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Attenuation of Kidney Injuries Maintains Serum Sulfatide Levels Dependent on Hepatic Synthetic Ability: A Possible Involvement of Oxidative Stress

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Serum sulfatides are the major glycosphingolipids in lipoproteins. Although serum sulfatides are mainly synthesized and secreted by the liver, they are significantly decreased when the kidneys are impaired. Our recent experimental study using a murine protein-overload nephropathy model suggested a hypothetical mechanism whereby serum sulfatides were reduced due to kidney dysfunction. This was the result of decreased hepatic expression of a sulfatide synthetic enzyme, cerebroside sulfotransferase (CST), which is associated with systemic enhancement of oxidative stress. However, there is a possibility that the experimental process, protein-overload itself, directly affected the sulfatide metabolism and oxidative stress in the liver. To determine whether kidney dysfunction actually reduces the hepatic synthesis of sulfatides via oxidative stress, we examined sulfatide levels, the hepatic content of metabolic sulfatide enzymes, and the degree of oxidative stress in protein-overload mice subjected to renoprotective therapy using clofibrate, a representative hypolipidemic medicine. Protein-overload mice exhibited marked kidney injuries, enhancement of hepatic oxidative stress, decreased levels of serum and hepatic sulfatides, and decreased expression of hepatic CST. The clofibrate treatment attenuated kidney damage and hepatic oxidative stress while maintaining serum/hepatic sulfatide levels and hepatic CST content in the mice. Because clofibrate monotherapy without protein-overload treatment only minimally affected these hepatic parameters, the hepatic synthesis of sulfatides appeared to be strongly influenced by kidney dysfunction and subsequent oxidative stress. This study suggests that the crosstalk between kidney dysfunction and hepatic sulfatide metabolism is mediated by oxidative stress. These results should help to understand the phenomenon in patients with end-stage kidney disease.

Keywords: cerebroside sulfotransferase; clofibrate; kidney injury; oxidative stress; sulfatides
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Sulfatides, which belong to glycosphingolipids, are esters of sulfuric acids with galactosylceramides at the C3 position of the galactosyl residue (Ishizuka 1997). Sulfatides are found in various mammalian tissues such as brain, kidney, and liver (Ishizuka 1997; Tadano-Aritomi et al. 2001; Honke et al. 2004; Nagai et al. 2008). Sulfatides are also present in lipoproteins as major glycosphingolipids, and their antithrombotic potential has been proposed in several studies using atherosclerosis animals (Hara and Taketomi 1987, 1991; Hara et al. 1993, 1996; Kyogashima 2004). We have reported that the serum levels of sulfatides are significantly lower in human patients with end-stage renal failure than in normal subjects (Hu et al. 2007), and that these lower levels recover after kidney transplantation (Wang et al. 2011). Interestingly, the lower levels of serum sulfatides in these patients are related to the development of

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cardiovascular disease (Hu et al. 2007), which is a major risk factor for mortality (Sarnak et al. 2003). Therefore, understanding the mechanism of the relationship between the serum sulfatide levels and kidney function may lead to better understanding of the pathogenesis of cardiovascular disease in these patients as well as help to plan new therapeutic strategies for treating the disease.

To investigate the mechanism, we conducted an experimental study using murine protein-overload nephropathy, an established mouse model of acute kidney injury (Li et al. 2009; Zhang et al. 2009). In these mice, reductions in the levels of serum sulfatides were identified as well as decreases in hepatic levels of sulfatides and cerebroside sulfotransferase (CST), a key enzyme of sulfatide synthesis (Honke et al. 2002). In addition, increased markers of oxidative stress, such as malondialdehyde (MDA), were detected in their livers. Because serum sulfatides are considered to be secreted from the liver together with lipoproteins, these findings suggested that the reduction of serum sulfatide levels were due to the decrease in hepatic sulfatide synthesis, which was influenced by the oxidative stress involved in the kidney injury. However, it was unclear if these functional changes in hepatic sulfatide metabolism were really induced by kidney injury. The specific experimental process, protein-overload itself, might directly suppress the hepatic sulfatide synthesis.

To clarify whether kidney dysfunction actually reduces the hepatic synthesis of sulfatides via oxidative stress, we examined the levels of sulfatides, the hepatic content of metabolic sulfatide enzymes, and the degree of oxidative stress in protein-overload mice subjected to renoprotective therapy. It has been reported that the acute kidney injury induced by protein-overload nephropathy is caused by the toxicity of free fatty acids (FFA) bound to overloaded bovine serum albumin (BSA) (Kamijo et al. 2007a) and that this renal FFA toxicity is attenuated by pretreatment with clofibrate, a lipid-lowering agent which enhances the fatty acid-catabolizing activity in mouse kidney, resulting in less kidney injury (Takahashi et al. 2011). In the present study, we used clofibrate treatment as an established renoprotective therapy.

Methods

Mice and experimental design

All animal experiments were conducted in accordance with animal study protocols approved by the Shinshu University School of Medicine. Female Sv/129 mice (26 weeks of age; 26-30 g of body weight) were used in this study. They were maintained in a specific pathogen-free environment under controlled conditions (25°C; 12 h light/dark cycle) and had access to tap water and food *ad libitum*. The mice were divided into two groups, the regular-diet and clofibrate-pretreatment groups. The former group was fed a standard rodent diet (regular diet) throughout the experimental period. The latter group was treated with a regular diet containing 0.1% (w/w) clofibrate from two weeks prior to protein overload to the end of the study. Clofibrate and its metabolites are excreted from the body mainly via urine, therefore they are accumulated during a state of kidney dysfunction. High-dose clofibrate administration (0.5% in diet) to the protein-overloaded mice causes excess drug accumulation and related adverse effects, leading to aggravation of kidney injury (Kamijo et al. 2007a). On the other hand, the low-dose pretreatment regimen in this study is reported to be effective in preventing the kidney injury caused by protein overload and the resultant undesirable increase in plasma drug concentration (Takahashi et al. 2011). Therefore, we used this low-dose regimen in the current study as a form of renoprotective therapy. Clofibrate (ethyl 2-[4-chlorophenoxy]-2-methylpropionate) was purchased from Wako Pure Chemical Industries (Osaka, Japan). For the protein overload study, FFA-binding BSA was used since overloading of this material, but not FFA-free BSA, to mice caused obvious proximal tubular injury in our previous study (Kamijo et al. 2007a). Some mice were selected from each group, and 0.375 g of FFA-binding BSA was administered to each mouse via intraperitoneal bolus injections once a day for 17 days. The FFA-binding BSA was obtained from Sigma-Aldrich Corporation (St. Louis, MO, USA; catalog number, A4503) and diluted with sterile saline (Otsuka Pharmaceutical, Tokyo, Japan) just prior to the injection. None of mice died during the experimental period. At days 0 and 17 of the BSA treatment, the mice were sacrificed under anesthesia for collection of blood and tissues. Serum, livers, and kidneys obtained from each mouse were stored at −80°C until analyses. The numbers of mice subjected to analyses were as follows: $n = 3$ in each group at day 0 (no BSA injection) and $n = 6$ in each group at day 17.

