Synergistic antifibrotic efficacy of statin and protein kinase C inhibitor in hepatic fibrosis

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Yang JI, Yoon J-H, Bang Y-J, Lee S-H, Lee S-M, Byun HJ, Myung S-J, Kim W, Lee H-S. Synergistic antifibrotic efficacy of statin and protein kinase C inhibitor in hepatic fibrosis. Am J Physiol Gastrointest Liver Physiol 298: G126–G132, 2010. First published November 12, 2009; doi:10.1152/ajpgi.00299.2009.—Statin has antifibrotic efficacy in human fibrosing diseases, such as pulmonary and renal fibrosis, and is therefore implicated in hepatic fibrosis. However, statin can also activate protein kinase C (PKC), which augments hepatic fibrogenesis and is thereby likely to reduce the antifibrotic efficacy of statin. This study was designed to explore the hypothesis that simultaneous treatment with statin and PKC inhibitor may synergistically enhance antifibrotic efficacy in hepatic fibrosis. Hepatic fibrosis models were established in BALB/c mice by intraperitoneal injection of carbon tetrachloride or thioacetamide for 6 wk. Pravastatin and enzastaurin (PKC inhibitor) were administered by gavage for 5 wk. Cellular apoptosis was explored using 4',6-diamidino-2-phenylindole or terminal deoxyribonucleotidyl transferase-mediated deoxyuridine triphosphate biotin nick end-labeling (TUNEL) staining and immunoblot analysis. Hepatic fibrosis and hepatic stellate cell (HSC) activation were assessed by morphometric analysis of histological findings and immunohistochemistry for α-smooth muscle actin. In vitro, the addition of PKC inhibitor significantly increased statin-induced LX-2 cell apoptosis by enhancing the activation of mitochondrial apoptotic signals. TUNEL-positive HSCs were significantly increased in mice treated with statin + PKC inhibitor compared with those in control or single compound-treated mice. The percentage of area occupied by activated HSCs and the extent of collagen deposition were significantly decreased in mice treated with statin + PKC inhibitor compared with those in control or statin-treated mice. In conclusion, simultaneous treatment with statin and PKC inhibitor synergistically enhanced the antifibrotic efficacy in both in vitro and in vivo models of hepatic fibrosis and may therefore have therapeutic implication for reducing hepatic fibrosis.

Hepatic fibrosis develops as a healing response to all causes of chronic hepatic injury; however, hepatic fibrosis also causes many clinically important problems that are associated with the progression of portal hypertension and liver failure (3, 11). Clinically, liver cirrhosis is the most common noncancerous cause of death among hepatobiliary and digestive diseases worldwide (11, 23). Considering that liver cirrhosis increases the risk of hepatocellular carcinoma, the mortality associated with liver cirrhosis may increase even further (10). Despite the high mortality associated with liver cirrhosis, liver transplantation is presently the only effective therapy for cirrhosis. However, liver transplantation has many limitations attributable to organ shortages and complications associated with long-term immunosuppression (14). Therefore, the development of effective therapies to reduce or reverse hepatic fibrosis is important clinically for reducing the morbidity and mortality associated with liver cirrhosis and the need for liver transplantation. Much recent evidence suggests that hepatic fibrosis is reversible and that even cirrhosis can be reversed (3, 4, 11, 14). However, data regarding effective antifibrotic therapies are limited.

Hepatic stellate cells (HSCs) act as the major fibrogenic cells in the liver and are therefore an excellent target for antifibrotic therapy (3, 4, 34). Hepatic fibrosis is characterized by the activation of HSCs and the accumulation of extracellular matrix (ECM). Once HSCs are activated by chronic hepatic injury, activated HSCs not only secrete large amounts of ECM but also regulate the degradation of the ECM. Because apoptosis of activated HSCs can lead to the elimination of the main fibrogenic cells and increase the ECM degradation, induction of activated HSC apoptosis is an effective therapeutic method for targeting and removing activated HSCs (3, 11, 25).

