Expression of thioredoxin and thioredoxin-binding protein-2 in the liver of patients with chronic hepatitis C as a predictor of response to interferon therapy

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Abstract. Oxidative stress contributes to the pathogenesis of various hepatic injuries. Thioredoxin (TRX) is an indicator of oxidative stress, reported to be increased in the serum of patients with chronic hepatitis C with the progression of fibrosis. The aim of this study was to evaluate the clinical significance of the expression of TRX and thioredoxin-binding protein-2 (TBP-2), which is a negative regulator of TRX function, in the liver of patients with chronic hepatitis C and the relationship of this to the efficacy of interferon (IFN) treatment. A retrospective study was performed using the liver biopsy specimens obtained before IFN treatment from 69 patients with chronic serotype 1 hepatitis C virus (HCV) infection. TRX and TBP-2 mRNA levels in the liver biopsy specimens were amplified by real-time RT-PCR. The serum TRX protein level was estimated with a sandwich enzyme-linked immunosorbent assay kit, and the expression of TRX protein in the liver was examined immunohistochemically in 19 patients. There was no association between the serum TRX level and the TRX level in the liver. There was a significant correlation between the expression level of TRX protein in the liver and the TRX mRNA level in the liver. TRX and TBP-2 levels in the liver tended to decrease slightly with increased fibrosis stage, although not significantly. The TRX level in the liver tended to increase with hepatitis activity index, although not significantly. TBP-2 mRNA levels in the liver were significantly higher in responders than non-responders to the IFN therapy ($p<0.05$). Among patients who had a high viral load of >850 KIU/ml, the TRX level in the livers of non-responders was significantly lower than that in the livers of responders ($p<0.05$). TRX and TBP-2 mRNA levels in the liver before IFN therapy may predict the outcome of IFN therapy in patients with chronic serotype 1 HCV infection.

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Introduction

Oxidative stress is considered to be involved in many pathological conditions, such as aging, atherosclerosis, cancer, inflammatory diseases, and acquired immune deficiency syndrome. Imbalance in the oxidant-antioxidant status is also suggested to play a major role in viral hepatitis, such as that caused by hepatitis C virus (HCV) infection (1). There are many oxidative stress markers: a) oxygen radicals such as reactive oxygen molecules; b) 8-hydroxydeoxyguanosine (8-OHdG), a DNA base-modified product generated by reactive oxygen species produced by activated macrophages, and an indicator of DNA damage; c) reactive aldehydes arising as a consequence of lipid peroxidation, such as 4-hydoxy-2-nonenal (HNF) and malon-dialdehyde (MDA); and d) antioxidant molecules such as thioredoxin (TRX). TRX is a stress-inducible thiol-containing protein, reported to be increased in the serum of HCV patients with the progression of fibrosis. TRX was originally discovered in *Escherichia coli* as a proton donor to ribonucleotide reductase (2). Human TRX was cloned as adult T-cell leukemia-derived factor (ADF), which was initially described as an interleukin-2 receptor/α chain inducer detected in the culture supernatant of human T-lymphotropic virus type-I (HTLV-1) transformed cells (3). TRX contains a dithiol-active site (-Cys-Gly-Pro-Cys-) (4) and has a variety of biological activities (5), including scavenging of active oxygen radicals (6), and regulation of redox-sensitive molecules (7). Since TRX is induced by many forms of oxidative stress (5,8), serum TRX levels are believed to be a clinically useful indicator of oxidative stress. It was reported that serum TRX levels were elevated in patients with chronic hepatitis C and related to the histological stage and the efficacy of interferon (IFN) treatment (9). However, there is no report on the association of the TRX level in the liver and the histological findings or the efficacy of IFN therapy.

Human thioredoxin-binding protein-2 (TBP-2)/vitamin D_3 up-regulated protein, originally reported as an up-regulated gene in HL-60 cells treated with 1α,25-dihydroxyvitamin D3 (10), is a negative regulator of TRX function (11). Recent

studies have gradually clarified the involvement of TBP-2 in biologically important cellular events such as differentiation and apoptosis. However, there are no reports on the expression of TBP-2 in the liver. In this study, we examined the mRNA levels of TRX and TBP-2 in the livers of patients with HCV-related liver diseases, and compared them with histological findings of the liver and clinical laboratory data. We also investigated whether there was a correlation between the mRNA levels of TRX and TBP-2 in the liver and the efficacy of IFN treatment.