Quantitation and identification of sulfatides

Sulfatides were extracted from serum and tissue-homogenate of each mouse using the HIP method [*n*-hexane-isopropanol, 3:2 (v/v)] (Hara and Radin 1978). They were then converted to lysosulfatides (LS; sulfatides without fatty acids) by saponification with sodium hydroxide (Li et al. 2007). The LS samples were purified through mono-tip C18 cartridges (GL Sciences, Tokyo, Japan). Following the addition of the internal standard, *N*-acetyl LS-sphinganine, the samples were analyzed by matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) using a Voyager Elite XL Biospectrometry Workstation (6.5-m flight length in the negative ionization mode) (PerSeptive Biosystems, Framingham, MA, USA) (Li et al. 2007). The levels of sulfatides were calculated as the sum of the seven LS molecular species shown in Figure 1 (Ishizuka 1997; Li et al. 2007).

Analysis of mRNA

Total RNA from the liver and kidney was extracted using an RNeasy Mini Kit (QIAGEN, Hilden, Germany), and mRNA was reverse-transcribed using oligo-dT primers with SuperScript II reverse transcriptase (Invitrogen Corporation, Carlsbad, CA, USA). Quantitative real-time polymerase chain reaction (PCR) was performed using a SYBR Premix Ex Taq II (Takara Bio, Otsu, Japan) on a Thermal Cycler Dice TP800 system (Takara Bio). The primer pairs shown in Table 1 were designed with Primer Express software (Applied Biosystems, Foster City, CA, USA) and ordered from Sigma-Aldrich. The level of target mRNA was normalized to that of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and then normalized to that of mice in the regular-diet group at day 0.

Table 1. The primer pairs used for quantitative real-time PCR.

Gene name	Primer sequence (5′ to 3′)	NCBI GenBank
ARSA	F ACCACCCCTAACCTGGATCAGT R ATGGCGTGCACAGAGACACA	NM_009713
Catalase	F CGACCAGGGCATCAAAAACTT R AACGTCCAGGACGGGTAATTG	NM_009804
CGT	F TGGGTCCAGCCTATGGATGT R GCAGCGTTGGTCTTGGAAAC	NM_011674
CST	F ATGGCCTTCACGACCTCAGA R CGGTCTTGTGCGTCTTCATG	NM_016922
Cu, Zn-SOD	F TCCCAGACCTGCCTTACGACTAT R AGCCTTGTGTATTGTCCCCATACT	NM_011434
GALC	F GAGTGAGAATCATAGCGAGCGATA R AGTTCCTGGTCCAGCAGCAA	NM_008079
GAPDH	F TGCACCACCAACTGCTTAG R GGATGCAGGGATGATGTTCTG	M32599
GPx-1	F CCAGGAGAATGGCAAGAATGA R TCTCACCATTCACTTCGCACTT	NM_008160
Mn-SOD	F TCCCAGACCTGCCTTACGACTAT R GGTGGCGTTGAGATTGTTCA	NM_013671
NOX2	F GAAAACTCCTTGGGTCAGCACT R ATTTCGACACACTGGCAGCA	NM_007807
NOX4	F AGCATCTGCATCTGTCCTGAAC R GACTGTCCGGCACATAGGTAAAA	NM_015760

F, forward sequence; R, reverse sequence.

Immunoblot analysis

Liver and kidney lysates were prepared respectively as described earlier (Aoyama et al. 1989, 1993, 1994), and their protein concentrations were measured colorimetrically with the BCA Protein Assay Kit (Pierce Biotechnology, Rockford, IL, USA). The lysates were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transferred to nitrocellulose membranes, which were incubated with primary antibodies and then with alkaline phosphatase-conjugated secondary antibodies. Mouse polyclonal primary antibodies against CST were purchased from Abnova Corporation (Jhouzih St., Taiwan). Rabbit polyclonal primary antibodies against actin were obtained from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Rabbit polyclonal antibodies against catalase were prepared as described earlier (Furuta et al. 1986). The protein band positions were determined by co-electrophoresis of molecular weight standards (Bio-Rad Laboratories, Hercules, CA, USA). Band intensities were measured densitometrically, normalized to those of actin, and then normalized to those of mice in the regular-diet group at day 0.

Other methods

The concentrations of serum urea nitrogen and creatinine were measured with a clinical analyzer (JCA-BM2250; JEOL, Tokyo, Japan). The MDA concentrations were determined using a LPO-586 kit (OXIS International, Beverly Hills, CA, USA). Serum transaminase levels were measured with a Transaminase CII-test kit (Wako). For histological examinations, liver and kidney tissues from each group were fixed in 4% paraformaldehyde (Wako) and embedded in paraffin. The deparaffinized sections were stained with hematoxylin and eosin, periodic acid Schiff, or periodic acid-methenamine-silver, as noted previously (Takahashi et al. 2011).

Statistical analysis

Analysis of significant differences with respect to the interactive effects of two factors (clofibrate and BSA treatments) was performed using two-way ANOVA. Data were expressed as mean ± standard error of the mean (SEM). Probability values < 0.05 were considered to be statistically significant.