Statin can be an effective antifibrotic agent in hepatic fibrosis because it has exhibited antifibrotic efficacy in many different organs by inhibiting the activation and proliferation of fibrogenic cells and ECM production and by inducing the apoptosis of fibrogenic cells (17, 21, 22, 28, 29, 32). Among the mechanisms of the antifibrotic effects of statin, induction of activated HSC apoptosis might be the most important mode of action because activated HSC apoptosis is essential for the reversal of fibrosis (3, 4, 11). However, activated HSC apoptosis by statin may be interrupted by protein kinase C (PKC) activation, which can be induced by statin treatment itself. Statin has been shown to activate intracellular PKC during apoptosis induction in cells from various organs (2, 33). Because PKC-dependent pathways are important for regulating the activation and proliferation of HSCs (9, 26, 31, 35), PKC activation may make activated HSCs resistant to statin-induced apoptosis and therefore attenuate the antifibrotic efficacy of statin in hepatic fibrosis.Thus we hypothesized that simultaneous treatment with statin and PKC inhibitor may synergistically enhance the antifibrotic efficacy of hepatic fibrosis by enhancing statin-induced, activated HSC apoptosis.

MATERIALS AND METHODS

Cell culture and treatments. LX-2 cells, an immortalized human HSC line (34), were grown in DMEM supplemented with 10% fetal bovine serum, 100,000 U/l penicillin, and 100 mg/l streptomycin at 37°C in a humidified atmosphere of 5% CO2 and 95% air. Cells were serum starved for 20 h before statin and/or PKC inhibitor treatment. Pravastatin, an inhibitor of 3-hydroxy-3-methylglutaryl coenzyme A

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Thirty mice were used for each experiment using CCl4 or TAA and normal saline solution; Sigma) three times per week for 6 wk (13, 18). Enzastaurin (125 mg/kg body wt, in 5% dextrose by gavage 5 days per week for 5 wk) and enzastaurin (125 mg/kg body wt, in 5% dextrose by gavage 5 days per week for 5 wk) and pravastatin (5 mg/kg body wt, in 5% dextrose by gavage 5 days per week for 5 wk) and enzastaurin (125 mg/kg body wt, in 5% dextrose by gavage 5 days per week for 5 wk) for statin inhibitor group. Each treatment was begun 1 wk after starting CCl4 or TAA injection and given for 5 wk with the CCl4 or TAA injection.

Measurement of PKC activity. After treating LX-2 cells with statin (50 μM) for 0.5 h in the presence or absence of enzastaurin (5 μM), intracellular PKC activity was assessed using the Protein Kinase Assay Kit (EMD Chemicals, Darmstadt, Germany). Briefly, cells were collected in ice-cold PBS and sonicated on ice; the lysates were centrifuged, and the supernatants were collected and mixed with the substrates provided in the kit. The phosphorylated substrates were bound by biotinylated antibodies, which subsequently bound peroxidase-conjugated streptavidin. The peroxidase-conjugated streptavidin was reacted with 2-phenylindole (DAPI; Molecular Probes, Eugene, OR), and fluorescent images were captured.

Apoptosis assays. In vitro study, after treating both the 2-days-passed LX-2 cells and the 10-days-passed LX-2 cells with statin (50 μM) for 4 h in the presence or absence of enzastaurin (5 μM), apoptosis was quantified by assessing the characteristic nuclear changes of apoptosis using the nuclear binding dye, 4’,6-diamidino-2-phenylindole (DAPI; Molecular Probes, Eugene, OR), and fluorescent microscopy (Zeiss, Oberkochen, Germany) (24). A minimum of 400 cells was counted for each treatment, and the percentage of apoptotic cells was calculated. Apoptosis was assessed in vivo by terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick end-labeling (TUNEL) staining of formalin-fixed liver tissue using the ApopTag peroxidase in situ apoptosis detection kit (Chemicon, Temecula, CA). The extent of HSC apoptosis was quantified by counting a minimum of 50 α-smooth muscle actin (α-SMA)-positive cells and calculating the percentage of HSC apoptosis in three separate magnified fields (>400).

Immunoblot analysis. After having been treated with statin (50 μM) for each of 0, 1-, 2-, or 3-h period in the presence or absence of enzastaurin (5 μM), LX-2 cells were lysed for 20 min on ice with lysis buffer (50 mM Tris·HCl, pH 7.4; 1% Nonidet P-40; 0.25% sodium deoxycholate; 150 mM NaCl; 1 mM EDTA; 1 mM PMSF; 1 μg/ml aprotinin, leupeptin, pepstatin; 1 mM Na3VO4; and 1 mM NaF) and centrifuged at 14,000 g for 10 min at 4°C. Samples were resolved by SDS-PAGE, transferred to nitrocellulose membranes, and blotted with appropriate primary antibodies. Blots were incubated with peroxidase-conjugated secondary antibodies (Santa Cruz Biotechnology, Santa Cruz, CA), and bound antibodies were visualized using a chemiluminescent substrate (enhanced chemiluminescence; Amersham, Arlington Heights, IL). The following primary antibodies were used: rabbit anti-caspase 9 from Cell Signaling Technology (Danvers, MA), rabbit anti-caspase 8 from BD Pharmingen (San Diego, CA), and goat anti-actin from Santa Cruz Biotechnology.