Materials and methods

Patients. The subjects of this study were 69 patients (41 males and 28 females, age: 51.4±10.4 years, mean ± SD) with chronic hepatitis C. The HCV serotype infected were all in group 1. Twenty-five patients received IFN treatment according to a common schedule for chronic hepatitis C in Japan: recombinant or natural IFN-α (6-10 million units/day) was administered subcutaneously daily for 2 weeks and then 3 times/week for the subsequent 22 weeks. Sixteen patients were given intravenous administration of 6 million units IFN-ß daily for 4 weeks followed by recombinant or natural IFN-α (6-10 million units/day), which was administered subcutaneously daily for 2 weeks and then 3 times/week for the subsequent 22 weeks. Twenty-eight patients were given twice-daily intravenous administration of 3 million units of IFN-ß for 2 weeks before combination treatment with IFN-α-2b (6 million units subcutaneously daily for 2 weeks and then 3 times/week for 22 weeks) and ribavirin (600-800 mg/day). Complete response was defined as undetectable HCV-RNA for 6 months after the end of treatment. Partial response was defined as undetectable HCV-RNA at the end of treatment, but relapse thereafter. No response was defined as continuously positive HCV-RNA during and after treatment.

Serum was obtained from venous blood drawn in the fasting state before breakfast, and it was stored frozen at -40˚C until used.

Clinical laboratory data and histological examination. Biochemical tests and complete blood cell counts were measured using routine automated techniques. We determined clinical laboratory parameters, such as alanine aminotransferase (ALT), platelet count, and indocyanine green retention rate at 15 min ($ICGR_{15}$). The $ICGR_{15}$ was determined in fasting subjects from samples of venous blood taken 5, 10, and 15 min after a single injection of 0.5 mg/kg ICG. The serum level of HCV-RNA was measured by RT-PCR using an Amplicor HCV-monitor kit (Roche, Tokyo, Japan) (12). The HCV serotype was determined using an enzyme-linked immunosorbent assay (ELISA) using C14-1 and C14-2 group-specific recombinant peptides from NS4 region (13).

All patients underwent a percutaneous liver biopsy before IFN therapy. A part of the liver specimen was stored frozen at -40˚C until used. The liver specimen were also used for hematoxylin and eosin (H&E) staining for scoring according to the histological activity index (HAI) scoring systems (14). The grading of fibrosis in the liver biopsy specimens was based on the system proposed by Ishak (modified HAI scoring system). The modified HAI score includes the category of fibrosis, architectural changes and cirrhosis referred to as stages 0-6 (0, no fibrosis; 1, fibrous expansion of some portal areas with or without short fibrous septa; 2, fibrous expansion of most portal areas with or without short fibrous septa; 3, fibrous expansion of most portal areas with occasional portal-portal (P-P) bridging; 4, fibrous expansion of portal areas with marked P-P bridging as well as portal to central (P-C) bridging; 5, marked bridging (P-P and/or P-C) with occasional nodules; 6, cirrhosis, probable or definite). The modified HAI score includes four categories of necroinflammatory activity referred to as grade (A) periportal or periseptal interface hepatitis, 0-4 (0, negative; 1, mild: focal at few portal areas; 2, mild/moderate: focal at most portal areas; 3, moderate: continuous in <50% of tracts of septa; 4, severe: continuous in >50% of tracts of septa); (B) confluent necrosis, 0-6 (0, negative; 1, focal confluent necrosis; 2, zone-3 necrosis in some areas; 3, zone-3 necrosis in most areas; 4, zone-3 necrosis + occasional portal-central (P-C) bridging; 5, zone-3 necrosis + multiple P-C bridging; 6, panacinar or multiacinar necrosis); (C) focal (spotty) lytic necrosis, apoptosis and focal inflammation, 0-4 (0, negative; 1, one focus or less per 10x objective; 2, two to four foci per 10x objective; 3, five to ten foci per 10x objective; 4, >10 foci per 10x objective); (D) portal inflammation, 0-4 (0, negative; 1, mild: some or all portal areas; 2, moderate: some or all portal areas; 3, moderate/marked: all portal areas; 4, marked: all portal areas). The sum of these four (A-D) scores constitutes the grading score.