Results

Sulfatide levels and compositions in BSA-injected mice with and without clofibrate pretreatment

As in the previous study (Takahashi et al. 2011), intraperitoneal FFA-binding BSA injections for 17 days caused obvious hyperproteinemia, followed by vast increase of proteinuria in the regular-diet group of mice. The huge amount of proteinuria exerted FFA toxicity, and caused proximal tubular cell damage and kidney dysfunction. On the other hand, the BSA injections did not affect serum concentrations of transaminases or pathological findings of liver tissues (data not shown), suggesting the absence of apparent liver injury resulting from the injections. There was no difference in the levels of hyperproteinemia and proteinuria between the regular-diet and clofibrate-

pretreatment groups; however, the clofibrate pretreatment apparently decreased representative serum kidney dysfunction markers (serum urea nitrogen at day 0, 17.1 ± 5.3 vs. 16.5 ± 2.6 mg/dl; day 17, 50.2 ± 6.4 vs. 34.4 ± 5.2 mg/dl; serum creatinine at day 0, 0.22 ± 0.06 vs. 0.21 ± 0.04 mg/dl; day 17, 0.51 ± 0.13 vs. 0.38 ± 0.11 mg/dl for the regular-diet and clofibrate-pretreatment groups, respectively), and attenuated tubulointerstitial pathological findings including tubular atrophy, dilatation, and hyaline cast formation. The tubular protective effects of clofibrate appeared to be associated with the counteraction of peroxisome proliferator-activated receptor α deterioration, resulting in the decrease of FFA influx to the kidney, maintenance of renal fatty acid oxidation, diminution of intracellular accumulation of undigested FFA, and attenuation of disease developmental factors including oxidative stress, apoptosis, and nuclear factor-κB (NF-κB) activation. These findings suggest that clofibrate pretreatment has a considerable potential protecting tubular damage.

The amounts and compositions of sulfatides in serum, liver, and kidney are shown in Fig. 1. In the regular-diet group, the BSA injection caused marked reduction of serum and liver sulfatide levels (Fig. 1A and B, bar graphs), whereas it increased the amount of renal levels (Fig. 1C, bar graph). In contrast, these changes were less in the clofibrate-pretreatment group (Fig. 1, bar graphs). Clofibrate treatment without the BSA injection did not affect the levels of any types of sulfatides. The sphingoid composition of serum sulfatides was similar to that of hepatic ones, but not renal ones, in both groups (Fig. 1, pie charts), which strengthened the concept that serum sulfatides are secreted from the liver together with lipoproteins. Neither BSA nor clofibrate treatment affected the sphingoid composition of any types of the sulfatides (Fig. 1, pie charts). These findings indicate that the reduction of serum and liver sulfatide levels resulting from BSA injection was due to kidney injury and could be prevented by reinforcing kidney function with clofibrate pretreatment.