Histological analysis. Hepatic fibrosis was evaluated through hematoxylin and eosin (H & E) and Masson’s trichrome staining. Liver tissues were fixed in 10% formalin, embedded in paraffin, and then sliced into serial sections of 4-μm thickness. The first and second sections were stained with H & E and Masson’s trichrome according to standard protocols, and the third and/or fourth sections were used for TUNEL and/or immunohistochemical staining. Immunohistochemistry was performed using a monoclonal antibody for α-SMA (BioGenex, San Ramon, CA) on 10-days-passed LX-2 cells and the 10-days-passed LX-2 cells with statin (50 μM) for 0.5 h in the presence or absence of enzastaurin (5 μM).

Results

PKC inhibitor enhances statin-induced HSC apoptosis. We first examined whether PKC in activated HSCs was suppressed by PKC inhibitor. As shown in Fig. 1, statin slightly activated PKC in LX-2 cells, and the intracellular and statin-activated PKC were markedly suppressed by PKC inhibitor. Next, we examined whether statin or PKC inhibitor, singly or in combination, induced HSC apoptosis. As shown in Fig. 2, statin significantly induced LX-2 cell apoptosis, and the addition of PKC inhibitor significantly enhanced statin-induced apoptosis, whereas PKC inhibitor alone did not significantly induce apop-
Fig. 2. PKC inhibitor synergistically enhances statin-induced HSC apoptosis in an activation-dependent manner. LX-2 cells were treated with statin (50 μM) for 4 h in the presence or absence of enzastaurin (5 μM). Apoptosis was quantified using 4',6-diamidino-2-phenylindole staining and fluorescent microscopy. The extent of apoptosis in 10-days-passed LX-2 cells was compared with that in 2-days-passed LX-2 cells. In both 2-days- and 10-days-passed cells, statin significantly induced apoptosis (α: vs. control), and the addition of PKC inhibitor significantly enhanced statin-induced apoptosis (β: statin only or control), whereas PKC inhibitor alone did not significantly induce apoptosis (α and β; one-way ANOVA test with Tukey’s HSD test, P < 0.001). In addition, statin or statin + PKC inhibitor induced apoptosis more potently in 10-days-passed cells than in 2-days-passed cells (γ; two-way ANOVA test with Tukey’s HSD test, P < 0.001). Data are expressed as the means ± SD.

Fig. 3. PKC inhibitor enhances the statin-activated mitochondrial apoptotic pathway. LX-2 cells were treated with statin (50 μM) for the indicated time period in the presence or absence of enzastaurin (5 μM). At each time point, cells were lysed, and equivalent amounts of proteins were immunoblotted for caspase 9, caspase 8, and β-actin.

different treatments (Table 1). In the CCl4 experiment, 7 control mice, 6 mice treated with statin, and 5 mice treated with statin + PKC inhibitor survived (10 mice were assigned per each of the 3 different treatment groups initially), and there was also no significant difference in hepatic enzyme levels or body and liver weights among mice receiving three different treatments (Table 2). We first examined whether statin or statin + PKC inhibitor treatment attenuated hepatic fibrosis in vivo. The extent of collagen deposition was markedly decreased in TAA-injected mice treated with statin + PKC inhibitor than that in control mice or mice treated with statin only (Fig. 4), and this effect was also confirmed in CCl4-injected mice (Fig. 5). These results implicate the potent antifibrotic efficacy of statin + PKC inhibitor treatment in hepatic fibrosis.

