, fenofibrate has been also shown to modify the expression of the ALT and AST genes in animal and human hepatocytes and to increase the activities of these enzymes in a PPARα-dependent manner in human hepatocytes (Edgar et al., 1998; Tomkiewicz et al., 2004; Thulin et al., 2008). The gene modification and elevations of the enzyme activities of ALT and AST in the hepatocytes are considered to be related to the pharmacological action of fenofibrate, independently of any toxic phenomenon (Edgar et al., 1998; Tomkiewicz et al., 2004). Despite several in vitro approaches for the non-hepatotoxic elevations of transaminase activities with PPARα agonism, in vivo trials have not been reported for the study of the mechanism of the elevation in the transaminase activities in animals or humans treated with PPARα.
Effects of fenofibrate on plasma and hepatic transaminases

Fig. 4. Effects of fenofibrate on specific transaminase activities of ALT and AST in liver of rats. A: mitochondrial ALT activities, B: cytosolic ALT activities, C: mitochondrial AST activities, D: cytosolic AST activities. Fenofibrate, at a dose level of 400 mg/kg, or vehicle was given once orally to rats. Mitochondria and cytosol fractions in rat liver were prepared by the differential centrifugation method. Mitochondria and cytosol fractions (n = 5/group/point) were used for the determination of transaminase activities in the control and fenofibrate treated-groups. Data are shown as mean ± S.D. * p < 0.05, ** p < 0.01 relative to the control group (Student’s t-test).

activators. In the present study, we investigated the relationship between the pharmacological effects, plasma and hepatic transaminase levels and the gene expression of transaminases in the liver of rats treated with fenofibrate. After a single oral dose of fenofibrate at a dose level of 400 mg/kg, plasma total cholesterol and triglyceride levels decreased from 24 hr after dosing, indicating the pharmacological action of fenofibrate on lipid metabolism. Plasma ALT and AST activities were increased at 24 and 48 hr after dosing, respectively, indicating that plasma ALT is more sensitive than AST to treatment with fenofibrate. The increases in plasma transaminase activities were slight (1.2-1.3 fold increases for ALT and 1.2 fold increases for AST versus the control levels) and were not accompanied by any changes in other liver function parameters including LDH, ALP and total bilirubin levels. The profile of the elevations of the plasma transaminase activities was similar to that reported in humans in terms of the magnitude of the elevation, the relatively high sensitivity of ALT compared with AST and the lack of alteration of other liver function parameters (Balfour et al., 1990; Blane, 1987). The results of biopsy of the liver from patients who received fenofibrate over a long time have revealed that there were no histopathological findings suggestive of DILI although plasma transaminase levels had been increased before biopsy (Blane, 1987). In our study, histopathological findings in the liver from the fenofibrate-treated rats were limited to those related to PPARα agonist, i.e. slight granular eosinophilic cytoplasm, indicating peroxisome proliferation, while any his-
Fig. 5. Effects of fenofibrate on transaminase activities in whole liver of rats.
A: ALT activities in the whole liver, B: AST activities in whole liver. Fenofibrate, at a dose level of 400 mg/kg, or vehicle was given once orally to rats. Mitochondria and cytosol fractions in rat liver were prepared by differential centrifugation method. Mitochondria and cytosol fractions (n = 5/group/point) were used for the determination of transaminase activities in the control and fenofibrate treated-groups. Total activities of ALT or AST in the whole liver were calculated from the following formula: activities in the whole liver (IU/liver) = (activities/g tissue in the mitochondria fraction + activities/g tissue in the cytosol fraction) × liver weights. Data are shown as mean ± S.D. * p < 0.05, ** p < 0.01 relative to the control group (Student’s t-test).

Fig. 6. Correlation between the plasma transaminase activities and transaminase activities per whole liver in fenofibrate-treated rats.
A: plasma ALT activities versus hepatic ALT activities, B: plasma AST activities versus hepatic AST activities. Correlation analyses were conducted using the individual data from the fenofibrate-treated rats at all sampling points (n = 15).

topathological finding suggestive of hepatotoxicity was not observed in the liver. Our study further revealed that the increases in plasma AST activities were attributable to increases in plasma mitochondria-type AST activities in the fenofibrate-treated rats.

In the measurements of transaminase activities in the liver, the activity of mitochondrial ALT and AST was increased at 24 and 48 hr after dosing, respectively, indicating that the onset of the elevation of these enzymes in the liver was consistent with that of their elevation in the plasma. Further, the increased hepatic mitochondrial AST activity was accompanied by increased mRNA levels of this enzyme in the hepatocytes. In the case of hepatocellular injury, transaminase activities in the liver should be increased due to leakage of the enzymes from the hepatocytes into the peripheral blood where they are meas-
Effects of fenofibrate on plasma and hepatic transaminases

**Fig. 7.** Correlation between plasma transaminase activities and triglyceride levels in fenofibrate-treated rats. A: plasma ALT activities versus plasma triglyceride levels, B: plasma mitochondria-type AST versus triglyceride levels. Correlation analyses were conducted using the individual data from the fenofibrate-treated rats at all sampling points (n = 15).