Measurement of TRX and TBP-2 in the liver by relative quantitative real-time polymerase chain reaction. The liver specimens were partially homogenized and RNA was isolated using Ficoll-Paque Plus (Amersham Biosciences Corp., Piscataway, NJ, USA) according to the manufacturer's instructions. Then, mRNA was amplified by the real-time one-step RT-PCR method with a Quantitect SYBR Green RT-PCR kit (Qiagen, Tokyo, Japan) with the ABI Sequence Detection System (e.g., ABI PRISM-7700, Takara Bio Inc., Ohtsu, Japan) according to the manufacturer's instructions using the following primers: TRX; 5'-AGCAGATCGAG AGCAAGACT (sense), 5'-CTCTGAAGCAACATCCT GAC (antisense), TBP-2; 5'-GGTGATAGTGGAGGTGT GTG (sense), 5'-ATAGCGCAGGTACTCCGAAG (antisense).

Serum TRX measurement. Serum was obtained from 19 patients who received IFN-ribavirin combination therapy. Serum TRX levels were measured with a sensitive sandwich ELISA kit (Redox Bioscience, Inc., Kyoto, Japan) (15). Briefly, ADF 21-antibody-precoated 96-microwell plates were incubated for 2 h at room temperature with 20 μ1 of serum or standard solution (ADF; 0, 30, 60, 120 rig/ml) in the presence of 200 μ1 of 50 mM sodium phosphate buffer (pH 6.0) containing 150 mM NaCl, 1.0 mM $MgCl_2$, 1.0% BSA and 0.1% NaN. The plates were washed 5 times with 10 mM sodium phosphate buffer (pH 7.5) containing 0.05% Tween-20 and 150 mM NaCl (wash solution), and 200 μ1 of the horseradish peroxidase labeled anti-ADF antibody was added to the plates, which were then incubated at room temperature for 2 h. After the plates were washed 5 more times with the wash solution, 100 μ1 of 100 mM triethanolamine-succinate buffer (pH 4.4)

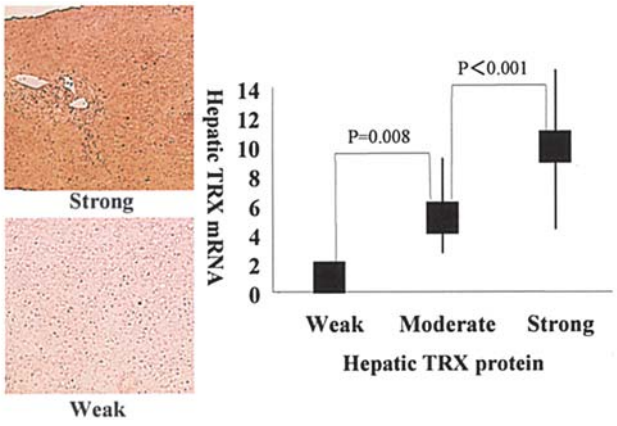

Figure 1. The relationship between the expression levels of TRX protein and TRX mRNA levels in the liver. Sixty-nine Japanese patients with chronic serotype 1 HCV infection underwent a percutaneous liver biopsy before IFN therapy. The expression of TRX protein in the tissue section was examined immunohistochemically with monoclonal antibody to TRX. A part of the liver specimen was partially homogenized and used to isolate RNA, and then mRNA was amplified by the real-time one-step RT-PCR method.