Next, we examined whether statin or statin + PKC inhibitor treatment led to a reduction of activated HSCs in vivo. Immunohistochemical staining for α-SMA and morphometric analysis in CCl4-injected mice showed that the percentage of area occupied by activated HSCs was significantly decreased in mice treated with statin + PKC inhibitor compared with that in

Table 1. Body and liver weights and hepatic enzymes were not significantly different among the 3 treatment groups in TAA-injected mice

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Statin</th>
<th>Statin + PKC Inhibitor</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Numbers</td>
<td>8</td>
<td>8</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>Weight, g</td>
<td>21.8±0.1</td>
<td>22.3±0.9</td>
<td>20.7±2.4</td>
<td>0.65</td>
</tr>
<tr>
<td>Liver weight, g</td>
<td>2.0±0.3</td>
<td>2.0±0.2</td>
<td>1.8±0.3</td>
<td>0.36</td>
</tr>
<tr>
<td>Liver weight,%</td>
<td>9.0±0.7</td>
<td>9.0±0.6</td>
<td>8.5±0.7</td>
<td>0.29</td>
</tr>
<tr>
<td>Albumin</td>
<td>16±0.8</td>
<td>20±0.5</td>
<td>23±0.8</td>
<td>0.08</td>
</tr>
<tr>
<td>AP</td>
<td>141.3±55.5</td>
<td>164.3±49.7</td>
<td>166.1±47.4</td>
<td>0.21</td>
</tr>
<tr>
<td>ALT</td>
<td>150.0±74.9</td>
<td>157.1±87.1</td>
<td>173.9±118.3</td>
<td>0.60</td>
</tr>
</tbody>
</table>

Data are expressed as the means ± SD. Body and liver weights were not significantly different among the 3 treatment groups, suggesting similar hepatic injuries by thioacetamide (TAA) in the 3 groups. Though statistically insignificant, the albumin level was higher in mice treated with the combination of statin and PKC inhibitor than in control or statin-treated mice, which suggests that the combination treatment is beneficial to liver function. Other hepatic enzymes showed no significant differences among the 3 treatment groups, suggesting similar hepatic injuries in the 3 groups. AP: alkaline phosphatase; AST: alanine aminotransferase; ALT: aspartate aminotransferase.
The abundant fibrotic area at the same location for the necrotic debris without TUNEL-positive cells were found in the area with α-SMA-positive cells in control mice or mice treated with statin only. Therefore, these findings collectively indicate that enhanced activated HSC apoptosis by treatment with statin + PKC inhibitor might lead to more potent antifibrotic efficacy than treatment with statin alone in vivo models of hepatic fibrosis.

**DISCUSSION**

The principal finding of this study relates to the enhanced antifibrotic efficacy of the combination of statin and PKC inhibitor. Statin induces activated HSC apoptosis by activating mitochondrial apoptotic signals while it also activates PKC in these cells. The combination of statin with PKC inhibitor, then, enhances statin-induced activated HSC apoptosis and leads to a reduction of hepatic fibrosis in animal models.

Statin has antifibrotic efficacy in many organs by inhibiting the activation and proliferation of fibrogenic cells, attenuating ECM production, and inducing apoptosis of fibrogenic cells (1, 17, 21, 22, 28, 29, 32). Statin-induced activated HSC apoptosis was also confirmed in our present study. Recently, statin was reported to induce apoptosis in rat HSCs by activating the mitochondrial apoptotic pathway (1), which was consistent with our findings.

Statin, however, has been shown to activate intracellular PKC (2, 33). PKC-dependent pathways are important for regulating the activation and proliferation of HSCs by such mechanisms as mediating platelet-derived growth factor-induced HSC proliferation, angiotensin-II-induced tissue inhibitor of metalloproteinases-1 upregulation, acetaldehyde-induced increase in pp70(S6K), and extracellular signal-regulated kinase activation (9, 26, 31, 35). Therefore, statin-induced PKC activation in activated HSCs may interrupt statin-induced HSC apoptosis, thereby reducing its antifibrotic efficacy. The addition of PKC inhibitor is thus expected to block this antia apoptotic effect induced by statin. Indeed, our present data demonstrate that statin-induced activation of mitochondrial apoptotic signals might be enhanced by the combination of statin and PKC inhibitor.