**Fig. 8.** Effects of fenofibrate on mitochondrial and cytosolic AST mRNA levels in the liver of rats. A: mitochondrial AST mRNA levels, B: cytosolic AST mRNA levels. Fenofibrate, at a dose level of 400 mg/kg, or vehicle was given once orally to rats. Liver samples (n = 5/group/point) were used for the measurement of mRNA. mRNA levels of mitochondrial and cytosolic AST were measured by the real time RT-PCR method. Data are shown as mean ± S.D.. * p < 0.05, ** p < 0.01 relative to the control group (Student's t-test).

Measured and the plasma ALT and AST levels can increase up to 10-100 fold. The results of our study indicate that the elevations of plasma ALT and AST activities after fenofibrate treatment were related to increased hepatic enzyme synthesis, which was mainly noted in the mitochondria, and not directly linked to cell loss of intracellular enzyme proteins.

In our study, plasma cytosol-type AST activities, cytosolic AST activities in the liver and cytosolic AST mRNA levels were unchanged or were decreased in the fenofibrate-treated rats. These results are consistent with those previously reported. Edgar et al. (1998) have shown that treatment with fenofibrate decreased liver cytosolic AST mRNA in wild-type mice, while no change was noted in PPARα deficient mice. Another work by Tomkiewicz et al. (2004) has demonstrated that fenofibrate increased the expression of the cytosolic AST gene in human liver cells, while in rat liver cells, fenofibrate repressed the
expression of the cytosolic AST gene. Edgar et al. (1998) also reported that activities and mRNA levels of cytosolic AST were increased in HepG2 cells and human hepatocytes treated with fenofibrate. Although the etiology of the opposite direction of the changes in cytosolic AST activities and mRNA levels in human and rat cells is not well known, differences in the regulation of the cytosolic AST genes by PPARα activation between rodents and human have been proposed.

Correlation analyses were also conducted using individual values of plasma transaminase activities and total transaminase activities per whole liver on the assumption that the elevation of plasma transaminase activities reflects that of total transaminase activities in the whole liver. A good correlation was obtained between these two parameters both for ALT (r = 0.839, p < 0.01) and AST (r = 0.702, p < 0.01). The basal levels of transaminases in the blood are considered to be explained by the release of hepatocellular enzyme proteins into the circulation during normal cell turnover (Schmidt et al., 1989) and blood transaminase levels increase up to 10-100 fold when marked hepatocyte cell lysis occurred as the results of DILI. The results of the correlation analyses indicate that the slight increases in plasma transaminase activities in the fenofibrate-treated rats are the consequences of the release of increased hepatocellular enzyme proteins into the circulation during normal cell turnover.

Fibrates have been shown to modify a variety of aspects of lipid metabolism leading to reductions in plasma lipid levels (Balfour et al., 1990; Blane, 1987). Possible mechanisms of the lipid-lowering action of fenofibrate include an increase in lipoprotein-lipase synthesis, activation of β-oxidation of free fatty acids and repression of the Apo C-III gene, through PPARα activation, which lead to acceleration of triglyceride catabolism and repression of triglyceride synthesis. Triglyceride metabolism is more closely linked to amino acid and glucose metabolism and there is a possibility that fenofibrate secondarily modifies the gene expression of enzymes involved in the amino acid-glucose metabolism pathways. ALT and AST are some of the key enzymes involved in this pathway and the increased gene expression of mitochondrial AST in the hepatocytes after fenofibrate treatment noted in our study may be related to alteration of amino acid-glucose metabolism due to the alteration of triglyceride metabolism as the pharmacological action of fenofibrate. The good negative correlation between plasma triglyceride levels and plasma transaminase activity noted in our study strongly supports this idea (Figs. 7A and B).

In conclusion, treatment with fenofibrate slightly increased plasma transaminase activities in rats with the findings directly related to the pharmacological action of the drug, i.e. decreases in plasma lipid parameters. The increases were in parallel with the increases in hepatic transaminase activities associated with increases in the transaminase genes in the liver and were considered not to be a consequence of hepatotoxicity from the drug. The modification of transaminase gene expression is likely to be secondary to the pharmacological action of fenofibrate. The evidence obtained in our study underlines the importance of gene regulation as a possible alternative mechanism for increased blood transaminase activities.

ACKNOWLEDGMENTS

The author would like to thank the invaluable contributions of Dr. Yoshifumi Miyakawa, Dr. Akemi Takahashi, Kazuma Kondo and Hideaki Yokoyama and the staff at the Toxicology Research Lab., Central Pharmaceutical Research Institute, JAPAN TOBACCO INC.. We would like to also acknowledge Dr. Kazuhiko Matsumoto, Torii Pharmaceutical Co., Ltd., for their interest and support during this study.

REFERENCES


Effects of fenofibrate on plasma and hepatic transaminases