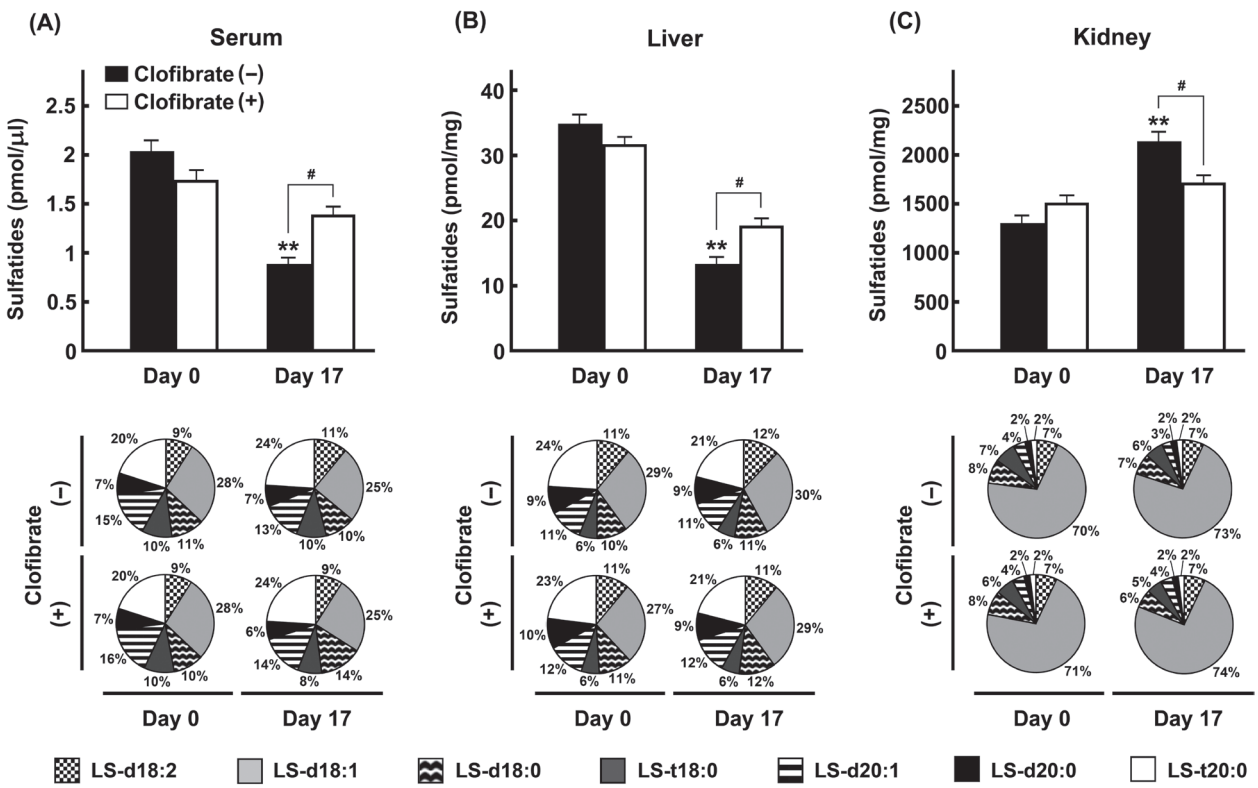

Fig. 1. Sulfatide levels and sphingoid compositions in serum, liver, and kidney from mice receiving BSA injection with and without clofibrate pretreatment.
Sulfatide levels (bar graphs) and sphingoid compositions (pie charts) in serum (A), liver (B), and kidney (C) were measured by MALDI-TOF MS. The levels of sulfatides were calculated as the sum of the seven LS molecular species as follows: LS-sphingadienine (d18:2), -(4*E*)-sphingenine (d18:1), -sphinganine (d18:0), -4D-hydroxysphinganine (t18:0), -(4*E*)-icosasphingenine (d20:1), -icosasphinganine (d20:0), and -4D-hydroxyicosasphinganine (t20:0), which are indicated in the bottom of the figure. Data are expressed as mean ± SEM. Black bar, regular-diet group; white bar, clofibrate-pretreatment group. The numbers of mice used for the analysis were as follows: $n = 3$ in each group at day 0 and $n = 6$ in each group at day 17 of the BSA treatment. $^{**}P < 0.01$, between mice at days 0 and 17 in each group. $^{\#}P < 0.05$, between the regular-diet and clofibrate-pretreatment groups.

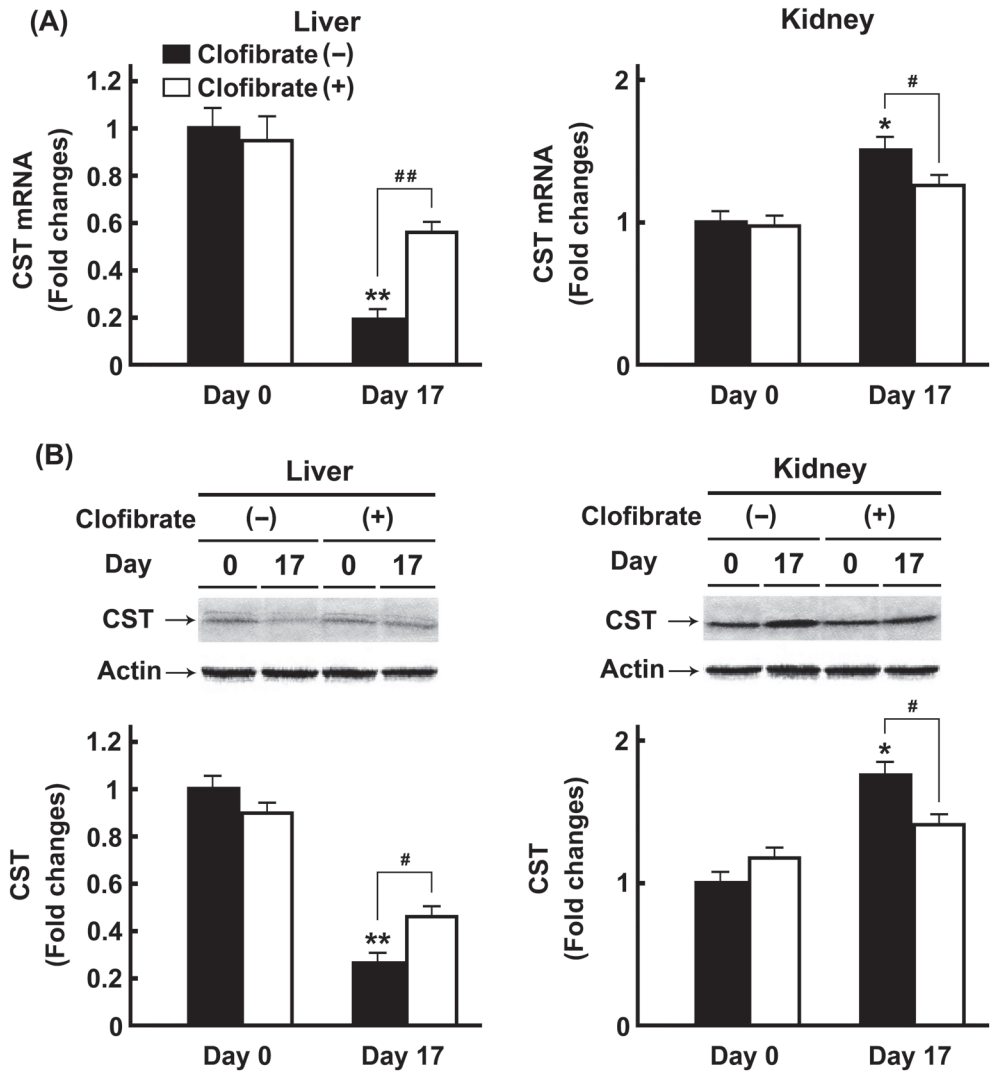

Fig. 2. Expression of the sulfatide biosynthetic enzyme CST in the liver and kidney from mice receiving BSA injection with and without clofibrate pretreatment.
(A) Analysis of mRNA. Hepatic and renal expressions of mRNA were measured using quantitative real-time PCR and normalized to those of GAPDH mRNA, respectively. The mRNA levels are shown as fold changes to those of mice in the regular-diet group at day 0. (B) Immunoblot analysis. One hundred micrograms of whole-liver or whole-kidney lysate protein from each mouse were loaded into each well for electrophoresis. Actin was used as the loading control. Band intensities were measured densitometrically, normalized by those of actin, and then normalized to those of mice in the regular-diet group at day 0. Data are expressed as mean ± SEM. The groups and numbers of mice were the same as Fig. 1. $^{*}P < 0.05$, $^{**}P < 0.01$, between mice at days 0 and 17 in each group. $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, between the regular-diet and clofibrate-pretreatment groups.