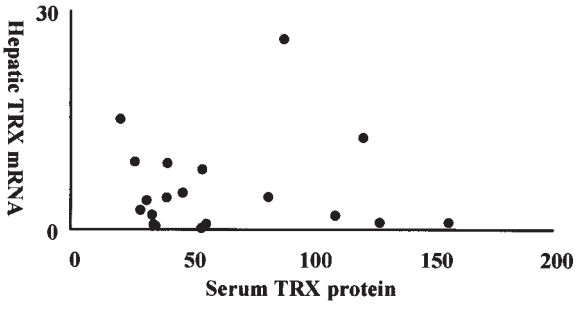

Figure 2. The relationship between serum TRX levels and TRX mRNA levels in the liver. Serum before IFN therapy was obtained from 19 patients who received IFN-ribavirin combination therapy. Serum TRX levels were measured with a sensitive sandwich ELISA kit. TRX mRNA levels in the liver tissue were determined by the real-time one-step RT-PCR method (see Fig. 1).

containing 1.5 mM H_2O_2 and 0.13% ABTS was added, and they were incubated for 1 h at room temperature. The reaction was then stopped by the addition of 100 μl of 1% oxalic acid solution, and the optical density of the sample in the plates was measured at 415 nm with a microplate reader (Benchmark, Bio-Rad Laboratories, CA, USA). All measurements were made in duplicate, and the average value was adopted.

Immunohistochemical detection of TRX. The expression of TRX protein in the tissue sections was examined immunohistochemically with monoclonal antibodies to TRX (Redox Bioscience, Inc.) (16). All procedures were performed using a Dako LSAB kit according to the manufacturer's instructions. Briefly, formalin-fixed 5-mm sections were deparaffinized in xylene for 5 min 3 times, dehydrated in ethanol, and incubated with 3% hydrogen peroxide for 15 min. The sections were then incubated with a blocking solution for 30 min and with anti-TRX antibodies, that was diluted 1:100 in 0.01 M phosphate-buffered saline (pH 7.2), for 16 h at 4˚C. After the sections were washed with 0.05 M Tris-HCl (pH 7.6), visualization was carried out with aminoethylcarbazole chromogen

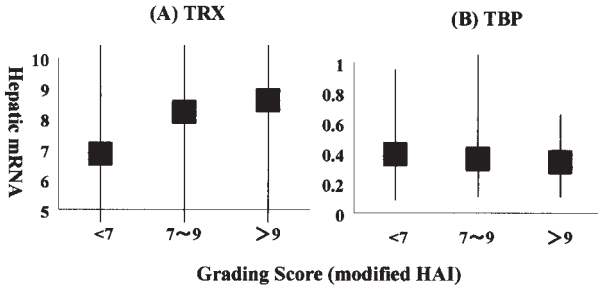

Figure 3. The relationship between TRX or TBP-2 mRNA levels in the liver and the histological necroinflammatory activity index. Sixty-nine Japanese patients with chronic serotype 1 HCV infection underwent percutaneous liver biopsy before IFN therapy. (A) TRX or (B) TBP-2 mRNA levels in the liver tissue were determined by the real-time one-step RT-PCR method (see Fig. 1). The grading of necroinflammatory activity in the liver biopsy specimens was based on the system proposed by Ishak (modified histological activity index scoring system).

dissolved in hydrogen peroxide. Thereafter, the sections were counter-stained with hematoxylin and mounted. Liver sections in which most lobules had diffusely or partly positive staining were designated as strong; sections in which less than half of the lobules were positively stained were designated as moderate; and sections in which throughout the specimen some hepatocytes were stained but the staining was not diffuse or localized were designated as weak. Using the above detection system, the extent of TRX in liver biopsy specimens was evaluated. The intensity of staining was evaluated by more than two observers.

Statistical analysis. The χ^2 test, Fisher's exact test, Mann-Whitney U test or unpaired t-test was used as appropriate to determine the statistical significance of differences between clinical data. A p-value of <0.05 was considered to indicate statistical significance.

Results

TRX and TBP-2 mRNA levels in the liver and clinical laboratory data. The correlations between TRX or TBP-2 levels in the liver and the value of each of the clinical laboratory parameters were examined to identify the variables that are associated with high TRX and TBP-2 levels in the liver of patients with HCV infection. There was no correlation between TRX or TBP-2 mRNA levels in the liver and clinical features or laboratory parameters including; patient age, gender, serum ALT level, platelet count, $ICGR_{15}$, or serum HCV-RNA level (data not shown).

