Comparison of the liver findings after simvastatin-treatment between Spontaneously Diabetic Torii-Lepr\textsuperscript{fa} (SDT fatty) rats and Sprague-Dawley rats

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(Received January 7, 2020; Accepted January 20, 2020)

ABSTRACT — One of the risk factors for drug-induced liver injury (DILI) is the diabetic state. Our previous investigation showed that liver injury after repeated oral dosing with allyl alcohol and carbamazepine was enhanced more in the Spontaneously Diabetic Torii-Lepr\textsuperscript{fa} (SDT fatty) rats than in the Sprague-Dawley (SD) rats. It was caused by lower hepatic detoxification due to depleted hepatic glutathione synthesis. This is because simvastatin, frequently used in diabetic patients, shows a positive high reaction in a GSH adduct assay \textit{in vitro} although the GSH adduction is considered not to be a major metabolic pathway of simvastatin. Therefore, in the present study, effects of simvastatin-treatment on the liver were compared between the Sprague-Dawley (SD) rats and SDT fatty rats in order to obtain additional information to estimate the potential risk of DILI in the diabetic state. There were no effects with simvastatin on the liver in the SD or SDT fatty rats after 13-week oral dosing of simvastatin. These results indicate that simvastatin does not have potential to induce liver injury in diabetic state and are consistent with the reports for the clinical use of simvastatin in the diabetic patients.

Key words: SDT fatty rats, Type 2 diabetes, Hepatotoxicity, Glutathione, Simvastatin

INTRODUCTION

Drug-induced liver injury (DILI) is a significant public health issue because of its potential impact on both the patients and development of new drugs. DILI events are also the main cause of regulatory action pertaining to drugs, and the regulatory action includes denial of marketing approval, restrictions with respect to clinical indications and withdrawal from the marketplace (Lee, 2003; Smith and Schmid, 2006).

One of the risk factors for DILI is the diabetic state (Chalasani et al., 2014; Gaude et al., 2015; Ortega-Alonso et al., 2016; Lu et al., 2016) and diabetic patients have a nearly 2-fold higher risk of acute liver failure, chronic liver failure and hepatocarcinogenesis compared with non-diabetic patients (El-Serag and Everhart, 2002; Huerta et al., 2002; El-Serag et al., 2004). Decreased levels of glutathione have been observed in the blood of diabetic patients, possibly due to compromised levels of GSH synthesis and metabolism enzymes (Illing et al., 1951; Lal and Kumar, 1967; Awadallah et al., 1978; Lagman et al., 2015). A potential risk for the DILI related to acetaminophen toxicity is considered to be lower levels of glutathione in humans (Sun et al., 2009) and this is unex-
pected because reactive metabolites, which often cause DILI, are detoxified by conjugation with glutathione. From these observations, one of the reasons for the higher risk of DILI in diabetic patients is related to lower glutathione levels. Thus, it is important to estimate the risk of DILI for drugs which are frequently used in diabetic patients and which are detoxified by glutathione.

Many nonclinical studies have been conducted with diabetes model animals in order to estimate the risk of DILI in diabetic patients (Wang et al., 2007). In our recent studies, we have demonstrated that the Spontaneously Diabetic Torii-Leprα (SDT fatty) rats have lower levels of hepatic reduced-form (GSH) and oxidized-form (GSSG) glutathione when compared with Sprague-Dawley (SD) rats, and the profiles of glucose metabolism, hepatic function tests and glutathione synthesis in the SDT fatty rats were like those in patients with type 2 diabetes (T2DM) (Takahashi et al., 2019a, 2019b). In addition, we have also demonstrated that liver injury is induced in the SDT fatty rats using allyl alcohol and carbachamepine, both of which are detoxified by glutathione conjugation in the liver (Takahashi et al., 2019a, 2019b). These results indicate that the potential risk of chemical or drug induced liver injury is higher in diabetic patients than in healthy humans (Takahashi et al., 2019a, 2019b).

This is because statins are frequently used in patients with T2DM to treat hyperlipidemia and prevent cardio-vascular events (Tolman et al., 2007). In addition, statin therapy, like all cholesterol-lowering therapy including bariatric surgery (Andersen et al., 1991; Drenick et al., 1970), causes slight and transient elevations in liver enzymes (Tolman, 2000, 2002). However, severe liver damage and liver failure are very rare with statins (Tolman, 2002) and the liver seems to adapt with continuing therapy and there are no long-term consequences from these effects. On the other hand, among the statins, simvastatin shows a positive high reaction in a GSH adduct assay in vitro (Sakatis et al., 2012) although the GSH adduction is considered not to be a major metabolic pathway for simvastatin in humans. In the present study, in order to obtain additional information to estimate the potential risk of DILI in T2DM patients, simvastatin was used as a model drug and effects with simvastatin on the liver were compared between the Sprague-Dawley (SD) rats and SDT fatty rats after 13-week oral dosing of simvastatin.