### Table 2. Body and liver weights and hepatic enzymes were not significantly different among the 3 treatment groups in CCl4-injected mice

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>Control</th>
<th>Statin</th>
<th>Statin + PKC Inhibitor</th>
<th>P Value</th>
</tr>
</thead>
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<tr>
<td>Numbers</td>
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<td>6</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Weight, g</td>
<td>26.4 ± 2.0</td>
<td>24.5 ± 2.3</td>
<td>24.4 ± 2.1</td>
<td>0.19</td>
</tr>
<tr>
<td>Liver weight, g</td>
<td>2.0 ± 0.2</td>
<td>1.7 ± 0.2</td>
<td>1.9 ± 0.2</td>
<td>0.06</td>
</tr>
<tr>
<td>Liver weight, %</td>
<td>7.8 ± 0.4</td>
<td>7.1 ± 0.8</td>
<td>7.8 ± 0.4</td>
<td>0.11</td>
</tr>
<tr>
<td>Albumin</td>
<td>18 ± 0.2</td>
<td>18 ± 0.1</td>
<td>19 ± 0.2</td>
<td>0.27</td>
</tr>
<tr>
<td>AP</td>
<td>106.7 ± 16.3</td>
<td>96.0 ± 19.0</td>
<td>98.8 ± 12.5</td>
<td>0.49</td>
</tr>
<tr>
<td>AST</td>
<td>169.4 ± 119.8</td>
<td>274.5 ± 284.9</td>
<td>203.8 ± 90.6</td>
<td>0.60</td>
</tr>
<tr>
<td>ALT</td>
<td>153.0 ± 83.3</td>
<td>107.2 ± 28.8</td>
<td>106.2 ± 13.2</td>
<td>0.26</td>
</tr>
</tbody>
</table>

Data are expressed as the means ± SD. Body and liver weights and hepatic enzymes were not significantly different among the 3 treatment groups, suggesting similar hepatic injuries by CCl4 in the 3 groups.
signaling is enhanced by the addition of PKC inhibitor in activated human HSCs, and the enhanced antifibrotic efficacy of this combination therapy was further confirmed in in vivo studies. As we reported previously, the inhibition of caspase-9 binding to X-linked inhibitor of apoptosis protein by PKC inhibitor may be responsible for this enhancement of statin-induced HSC apoptosis by PKC inhibitor (19).

We used two different models of murine hepatic fibrosis, CCl4- and TAA-induced models, which are relatively well established and frequently used for studying hepatic fibrosis (7, 8, 13, 18). In vivo experiments are regarded as the gold standard in hepatic fibrosis research, including HSC biology, and are a required preclinical step for clinical application of new therapeutic strategies in hepatic fibrosis (8). On the other hand, it is also possible that drugs affecting cytochrome P450 2E1 activity may falsely exhibit fibrosis-reducing activity by interfering with the fibrosis-inducing activity of CCl4, instead of by decreasing HSC activation or survival. However, because neither pravastatin nor enzastaurin, which were used in our study, interacts with or induces cytochrome P450 2E1, induction of hepatic fibrosis by CCl4 might not be affected by these drugs. Moreover, we also employed the TAA-induced fibrosis model, which is independent of cytochrome P450 2E1 activity for inducing hepatic fibrosis. In vivo experiments are also important because fibrogenic cells other than activated HSCs, such as myofibroblasts, may respond profibrogenically to experimental drugs and thereby interrupt the antifibrotic effect. However, because our results showed an overall fibrosis reduction in two different models, the observed antifibrotic efficacy of pravastatin + enzastaurin most likely resulted from enhanced activated HSC apoptosis. Regarding in vivo experiments, there were two limitations in our study. First, many CCl4-treated mice died although there was no significant difference between treatment groups. Survival of mice is one of the most important factors for interpreting therapeutic benefit. However, CCl4 is known to result in high mortality of 40–100% during hepatic fibrosis induction in mice (6). Moreover, no death occurred in TAA-treated mice with combined statin

**Fig. 5.** The combination of statin and PKC inhibitor attenuates hepatic fibrosis in CCl4-injected mice. Liver sections and morphometric analysis of collagen-deposited area from CCl4-injected mice. Hepatic fibrosis was examined by Masson’s trichrome staining for collagen deposition (×100 magnified fields), and gray area represents collagen deposition. The extent of collagen deposition was measured by image analysis in 3 randomly selected, separate, ×100 magnified fields from each slide, and the percentage of gray area was calculated to compare the differences in collagen deposition among mice receiving 3 different treatments. The extent of collagen deposition was markedly decreased in mice treated with statin + PKC inhibitor (b; vs. statin only or control) than that in mice treated with statin only (a; vs. control) or in control mice (a and b; one-way ANOVA test with Tukey’s HSD test, P < 0.001). Data are expressed as the means ± SD.