TRX level in the liver. The distribution of TRX protein in the liver tissue as shown by immunohistochemistry revealed that TRX was localized in the cytoplasm of the hepatocytes. TRX protein was found to be clustered around the portal areas with interface hepatitis (Fig. 1). There was a significant association between the expression level of TRX protein as determined by immunohistochemistry and the TRX mRNA level in the liver tissue (Fig. 1). However, there was no correlation between the serum TRX protein level and the TRX mRNA level in the liver tissue (Fig. 2). Although the relationship was not significant, the TRX mRNA level in the liver tended to increase

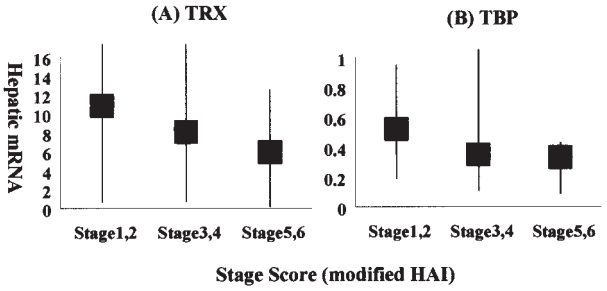

Figure 4. The relationship between TRX or TBP-2 mRNA levels in the liver and the histological fibrosis staging score. Sixty-nine Japanese patients with chronic serotype 1 HCV infection underwent a percutaneous liver biopsy before IFN therapy. (A) TRX or (B) TBP-2 mRNA levels in the liver tissue were determined by the real-time one step RT-PCR method (see Fig. 1). The grading of fibrosis in the liver biopsy specimens was based on the system proposed by Ishak (modified histological activity index scoring system).

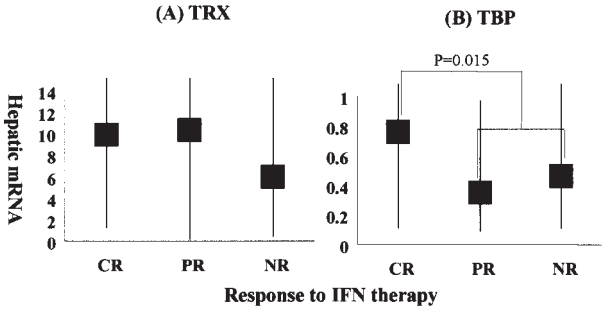

Figure 5. The relationship between TRX or TBP-2 mRNA levels in the liver and the response to the IFN therapy. Sixty-nine Japanese patients with chronic serotype 1 HCV infection underwent a percutaneous liver biopsy before IFN therapy. TRX and TBP-2 mRNA levels in the liver tissue were determined by the real-time one step RT-PCR method (see Fig. 1). The response to the IFN therapy was defined as followings: complete response was defined as undetectable HCV-RNA for 6 months after the end of treatment; partial response was defined as undetectable HCV-RNA at the end of treatment, but relapse thereafter; nonresponse was defined as continuously positive HCV-RNA during and after treatment. CR, complete responder; PR, partial responder; NR, non-responder.

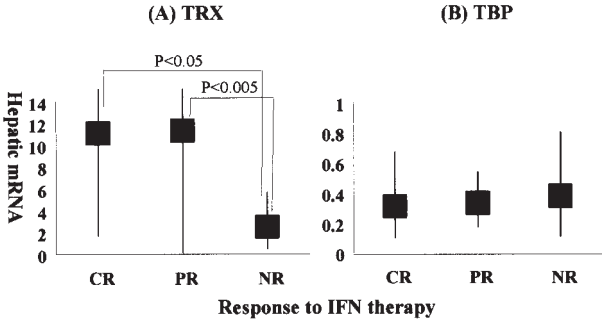

Figure 6. The relationship between TRX or TBP-2 mRNA levels in the liver and the response to IFN therapy in patients with a high viral load of >850 KIU/ ml of serum HCV-RNA. Sixty-nine Japanese patients with chronic serotype 1 HCV infection underwent a percutaneous liver biopsy before IFN therapy. TRX and TBP-2 mRNA levels in the liver tissue were determined by the real-time one-step RT-PCR method (see Fig. 1). Nineteen patients had a high viral load of >850 KIU/ml of HCV-RNA. The definitions and abbreviations regarding the response to IFN therapy are the same as in Fig. 5.