Expression of enzymes involved in sulfatide metabolism in BSA-injected mice with and without clofibrate pretreatment

To elucidate the mechanisms underlying the above-mentioned phenomena, the hepatic and renal expression of sulfatide-metabolizing enzymes were measured. According to our earlier findings (Zhang et al. 2009), the sulfatide-generating enzyme CST was first examined. The BSA injection caused a significant decrease of hepatic CST mRNA in the regular-diet group (Fig. 2A), while it increased the renal mRNA levels. In contrast, these effects of the BSA injection were lower in the clofibrate-pretreatment group (Fig. 2A). Clofibrate administration itself did not affect CST mRNA expression in either tissue. Immunoblot analysis confirmed similar expression patterns of CST at the protein level (Fig. 2B). BSA or clofibrate treatment did not influence the expression of arylsulfatase A (ARSA), ceramide galactosyltransferase (CGT), or galactosylceramidase (GALC), in either tissue (Fig. 3). These data suggest that the levels of serum and liver sulfatides were solely correlated with the expression level of hepatic CST.

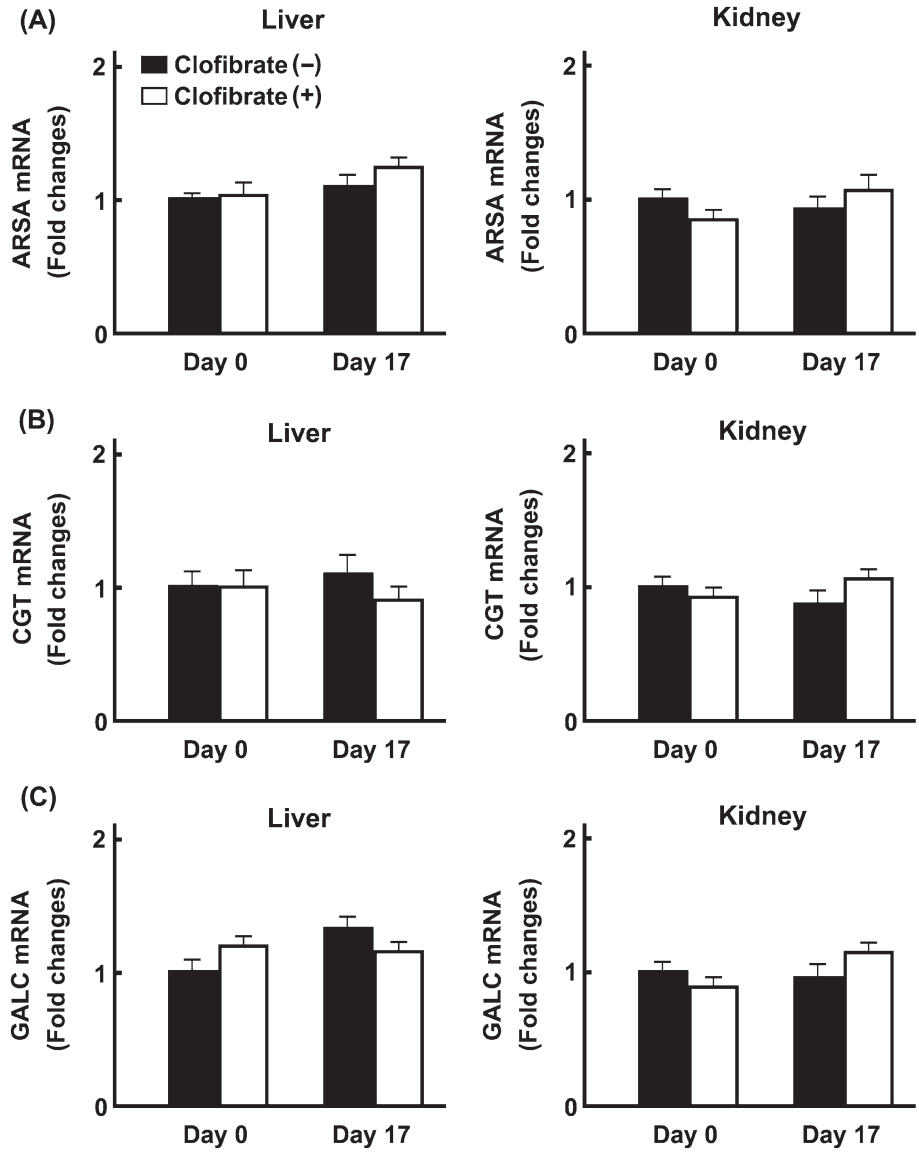

Fig. 3. Expression of the other sulfatide-metabolizing enzymes in the liver and kidney from mice receiving BSA injection with and without clofibrate pretreatment.
Hepatic and renal expressions of mRNA encoding ARSA (an enzyme degrading sulfatides to galactosylceramides) and CGT and GALC (enzymes for biosynthesis and degradation of galactosylceramides, respectively) were measured using quantitative real-time PCR and normalized to those of GAPDH mRNA, respectively. The mRNA levels are shown as fold changes to those of mice in the regular-diet group at day 0. Data are expressed as mean ± SEM. The groups and numbers of mice were the same as Fig. 1.

Oxidative stress in BSA-injected mice with and without clofibrate pretreatment

In our previous study, the decrease of hepatic CST expression by BSA treatment occurred simultaneously with an increase of oxidative stress (Zhang et al. 2009). To determine the degree of oxidative stress, the tissue levels of MDA, a representative lipid peroxidation marker, were analyzed. In the regular-diet group, the BSA injection increased MDA in the liver and kidney (Fig. 4A). However, these increases of MDA were slight in the clofibrate-pretreatment group (Fig. 4A). The expression of oxidative stress-related enzymes was then examined in both groups. The BSA injection to the regular-diet group resulted in lower levels of mRNA encoding catalase, a typical anti-oxidative enzyme, in the liver and kidney (Fig. 4B). On the contrary, the tissue levels of catalase mRNA were higher in the clofibrate-pretreatment group despite the BSA co-administration (Fig. 4B). This induction of liver and kidney catalase also occurred in the absence of BSA injection and the induced levels were similar to those determined with the injection (Fig. 4B), indicating that the effect was the result of the clofibrate treatment *per se*. Immunoblot analysis showed similar changes at the protein level (Fig. 4C). BSA or clofibrate treatment did not