**Fig. 6.** The combination of statin and PKC inhibitor reduces activated HSCs in vivo. Activated HSCs in the livers of CCl4-injected mice were examined by immunohistochemistry for α-smooth muscle actin (α-SMA), and the α-SMA-positive cells are shown in brown (×400 magnified fields). The extent of α-SMA-positive cells was measured by image analysis in 3 randomly selected, separate ×100 magnified fields from each slide. The percentage of area occupied by activated HSCs was significantly decreased in mice treated with statin + PKC inhibitor (b; median one-way ANOVA test, P < 0.001) compared with that in control mice or mice treated with statin only. Data are expressed as the means ± SD.
and PKC inhibitor in our present study, and no mice died in our previous study using combined statin and PKC inhibitor in subcutaneous tumor graft mice with normal liver (19); this death in CCl4-treated mice seems to be caused by CCl4 toxicity. Lower dose of CCl4 is expected to induce hepatic fibrosis successfully as reported in other studies (18). Second, only toxin-induced hepatic fibrosis models were explored in our study. Because bile duct ligation can induce hepatic fibrosis in mice, this model may also be used in our experiments. However, because most statins undergo biliary excretion, it would be difficult to get constant and safe dose achievements of statin in bile duct-ligated mice, and we, therefore, cautiously selected two different toxin-induced fibrosis models (30). Pravastatin has many beneficial characteristics for use in hepatic fibrosis (16, 30). Compared with other statins, pravastatin is active by itself, undergoes little hepatic metabolism, and is selectively distributed to the liver. The dose of pravastatin used in this study (5 mg/kg body wt) was comparable to the conventional therapeutic dose in human (about 24 mg in 60-kg adult). Enzastaurin is also a safe drug without serious adverse effects such as hepatic derangement (5). The dose of enzastaurin used in this study (125 mg/kg body wt) was also comparable to the conventional therapeutic dose in human (about 600 mg in 60-kg adult) (5). As reported previously, liver disease attributable to statin itself is rare; therefore, a cautious trial using a conventional therapeutic dose of statin with close monitoring may be safe in patients with hepatic fibrosis (20). Enzastaurin showed neither significant adverse systemic nor hepatic effects even in advanced cancer patients (5). During our in vivo studies, we observed that the mean body weights and the medians of hepatic enzymes did not differ significantly among mice receiving three different treatments in both the CCl4-treated and the TAA-treated groups. Moreover, although none of the treatments, including combined statin and PKC inhibitor, appeared to significantly improve liver function, there was a slightly favorable increase of serum albumin level, which is a clinically useful marker for the synthetic function of liver, in mice treated with combined statin and PKC inhibitor in both the CCl4-treated and the TAA-treated groups. The lack of statistical differences in serum albumin levels may be due to limited duration of our experiments and may thus become significant with longer duration of treatment. Another possibility may be due to relatively reserved hepatic function despite hepatic fibrosis. Considering human liver cirrhosis, despite the existing hepatic fibrosis, hepatic synthetic functions reflected by albumin decrease slowly until decompensation occurs. Because there was also no serious adverse effect such as animal death or hepatic dysfunction by combined statin and enzastaurin in our previous study using subcutaneous tumor graft mice with normal liver, combined pravastatin and enzastaurin may be considered to have no significant adverse effect in mice (19). Therefore, trials of combined pravastatin and enzastaurin could be considered in patients with hepatic fibrosis but still will require more detailed preclinical or clinical information about the effects of combined drugs on liver function.

Collectively, the results of the present study demonstrate that simultaneous treatment with statin and PKC inhibitor synergistically enhanced antifibrotic efficacy in both in vitro and in vivo models of hepatic fibrosis. Therefore, this combination strategy of statin and PKC inhibitor may have therapeutic implication for reducing hepatic fibrosis. Furthermore, because the progression of liver cirrhosis increases the risk for development of hepatocellular carcinoma, antifibrotic therapies may also be considered anticarcinogenic therapies. Moreover, because our previous study showed that statin + PKC inhibitor had selective antitumor efficacy for established hepatocellular carcinoma, this combination might be a powerful therapeutic strategy against both hepatic fibrosis and hepatocellular carcinoma (19).

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