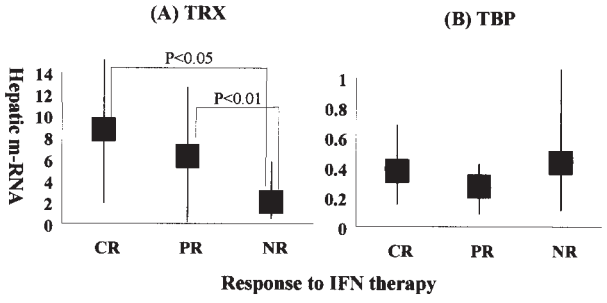

Figure 7. The relationship between TRX or TBP-2 mRNA levels in the liver and the response to IFN-ribavirin combination therapy. Sixty-nine Japanese patients with chronic serotype 1 HCV infection underwent a percutaneous liver biopsy before IFN therapy. TRX and TBP-2 mRNA levels in the liver tissue were determined by the real-time one-step RT-PCR method (see Fig. 1). Twenty-eight patients received IFN-ribavirin combination therapy. The definitions and abbreviations regarding the response to IFN therapy are the same as in Fig. 5.

in parallel with the activity of the hepatitis (Fig. 3A). Although the relationship was not significant, the TRX mRNA level increased in the liver tended to decrease as the staging score of the hepatic fibrosis increased (Fig. 4A).

TBP-2 level in the liver. There was no significant association of the TBP-2 mRNA level in the liver and the histological necroinflammatory activity score (Fig. 3B). Although the relationship was not significant, the TBP-2 mRNA level in the liver tended to decrease as the staging score of the hepatic fibrosis increased (Fig. 4B). There was no correlation between the TBP-2 mRNA level and TRX mRNA level in the liver (data not shown).

The relationship between TRX and TBP-2 mRNA levels in the liver and the efficacy of IFN therapy. Demographic and clinical characteristics of the IFN-treated patients are shown in Table I. Among the 69 patients, the response to the IFN therapy was as follows: 25 (36%) were complete responders (CRs) who had undetectable HCV-RNA for 6 months after the end of treatment; 22 (32%) were partial responders (PRs) who had undetectable HCV-RNA at the end of treatment, but relapsed thereafter; 22 (32%) were non-responders (NRs) who were continuously positive for HCV-RNA during and after treatment (Table I). No significant difference existed between responders and nonresponders in the clinical background before treatment. TRX mRNA levels in the liver tended to be lower in NRs than in CRs or PRs, although the relationship was not significant (Fig. 5). TBP-2 mRNA levels in the liver were significantly higher in CRs than in the other patients ($p<0.05$). Among the 19 patients who had a high viral load as indicated by >850K IU/ ml of serum HCV-RNA before IFN therapy, there was a significant difference of the TRX mRNA levels between NRs and CRs and between NRs and PRs ($p<0.05$, $p<0.005$) (Fig. 6A). That is, the TRX mRNA levels in the liver of patients who had a high viral load were significantly lower in nonreponders to the IFN therapy. Demographic and clinical characteristics of the patients treated with IFN-ribavirin combination therapy are shown in Table II. No significant difference existed between responders and no responders in the clinical background before treatment. Among the 28 patients

Table I. Baseline characteristics of patients and their response to IFN therapy.

	CR	PR	NR
No. of cases	25	22	22
Sex (M/F)	16/9	14/8	11/11
Age (years, mean ± SD)	48.5±10.6	50.5±10.8	55.5±9.0
Stage (modified HAI score, mean ± SD)	2.8±1.1	3.6±1.1	3.2±1.2
Activity (modified HAI score, mean ± SD)	8.7±3.5	8.6±2.4	9.1±2.3
ALT (IU/l, mean ± SD)	118±85	95±61	68±24
HCV-RNA (KIU/ml, mean ± SD)	450±626	701±670	730±1021

HAI, histological activity index; ALT, alanine aminotransferase, CR, complete responder; PR, partial responder; NR, non-responder.