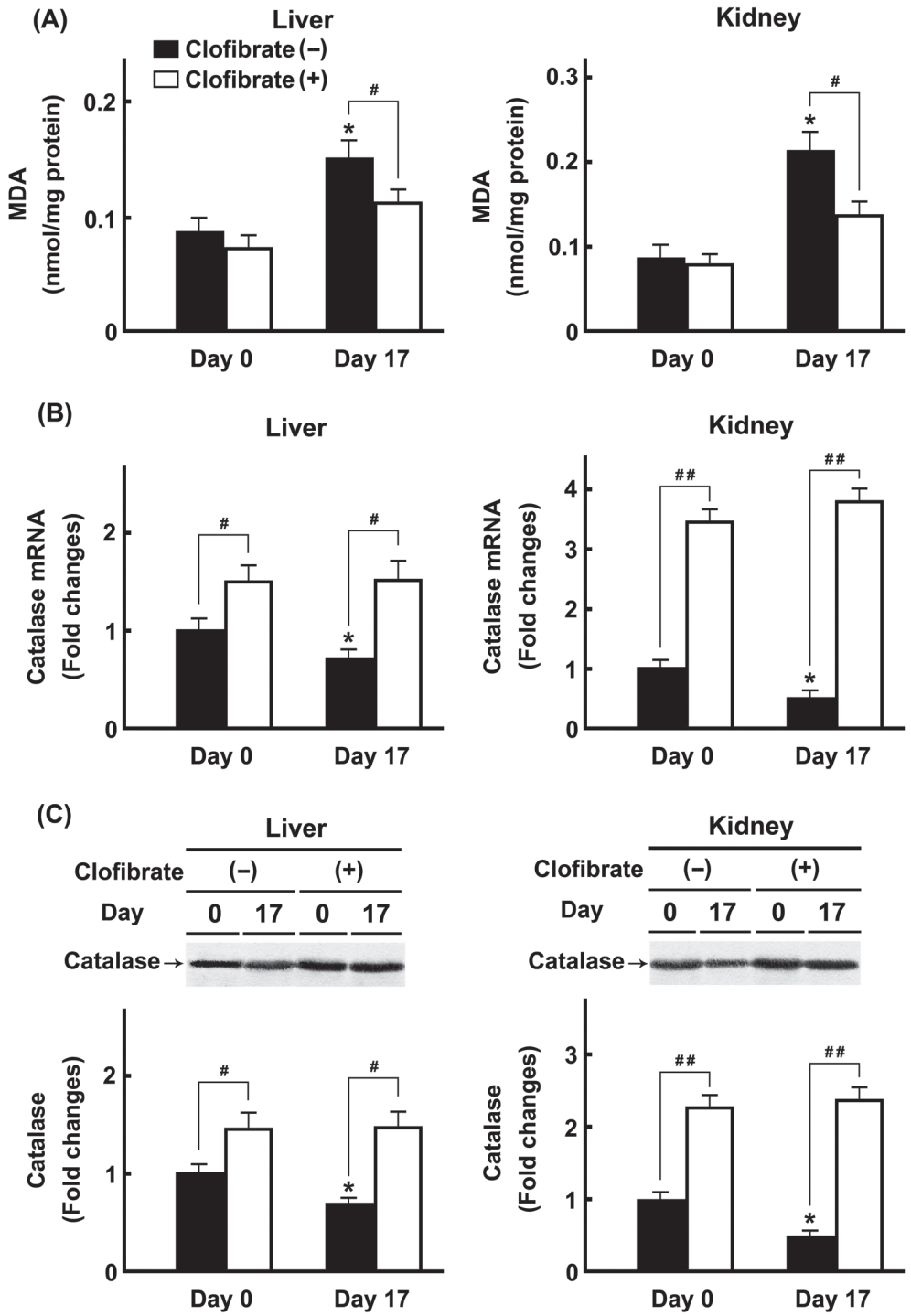

Fig. 4. Oxidative stress in the liver and kidney from mice receiving BSA injection with and without clofibrate pretreatment. (A) Tissue MDA contents. (B) Analysis of mRNA encoding catalase. Hepatic and renal expressions of mRNA were measured using quantitative real-time PCR and normalized to those of GAPDH mRNA, respectively. The mRNA levels are shown as fold changes to those of mice in the regular-diet group at day 0. (C) Immunoblot analysis of catalase. Twenty micrograms of whole-liver or whole-kidney lysate protein from each mouse were loaded into each well for electrophoresis. Actin shown in Fig. 2B was used as the loading control. Band intensities were measured densitometrically, normalized by those of actin, and then normalized to those of mice in the regular-diet group at day 0. All data are expressed as mean ± SEM. The groups and numbers of mice were the same as Fig. 1. $^{*}P < 0.05$, between mice at days 0 and 17 in each group. $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, between the regular-diet and clofibrate-pretreatment groups.