**MATERIALS AND METHODS**

**Animals**

Thirty male Jcl:SD (SD) rats and thirty male SDT.Cg Leprα/Jtucl (SDT fatty) rats at five weeks of age were obtained from CLEA Japan Inc. (Tokyo, Japan). The animals were housed individually in wire-mesh cages kept in an air-conditioned room with a 12-hr light-dark cycle (lighting from 7:00 a.m. to 7:00 p.m.) at a temperature of 23 ± 1°C, relative humidity of 55 ± 5% and a ventilation rate of about 15 times per hour. The SD and SDT fatty rats were quarantined and acclimated for more than 1 week and were allowed free access to a commercial pelleted diet (CRF-1, Oriental Yeast Co., Ltd., Tokyo, Japan) ad libitum during the quarantine/acclimation period. Tap water was available for drinking ad libitum. All animal experimental 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 2013, issued in October 1, 1973).

**Dosing of Simvastatin**

Simvastatin was purchased from TEVA Pharmaceutical Works Private Limited Company (Debrecen, Hungary) and was suspended in 0.5% methylcellulose (MC, Shin-etsu Chemical Co., Ltd., Tokyo, Japan) aqueous solution. The dose levels at 50 mg/kg and 150 mg/kg was selected for both strains as dose levels expected to little or no effect on the liver (slight increases in the plasma transaminase activities and hepatocellular damage) based on the results from a thirteen-week oral dose toxicity study of simvastatin in rats (Gerson et al., 1989). The animals in the matched control groups for each strain of rat were given the vehicle (0.5% MC aqueous solution). Each strain of rat was randomly allocated to each group on the day before the initiation of dosing so that the initial mean body weights of each group were equivalent. Simvastatin was administered orally once daily to the SD and SDT fatty rats (6 weeks of age, 10 rats of each strain per group) at the dose levels of 0 (vehicle control), 50 and 150 mg/kg for thirteen weeks. The dosing volume was set at 5.0 mL/kg. The dosing volume for each individual rat was calculated based on the most recently recorded body weight during the dosing period.

**Observations, Measurements and Examinations**

**Measurements of Body Weights, Food Consumption and Food Efficiency**

The animals were weighed immediately before the initiation of dosing on day 1, twice weekly during the initial 4 weeks and once weekly during the subsequent 9 weeks of the dosing period. Food consumption per ani-
mal was calculated once before the initiation of dosing (day -1) and twice weekly during the initial 4 weeks and once weekly during the subsequent 9 weeks of the dosing period. Food efficiency during the dosing period was calculated individually.

Blood Sampling
Blood sampling for the plasma was sampled from the subclavian vein into lithium heparin-treated syringes without anesthesia. Blood samples were collected from all the SD and SDT fatty rats in the control and simvastatin-treated groups under the fed condition between 8:00 a.m. and 10:00 a.m. during the pre-dosing period (in week -1) and in weeks 4, 8 and 12 of the dosing period (age of the animals: 5, 10, 14 and 18 weeks). Plasma samples, obtained as described previously (Kondo et al., 2012), were used for the measurement of plasma hepatic function parameters [aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyl-transpeptidase (GGT), lactate dehydrogenase (LDH), glutamate dehydrogenase (GLDH), sorbitol dehydrogenase (SDH), guanase (GU), alkaline phosphatase (ALP), and total bilirubin (T-BIL)], plasma parameters for glucose and lipid metabolism (glucose (GLU), triglycerides (TGL), total cholesterol (T-CH), phospholipid (PL) and total ketone bodies (TKB)).