Table II. Baseline characteristics of patients who received IFN-ribavirin combination therapy and their response to IFN therapy.

	CR	PR	NR
No. of cases	8	10	10
Sex (M/F)	7/1	6/4	3/7
Age (years, mean ± SD)	53.0±1.5	55.5±7.1	60.8±5.8
Stage (modified HAI score, mean ± SD)	2.6±1.1	3.9±1.2	3.6±1.1
Activity (modified HAI score, mean ± SD)	8.5±1.2	7.7±1.2	8.2±1.8
ALT (IU/l, mean ± SD)	156±101	107±74	67±21
HCV-RNA (KIU/ml, mean ± SD)	773±624	1075±672	2060±1713

HAI, histological activity index; ALT, alanine aminotransferase, CR, complete responder; PR, partial responder; NR, non-responder.

who received IFN-ribavirin combination therapy, TRX mRNA levels in the liver were significantly lower in NRs than in CRs or PRs ($p<0.05$, $p<0.01$) (Fig. 7A).

Discussion

Oxidative stress contributes to the pathogenesis of hepatic injury induced by alcohol, virus infection, hemochromatosis, ischemia/reperfusion injury, exposure to toxins, and abuse of drugs, such as acetaminophen overdose (17-19). The HCV core protein is thought to cause mitochondrial injury, leading to oxidative stress. Oxidative stress disturbs lipid metabolism and causes other damage that leads to steatosis and sometimes apoptosis (20). Serum TRX levels have recently been established to be an indicator of oxidative stress in various diseases. It was reported that serum TRX levels tended to increase parallel with the severity of chronic hepatitis C (9). However, there are very few reports about TRX levels in the liver. Moreover, there are no reports on the association of TRX levels in the liver and the efficacy of IFN therapy. Mahmood *et al* reported based on immunohistochemical analysis of liver biopsy specimens that TRX was distributed in hepatocytes around portal areas with piecemeal necrosis and cytoplasm of hepatocellular carcinoma cells (21). However, they did not report on the association of TRX expression in the liver and the efficacy of IFN therapy. In this study, we found that there was a significant association between the expression level of TRX protein as estimated by immunohistochemistry and TRX mRNA levels in the liver tissue (Fig. 1). However, there was no correlation between the serum TRX level and the TRX mRNA level in the liver tissue (Fig. 2). Previous immunohistochemical analysis demonstrated that TRX is distributed throughout the human body, including the liver, kidney, gastrointestinal epithelium, and gonads (22). In our immunohistochemical study of TRX expression in the liver, TRX protein was found to be clustered around portal areas with interface hepatitis, as previously reported (17). Moreover, TRX mRNA levels in the liver tended to increase with the activity of hepatitis, as previously reported for serum TRX. It is suspected that TRX protein in the liver may be produced by the hepatocytes and induced by inflammatory cells located among or around the hepatocytes. In view of the fact that TRX is produced by lymphocytes infected by viruses, such as HTLV-1 and Epstein-Barr viruses (23,24), and that HCV can infect lymphocytes and replicate inside them (25, 26), it has been suggested that serum TRX may originate in HCV-infected peripheral blood mononuclear cells. That may be the reason why there was no correlation between the serum TRX level and the TRX level in the liver.

It was reported that the serum TRX level of patients with HCV infection increased with the progression of liver fibrosis (9). In this study, however, we found that the TRX mRNA level in the liver tended to decrease as the stage of fibrosis increased. These contradictory results may be explained by

the differences between the serum TRX levels and TRX levels in the liver. Serum TRX levels are considered to reflect the total oxidative stress level of the entire body. Since the number of hepatocytes decreases with the progression of liver fibrosis, the production of TRX by hepatocytes may decrease with the progression hepatic fibrosis. The production of TRX in other organs or cells, such as HCV-infected peripheral blood mononuclear cells, may increase, leading to an increase in the serum TRX level. In addition, there is the difference of background in patients' fibrosis stage. Because all patients in this study received IFN therapy and underwent liver biopsy before IFN therapy, there was no stage of hepatic fibrosis of advanced without cirrhosis. Because the stage of hepatic fibrosis in the majority of patients was modified HAI stage score 3 or 4, there may have been no difference between stage and TRX levels. Further large-scale studies will be required to examine the levels of TRX in the liver at various hepatic fibrosis stages.