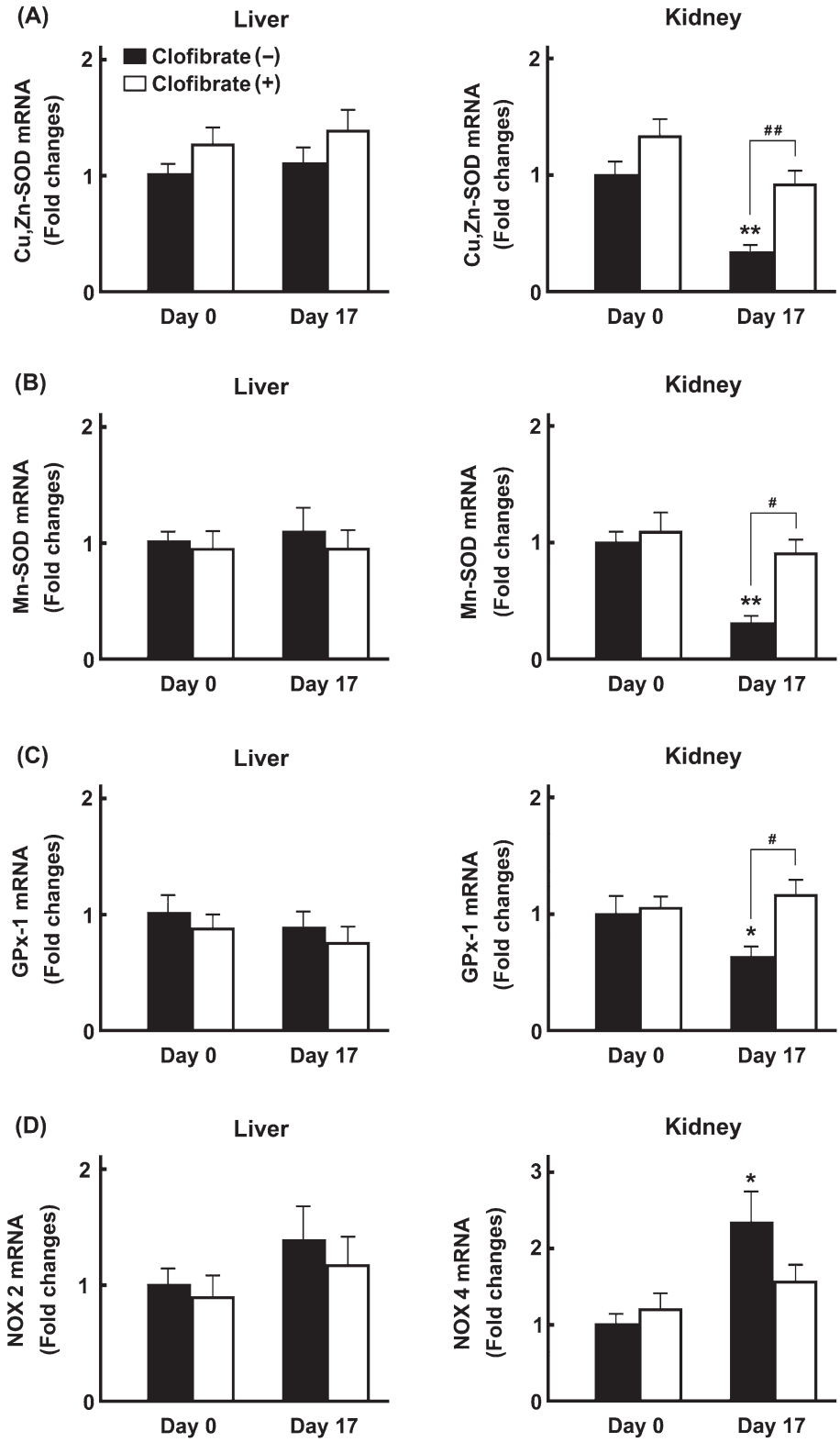

Fig. 5. Expression of oxidative stress-related enzymes in the liver and kidney from mice receiving BSA injection with and without clofibrate pretreatment.

Hepatic and renal expressions of mRNA encoding typical anti-oxidative enzymes (A-C) and tissue-specific NADPH oxidase (NOX2 for liver; NOX4 for kidney) (D) were measured using quantitative real-time PCR and normalized to those of GAPDH mRNA, respectively. The mRNA levels are shown as fold changes to those of mice in the regular-diet group at day 0. Data are expressed as mean ± SEM. The groups and numbers of mice were the same as Fig. 1. $^{*}P < 0.05$, $^{**}P < 0.01$, between mice at days 0 and 17 in each group. $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, between the regular-diet and clofibrate-pretreatment groups.

significantly alter the hepatic mRNA expression of other enzymes involved in oxidative stress elimination (Cu,Zn-superoxide dismutase [SOD], Mn-SOD, or glutathione peroxidase-1 [GPx-1]) and generation (NADPH oxidase) (Fig. 5). These results propose that the reduction of catalase contributed to the enhancement of hepatic oxidative stress during BSA injection, and that clofibrate treatment was related to the prevention of the oxidative stress.

Discussion

In the present study, we found that the alteration in hepatic sulfatide metabolism, including the reduction of the levels of serum sulfatides accompanied by BSA-induced kidney injury diminished in mice receiving clofibrate. This phenomenon was closely related to decreased kidney damage, since clofibrate treatment without BSA injection did not affect any parameters involving sulfatides. Thus, this study demonstrated the direct contribution of kidney dysfunction to the alteration of liver sulfatide metabolism in this acute kidney injury mouse model.

The reductions of hepatic sulfatide synthesis and serum sulfatide levels were previously proposed to be involved in the increase of systemic oxidative stress resulting from kidney injury (Zhang et al. 2009). In the present study, the elevations of both hepatic and renal MDA levels as found in mice with BSA-induced kidney injury were not detected in mice without the injury. This result was well correlated with the difference between the two groups in the levels of serum and liver sulfatides as well as hepatic CST. In addition, this correlates with our recent clinical observation (Wang et al. 2011), that a higher level of serum MDA detected in patients with end-stage renal disease was reduced following kidney transplantation, and it was statistically correlated with the changes in serum sulfatide levels. Therefore, oxidative stress might be a key mediator for the regulation of sulfatide metabolism in both humans and mice. To further understand the association between oxidative stress and sulfatide metabolism in liver, additional studies are undertaken, using the mice under special conditions which represent high levels of oxidative stress in liver without apparent kidney injury. For examples, the mice treated with ethanol (Nakajima et al. 2004), administered high level of bezafibrate (Nakajima et al. 2010), and fed with diet containing saturated fatty acids (Tanaka et al. 2010), will be examined, respectively.