Measurements of plasma parameters for hepatic function, glucose and lipid metabolism
Plasma activities of AST, ALT, LDH, GLDH, SDH, GU and ALP, and plasma concentrations of T-BIL, GLU, TGL, T-CH, PL and TKB were measured at 37°C with a TBA-120FR automated analyzer using standard reagents and the following principle: AST, ALT, LDH and ALP, UV method (FUJIFILM Wako Pure Chemical Corporation, Tokyo, Japan), UV method (RANDOX Laboratories Ltd., Antrim, UK), SDH, UV method (SEKISUI MEDICAL CO., Ltd., Tokyo, Japan), GU, UV method (Serotec Co., Ltd., Hokkaido, Japan), GGT, enzymatic method (Wako Pure Chemical, Tokyo, Japan), T-BIL, enzymatic method (KANTO CHEMICAL CO., INC., Tokyo, Japan), GLU, HX-G-6-PDH method (FUJIFILM Wako Pure Chemical Corporation, Tokyo, Japan), TGL and PL, enzymatic method (FUJIFILM Wako Pure Chemical Corporation, Tokyo, Japan), T-CH, enzymatic method (Kyowa Medex Co., Ltd., Tokyo, Japan), TKB, enzymatic method (calculated from the values of acetoacetic acid and 3-hydroxybutyric acid, Serotec Co., Ltd., Hokkaido, Japan).

Measurements of the liver weights
At the terminal sacrifice (19 weeks of age), the animals were fasted overnight on the last day of the dosing period. On the next day, the animals were anesthetized with isoflurane anesthesia and euthanized by exsanguination from the abdominal aorta. The final body weights from all the animals were recorded to calculate the relative weights of the liver to the body weights. The livers from the animals were weighed and the relative weights of each organ to the final body weights were calculated.

Histopathological examination in the liver and adrenal
At the terminal sacrifice (19 weeks of age), the left lobe of the liver was cut into longitudinal sections from the SD and SDT fatty rats in the control and simvastatin-treated groups. The liver slices and the intact right and left adrenals were embedded in paraffin. Sectioning and hematoxylin-eosin staining was performed according to routine histological procedures.

Statistical analysis
The mean values and standard deviations in each group were calculated for the body weights, food consumption, food efficiency, plasma parameters for glucose and lipid metabolism, hepatic function and, the liver weights at the end of the dosing period. A Student’s t test was conducted for comparison of the body weights, food consumption, food efficiency, plasma parameters for glucose and lipid metabolism, hepatic function, and the liver weights between the SD control rats and the SDT fatty control rats and between the vehicle control and simvastatin-treated groups each of the SD and SDT fatty rats. The levels of significance were set at 5% and 1% (two-tailed).

RESULTS
To confirm hepatic function, and glucose and lipid metabolism in the SDT fatty rats employed in this study, the following parameters were compared between the control SD and SDT fatty rats. From 5 to 19 weeks of age, the SDT fatty rats had higher values for body weights and food consumption, and lower values for food efficiency (Fig. 1) (p < 0.01). In the hepatic function parameters, the SDT fatty rats had higher plasma AST levels at 10 weeks of age (p < 0.01), higher plasma levels of ALT, GGT, GLDH, GU and ALP from 5 to 18 weeks of age (Figs. 2, 3 and 4) (p < 0.05 or p < 0.01). The magnitude of the differences in plasma ALT, GGT and GLDH levels was greater than that of the plasma AST levels. The SDT fatty rats had lower plasma T-BIL levels at 5 and 14 weeks of age (Fig. 4) (p < 0.01). In the plasma parameters

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for glucose and lipid metabolism, the SDT fatty rats had markedly higher plasma GLU levels from 5 to 18 weeks of age (Fig. 5) (p < 0.01), higher plasma levels of TGL, T-CH and PL from 5 to 18 weeks of age (p < 0.01), higher plasma TKB levels from 10 to 18 weeks of age (Figs. 5 and 6) (p < 0.01). The SDT fatty rats had higher levels for the liver weights at 19 weeks of age (Fig. 7) (p < 0.01).

To compare the effects of simvastatin on the liver between the SD and SDT fatty rats, the following examinations were conducted for the simvastatin-treated SD and SDT fatty rats during and after repeated dosing of simvastatin for 13 weeks.
In the simvastatin-treated SD rats, the following changes were noted in plasma parameters for hepatic function and lipid metabolism mainly at 150 mg/kg when compared with the control SD rats: slightly increased plasma AST and ALT levels in weeks 4 and 8 of the dosing period (Fig. 2) (p < 0.05 or p < 0.01), decreased plasma TGL levels from weeks 4 to 12 of the dosing period (Fig. 6) (p < 0.01) and decreased plasma PL levels in weeks 4.