Although IFN is the most widely approved agent for the treatment of chronic hepatitis C, its therapeutic efficacy is not always satisfactory. Factors such as sex, age, serum HCV-RNA level, HCV genotype, and stage of hepatic fibrosis have been suggested as independent predictors of IFN response (27). Sumida *et al* reported that the mean serum TRX value is lower in IFN responders than in nonresponders, and that oxidative stress must be an important factor responsible for resistance to IFN therapy (9). We found that the TRX mRNA level in the liver was significantly higher in responders than non-responders. These discrepant results may be due to the difference of the method of evaluation of the response to IFN therapy. These authors examined the early response to the IFN therapy; the clearance of HCV-RNA on day 14 following IFN administration. In this study, we examined the sustained virological response: the response to the IFN therapy for 6 months after the end of treatment. In addition, the difference may be explained by the difference between the use of serum TRX levels or TRX levels in the liver. Since TRX levels in the liver tended to increase with the severity of hepatitis, it is suspected that patients who had stronger hepatic inflammation may produce more TRX in the liver, and may become responders to the IFN therapy. Moreover, the administration of TRX in patients with chronic hepatitis C may improve the efficacy of the response to IFN therapy.

HCV serotype and serum HCV-RNA levels have been suggested to be independent predictors of IFN response. We examined the TRX levels in the liver of patients with the same clinical conditions of HCV serotype 1 and high viral load. We found that among patients who had a high viral load of >850 KIU/ml of HCV-RNA before IFN therapy, TRX levels in the liver were significantly lower in non-responders (Fig. 6A). Non-responders (NRs, namely, continuously resistant cases) who do not achieve HCV-RNA negativity during treatment are a clinically important problem. Factors responsible for nonresponse to IFN-ribavirin combination therapy have not been identified. Recently, it was reported regarding viral factors that substitutions of amino acid 70 in the core region, and substitutions of amino acid 91 are independent and significant factors associated with nonresponse to IFN-ribavirin combination therapy (28). We found that among the patients who received IFN-ribavirin combination therapy, TRX mRNA levels in the liver were significantly lower in NRs than in CRs or PRs (Fig. 7). Low TRX levels in the liver may be a host factor contributing to nonresponse to IFN-ribavirin combination therapy. Further large-scale studies will be required to examine the role of TRX in the liver in the efficacy of IFN therapy.

TBP-2 is reported to be a negative regulator of TRX function (11). However, there has been no report on the expression of TBP-2 in the liver, or its correlation with the efficacy of IFN therapy. We found that there was no correlation between the TBP-2 mRNA level and the TRX mRNA level in the liver. However, TBP-2 mRNA levels were significantly higher in responders than nonresponders. The association between TRX and TBP-2 in the liver, and the function of TBP-2 in the liver, have not been clarified. It was reported that TBP-2 is associated with the levels of growth factors and the cell cycle (29), and TBP-2 may be involved in the response to IFN. Further studies will be required to examine the role of TBP-2 in the liver and its relationship to the efficacy of IFN therapy.

The possible functions of TRX and TBP-2 in the anti-viral effect of IFN are unknown. Considering that TRX controls the activity of NF-κB, a nuclear factor that regulates the transcription of several genes involved in the inflammatory response (30), and that extracellular TRX can exhibit chemokine-like and cytokine-like activities (31), elevated serum TRX levels seem likely to have an active role in the production of oxidative stress, and decreased levels of TRX in the liver seem likely to have a negative role in the anti-viral effect of IFN. In conclusion, TRX and TBP-2 mRNA levels in the liver before IFN therapy may predict the outcome of IFN therapy in patients with chronic serotype 1 HCV infection.

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