Because of the small amount of information available on transcriptional regulators on the mouse CST gene, we searched its upstream regions (Match; http://www.gene-regulation.com/cgi-bin/pub/programs/match/bin/match.cgi) and found several antioxidant response element-like sequences: -915/-905 (GGTGACTCAGG), -788/-778 (TCTGACGTGGC), and -595/-585 (AGTGACTCGGC) on exon 1a; +33/+43 (AGTGACTCACA) on exon 1d; -1,256/-1,246 (TATGACGTCTG) and -526/-516 (AGTGAC TTCGC) on exon 1f; and -2,523/-2,513 (ACTGACTA AGC), -790/-780 (GGTGACGTGCC), and -361/-351 (GCTGACTCAAC) on exon 1g. These potential sites could be bound by NF-E2-related factor 2 (Motohashi and Yamamoto 2004), Bach1 (Dohi et al. 2008), and activating transcription factor 3 (Okamoto et al. 2006), all of which are responsive to oxidative stress. These factors might participate in the downregulation of hepatic CST gene expression found in the BSA-injected mice. In contrast to the liver, the level of renal CST mRNA in these mice was increased despite significant oxidative stress. Their kidneys exhibited inflammation and strong activation of NF-κB signaling (Takahashi et al. 2011), which did not occur in their livers. In our previous study, some potential NF-κB-binding sites were detected on the mouse CST gene (Zhang et al. 2009). Thus, the renal CST gene expression might be affected by NF-κB activation related to inflammation rather than the effect of oxidative stress. It has been reported that the mouse CST gene is expressed as multiple transcript variants in a tissue-specific manner (Hirahara et al. 2000; Suzuki et al. 2010), but the liver type of CST transcripts has not yet been investigated. Interestingly, the human CST gene is also expressed as multiple transcripts (Tsuda et al. 2000), implying similar transcriptional mechanisms between mouse and human CST genes. To presume the regulatory mechanisms governing liver-specific CST gene expression and their association with the above-mentioned transcription factors, further experiments such as reporter gene assay, electrophoretic mobility shift assay and immunoblot analysis using nuclear extracts, and chromatin immunoprecipitation assay, are necessary in future.

As discussed previously, acute kidney injury in BSA-injected mice is due to the toxicity from FFA bound to BSA, since overloading of FFA-free BSA does not cause the injury (Kamijo et al. 2007a; Takahashi et al. 2011). Under normal circumstances, FFA bound to albumin in blood are filtered through glomeruli and reabsorbed into proximal tubular epithelial cells, where they are metabolized as an important renal energy source. However, under conditions of continuous BSA overloading, excess amounts of bound FFA enter into the tubular cells and are accumulated due to insufficient catabolism. Because FFA are potent detergents, their excess accumulation may cause directly damage to tubular cells and organelles. Furthermore, accumulated FFA can easily react with oxidative molecules generated from the mitochondrial respiratory chain, leading to overproduction of lipid peroxides and induction of cellular oxidative stress. These toxic effects induce inflammation, apoptosis, and necrosis, which further exacerbate kidney injury (Kamijo et al. 2007a; Takahashi et al. 2011). Another group has also demonstrated the toxicity of albumin-derived FFA, such as elevation of oxidative stress, in human proximal tubular cells (Ishola et al. 2006). Moreover, the kidney dysfunction results in systemic accumulation of various uric toxins, which likely cause depression of internal functions, such as hepatic catalase levels, and induction of oxidative stress in the whole body, as noted in several clinical reports

(Kendrick and Chonchol 2008; Zanetti et al. 2008; Granata et al. 2009; Wang et al. 2011). The increased oxidative stress in the liver might affect the synthesis of sulfatides by modulating the CST level, leading to the reduction of the serum sulfatide level.

Clofibrate treatment is effective in inducing enzymes for not only fatty acid catabolism, but also anti-oxidative response, such as catalase, in rodents. These effects are mediated by peroxisome proliferator-activated receptor α, the nuclear receptor regulating fatty acid metabolism in the liver, kidney, and heart (Aoyama et al. 1998; Watanabe et al. 2000; Kamijo et al. 2002, 2007a,b; Nakajima et al. 2004; Tanaka et al. 2008). The clofibrate pretreatment enhances these cellular functions in the kidney and other tissues, including the liver, prior to BSA injection, and its continuous treatment maintains their enhancement throughout the BSA injection study. These effects contribute to the reduction of FFA-derived toxicity to the proximal tubules upon continuous BSA injection, resulting in a smaller amount of kidney injury (Takahashi et al. 2011). As a consequence, the decreased kidney injury in addition to the induction of hepatic catalase might prevent the generation of oxidative stress in the liver with BSA injection, leading to a slight modulation of hepatic sulfatide synthesis and serum sulfatide levels. The detailed mechanism accounting for the relationship between kidney dysfunction and hepatic sulfatide metabolism via oxidative stress requires further investigation.

The level of sulfatides in the kidney was increased in response to its dysfunction, which was inversely correlated with the levels in serum. In addition, the levels of sulfatides and CST activities were considerably higher in the kidney than in the liver (Hirahara et al. 2000). It is possible that sulfatides are produced in the kidney and secreted into the blood. However, there have been reports of organ specificities of glycosphingolipids (Ishizuka 1997). The sphingoid base structures of kidney sulfatides are quite different from those of liver and serum, but the latter two are almost identical. It is thus conceivable that serum sulfatides are mostly synthesized and secreted by the liver as a constituent of lipoproteins. The observed change of kidney sulfatide levels is likely dependent on CST. The significance of this phenomenon needs to be investigated in future work.

This study hypothesized that lower levels of liver sulfatides in mice with kidney injury were due to the decrease of their production, since our analysis clearly showed the significant reduction of hepatic CST expression and no alterations of other sulfatide-metabolizing enzymes. However, this study only focused on the expression levels of mRNA and protein. Additional analysis of the functional activities of these enzymes is important for better understanding our results. In addition, we have examined only one animal model of kidney injury. Similar experiments using other diseased animals would enhance our knowledge concerning the interactions between kidney injury and sulfatide metabolism.

In conclusion, the present study supports the idea that possible crosstalk exists between kidney function and liver sulfatide metabolism via oxidative stress, and suggests the importance of liver function for maintaining serum sulfatide levels. Our findings may provide useful information for considering new therapeutic approaches in human patients with end-stage renal disease, since their risk for cardiovascular disease is highly correlated with serum levels of sulfatides as compared with other known factors (Hu et al. 2007; Wang et al. 2011).

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Conflict of Interest

The authors declare that they have no conflicts of interest.

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