Fig. 2. Plasma levels of AST, ALT and GGT in the simvastatin-treated SD and SDT fatty rats. Abbreviation: AST; aspartate aminotransferase, ALT; alanine aminotransferase, GGT; gamma-glutamyltranspeptidaseBlood samples were collected at 9:00-10:00 a.m. from each rat on the days shown in the figure (n=9 or 10/group/point) under non-fasting conditions. “-1” indicates the pre-dosing period. Data are shown as means. ## < 0.01 significantly different between the control SD and SDT fatty rats (Student’s t-test), * p < 0.05, ** p < 0.01 significantly different from the matched control group (Student’s t-test).
and 8 of the dosing period (Fig. 6) (p < 0.01). There were some statistically significant differences in plasma parameters of hepatic function, glucose and lipid metabolism in the simvastatin-treated SD rats but they were transient or not dose-dependent.

In the simvastatin-treated SDT fatty rats, the following changes were noted in plasma parameters for hepatic function and lipid metabolism mainly at 150 mg/kg when...
compared with the control SDT fatty rats: increased plasma GGT levels in week 4 of the dosing period (Fig. 2) ($p < 0.01$), decreased plasma GLDH levels in week 4 and 8 of the dosing period (Fig. 3) ($p < 0.05$ and $p < 0.01$), increased plasma SDH levels in week 12 of the dosing period (Fig. 3) ($p < 0.05$ or $p < 0.01$), slightly decreased plasma ALP levels in weeks 4 and 8 of the dosing period (Fig. 4) ($p < 0.05$ or $p < 0.01$), increased plasma T-BIL levels in weeks 8 and 12 of the dosing period (Fig. 4) ($p < 0.05$ or $p < 0.01$), decreased plasma TGL and PL.

Fig. 4. Plasma levels of GU, ALP and T-BIL in the simvastatin-treated SD and SDT fatty rats. Abbreviation: GU; guanase, ALP; alkaline phosphatase, T-BIL; total bilirubin. Blood samples were collected at 9:00-10:00 a.m. from each rat on the days shown in the figure ($n=9$ or 10/group/point) under non-fasting conditions. “-1” indicates the pre-dosing period. Data are shown as means. ## < 0.01 significantly different between the control SD and SDT fatty rats (Student’s $t$-test), * $p < 0.05$, ** $p < 0.01$ significantly different from the matched control group (Student’s $t$-test).
levels from weeks 4 to 12 of the dosing period (Fig. 6) (p < 0.01), increased plasma TKB levels from weeks 4 to 12 of the dosing period (Fig. 5) (p < 0.05 or p < 0.01). There were some other statistically significant differences in the plasma parameters for hepatic function, glucose and lipid metabolism in the simvastatin-treated SDT fatty rats but they were transient or not dose-dependent.

In comparison of the magnitude of the changes between the simvastatin-treated SD and SDT fatty rats, plasma AST and ALT levels were slightly increased only in the simvastatin-treated SD rats (Fig. 2). Plasma GGT and TKB levels were increased only in the simvastatin-treated SDT fatty rats (Figs. 2 and 5). The magnitude of the decrease in plasma TGL and PL levels was greater in the simvastatin-treated SDT fatty rats than in the simvastatin-treated SD rats (Fig. 6).

The following treatment-related histopathological findings were observed in the liver in the simvastatin-treated SD rats at the end of the dosing period (Table 1): a very slight to slight periportal hepatocellular hypertrophy, very slight to slight single cell necrosis of the hepatocytes, very slight to slight lipofuscin deposition in the periportal Kupffer cells, very slight perportal inflammatory cell infiltration and very slight proliferation of the bile ducts. Treatment-related histopathological findings were not noted in the liver in the simvastatin-treated SDT fatty rats at the end of the dosing period. The absolute liver weights were slightly decreased only in the simvastatin-treated SDT fatty rats (Fig. 5) (p < 0.05).

**DISCUSSION**

Our previous investigation revealed that the SDT fatty rats have lower levels of hepatic GSH and GSSG, and
The characteristics of glucose metabolism, liver function and hepatic glutathione synthesis in the SDT fatty rats are like those in T2DM patients (Takahashi et al., 2019a). In addition, allyl alcohol and carbamazepine induced liver injury more prominently in the SDT fatty rats than in the SD rats (Takahashi et al., 2019a, 2019b). From these, the potential for allyl alcohol and carbamazepine to induce liver injury was assessed to be higher in diabetic patients.
Table 1. The histopathological findings in the liver in the simvastatin-treated SD and SDT fatty rats.

<table>
<thead>
<tr>
<th>Organ</th>
<th>Strain: SD rats</th>
<th>SDT fatty rats</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypertrophy, hepatocellular, periportal</td>
<td>- - - - - - - - - -</td>
<td>- - - - - - - - - -</td>
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<tr>
<td>Single cell necrosis, hepatocytes</td>
<td>- - - - - - - - - -</td>
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<tr>
<td>Deposit, lipofuscin, Kupffer cell, periportal</td>
<td>- - - - - - - - - -</td>
<td>- - - - - - - - - -</td>
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<tr>
<td>Infiltration, inflammatory cell, periportal</td>
<td>- - - - - - - - - -</td>
<td>- - - - - - - - - -</td>
</tr>
<tr>
<td>Proliferation, bile ductule</td>
<td>- - - - - - - - - -</td>
<td>- - - - - - - - - -</td>
</tr>
<tr>
<td>Granular eosinophilic cytoplasm, hepatocyte, (centrilobular)</td>
<td>- - - - - - - - - -</td>
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</tr>
<tr>
<td>Deposit, glycogen, hepatocyte</td>
<td>- - - - - - - - - -</td>
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±: Very slight, +: Slight, 2+: Moderate

The liver samples were obtained under fasted conditions at the end of the dosing period (n=9 or 10/group).
than in non-diabetics (Takahashi et al., 2019a, 2019b). In the present study, we investigated the potential of simvastatin to induce DILI using the SDT fatty rats to estimate the risk of DILI in diabetic patients because simvastatin is used in diabetic patients and shows a positive higher reaction in a GSH adduct assay in vitro (Sakatis et al., 2012) although major metabolic pathway for simvastatin in humans is considered to be oxidation by CYP3A.

Some differences were seen in the parameters for hepatic function and lipid metabolism between the control SD and SDT fatty rats in the present study. The control SDT fatty rats had higher body weights and food consumption, and lower food efficiency (Fig. 1) and this was considered to be related to excessive eating of the diet due to introducing the fa allele of the Zucker fatty rat into the genome of this rat model (Shinohara et al., 2000; Masuyama et al., 2005). The plasma GLU levels were markedly higher in the control SDT fatty rats (Fig. 5) and this could be due to the diabetic state in this animal model (Matsui et al., 2008). The plasma lipid metabolism-related parameters including TGL, T-CH, PL and TKB were higher in the control SDT fatty rats (Figs. 5 and 6) and these changes indicated that lipid metabolism was markedly impaired by the diabetic state of the animals. Higher blood levels of TGL, T-CH and TKB have been reported in T2DM patients and lipid abnormalities are prevalent in diabetes mellitus because insulin resistance or deficiency affects key enzymes and pathways in lipid metabolism (Taskinen, 2002; Krauss, 2004; Mahendran et al., 2013; Ozder, 2014). Thus, the lipid abnormalities noted in the control SDT fatty rats were like those in T2DM patients. The alterations in the hepatic function parameters including AST, ALT and GLDH were noted in the control SDT fatty rats when compared with the control SD rats. The liver weights were also higher in the SDT fatty rats than in the SD rats. These, however, were not related to damage to the hepatocytes based on the results of the histopathological examination (Table 1). The higher plasma levels of AST, ALT and GLDH in the SDT fatty rats (Figs. 2 and 3) were considered to be related to the acceleration of gluconeogenesis and protein catabolism in this animal model as seen in the results of our previous study (Takahashi et al., 2019a). The higher plasma GGT levels in the SDT fatty rats (Fig. 2) were adaptive changes to maintain hepatic glutathione synthesis, as seen in the results of our previous study (Zhang et al., 2005; Takahashi et al., 2019a). The higher plasma GU levels in the SDT fatty rats (Fig. 4) were related to the higher liver weights as noted in the results of our previous study (Takahashi et al., 2019a). The higher plasma ALP levels in the SDT fatty rats (Fig. 4) may be related to increased food consumption as well as discussed in our previous report (Takahashi et al., 2019a). The lower plasma T-BIL levels in the SDT fatty rats (Fig. 4) may be related to the induction of enzymes involved in the metabolism or excretion.

![Fig. 7. The liver weights in the simvastatin-treated SD and SDT fatty rats. The liver weights were measured at the end of the dosing period under fasted conditions (n=9 or 10/group). Data are shown as means ± S.D. ## < 0.01 significantly different between the control SD and SDT fatty rats (Student’s t-test), * p < 0.05, ** p < 0.01 significantly different from the matched control group (Student’s t-test).]
of bilirubin considering the higher liver weights in this strain.

As shown in Fig. 6, the magnitude of the decrease in plasma PL levels were greater in the simvastatin-treated SDT fatty rats than that in the simvastatin-treated SD rats. Statins lower plasma cholesterol by inhibiting 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase in the liver in humans (Singer et al., 1984, 1988). Plasma PL are also decreased after treatment with statins in humans and rabbits (Bard, 1989; Kobayashi et al., 1989), because PL are one of the components of lipoproteins and the change in plasma phospholipids is usually consistent with that in plasma cholesterol. The decrease in plasma PL levels in the simvastatin-treated SDT fatty rats was considered to be related to the pharmacology of simvastatin although there were no changes in plasma T-CH levels in the simvastatin-treated SDT fatty rats. Plasma cholesterol hardly changes in normal rats after treatment with statins because rats compensate for the inhibition of HMG-CoA reductase by inducing more of the target enzyme in the liver (Gerson et al., 1989). Table 1 demonstrated that hypertrophy of the periporal hepatocytes was noted in the simvastatin-treated fatty rats. Plasma AST and ALT levels were slightly increased only in the simvastatin-treated SD rats and the changes tended to disappear with prolongation of the dosing period (Fig. 2). The histopathological findings noted in the liver were slight in the simvastatin-treated SD rats in the present study (Table 1). Slight hepatotoxicity is caused by statins in rats and is considered to be related to the pharmacological action of statins because the liver findings are cancelled by supplementation with mevalonic acid (MacDonald and Halleck, 2004; Singer et al., 1984, 1988). Thus, the liver findings noted in rats with high dose levels of statin were could be mechanism-based and directly attributable to inhibition of mevalonate synthesis (MacDonald et al., 1988). In the simvastatin-treated SDT fatty rats, there were no histopathological findings suggestive of hepatotoxicity although plasma levels for the hepatic function tests (GGT, SDH and T-BIL) were slightly increased (Figs. 2, 3 and 4, Table 1). The slightly higher plasma GGT levels in the simvastatin-treated SDT fatty rats (Fig. 2) may not be adaptive changes to maintain hepatic glutathione synthesis because the GSH adduction is considered not to be a major metabolic pathway for simvastatin in vivo. From these results, there were no marked differences in the potential of simvastatin to induce liver injury between the SD and SDT fatty rats.

Considering all the results of the present study and our previous studies (Takahashi et al., 2019a, 2019b), the risk of hepatotoxicity with simvastatin in diabetic patients appears to be low although simvastatin is highly reactive in the glutathione binding assay in vitro (Sakatis et al., 2012). The low risk of simvastatin to humans can be explained in terms of the body burden of the drug in humans. There are many studies which try to correlate the daily dose, as an indicator of body burden of drugs, with the risk of DILI or other serious toxicity in humans and the risk of drugs with daily doses less than 50 mg/man/day or 100 mg/man/day has been regarded as low even if they have the ability to produce reactive metabolites (Walgren et al., 2005; Smith and Schmid, 2006; Lammert et al., 2008). The zone classification system, established by Nakayama et al. (2009), using covalent binding in human hepatocytes and the daily dose for the risk
assessment of idiosyncratic drug toxicity further supports this estimation. The maximum daily dose of simvastatin is 5 to 20 mg/man/day (less than 50 or 100 mg/man/day) and so the low risk of simvastatin for DILI may be explained by its daily dose. The very low bioavailability of simvastatin due to first pass effects at the intestines and liver may also contribute to the low risk of DILI of simvastatin (Garcia et al., 2003).

In conclusion, there were no effects with simvastatin on the liver in the SD or SDT fatty rats after 13-week oral dosing of simvastatin. These results indicate that simvastatin does not have potential to induce liver injury in diabetic state and are consistent with the reports for the clinical use of simvastatin in the diabetic patients.

ACKNOWLEDGMENTS

The author would like to thank the invaluable contributions of Toshiyuki Shoda, Takuya Matsui, Shinichi Oshida, Kazuma Kondo, Waka Shimizu, Masumi Takeda, Kazumi Ogawa, Eriko Sotomine, Risa Tsuchiya and the staff at the Toxicology Research Laboratories, Central Pharmaceutical Research Institute, JAPAN TOBACCO INC. The author also would like to thank the invaluable contributions of Michiko Yasuda at Graduate School of Integrated Pharmaceutical and Nutritional Sciences, Graduate Program in Environmental Health Sciences, University of Shizuoka.

Conflict of interest—-The authors declare that there is no conflict of interest.

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