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Serum thioredoxin reductase levels increase in response to chemically induced acute liver injury

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ABSTRACT

Background: Mammalian thioredoxin reductases (TrxR) are selenoproteins with important roles in antioxidant defense and redox regulation, principally linked to functions of their main substrates thioredoxins (Trx). All major forms of TrxR are intracellular while levels in serum are typically very low.
Methods: Serum TrxR levels were determined with immunoblotting using antibodies against mouse TrxR1 and total enzyme activity measurements were performed, with serum and tissue samples from mouse models of liver injury, as triggered by either thioacetamide (TAA) or carbon tetrachloride (CCl_4).
Results: TrxR levels in serum increased upon treatment and correlated closely with those of alanine aminotransferase (ALT), an often used serum biomarker for liver damage. In contrast, Trx1, glutathione reductase, superoxide dismutase or selenium-containing glutathione peroxidase levels in serum displayed much lower increases than TrxR or ALT.
Conclusions: Serum TrxR levels are robustly elevated in mouse models of chemically induced liver injury.
General significance: The exaggerated TrxR release to serum upon liver injury may reflect more complex events than a mere passive release of hepatic enzymes to the extracellular milieu. It can also not be disregarded that enzymatically active TrxR in serum could have yet unidentified physiological functions.

1. Introduction

All major isoforms of mammalian thioredoxin reductases (TrxR) are homodimeric selenocysteine-containing oxidoreductases, including ubiquitously expressed cytosolic TrxR1, mitochondrial TrxR2, and one variant (TGR) mainly found in testis [1]. The importance of these enzymes is mainly attributed to the catalysis of thioredoxin (Trx) reduction, whereby the active site disulfide of cytosolic Trx1 or mitochondrial Trx2 using NADPH is kept reduced by TrxR1 or TrxR2, respectively, thereby constituting the main players of the Trx system. This system, in turn, has a large number of redox functions in cells due to many diverse redox regulatory roles of Trx [1]. In addition to reduction of Trx, TrxR can also directly reduce several low molecular weight compounds [1], including dithio-bis-nitrobenzoic acid (DTNB) that is used as a model substrate in assays of TrxR activity [2]. TrxR is furthermore readily inhibited by several electrophilic compounds that easily react with the selenocysteine residue of the enzyme, e.g. with gold compounds such as aurothioglucose or auranofin being highly efficient inhibitors at virtually stoichiometric amounts [3]. TrxR enzymatic activity can thus be measured by NADPH dependent DTNB reduction using gold inhibition for specificity control [4–6], or, alternatively, by an insulin reduction assay that is based upon Trx as a TrxR substrate coupled to reduction of insulin disulfides by Trx [7].

Although TrxR1 was found to be secreted by certain cells, its activity in healthy human plasma is very low [8]. NADPH-dependent protein-disulfide reductase activity in serum of tumor-bearing mice was assumed early to be TrxR [9,10], but this assumption has not yet been further studied. Elevated levels of human serum Trx1, however, were identified early as ADF (Adult T-cell Derived Factor) [11] and is observed in numerous diseases, including several forms of liver pathology [12,13]. In contrast to cytokine-like effects of Trx1, no biological function of any TrxR isoform in serum has yet been reported. Its possible presence in serum has been much less studied and, hitherto, serum TrxR has not been studied in any toxicological models. Since TrxR1 is abundantly expressed in liver from where it was originally purified [10,14], we investigated here whether alterations of serum TrxR levels may occur as a response to chemically induced liver injury. We found that serum TrxR levels were indeed highly increased upon treatment with hepatotoxic agents in mice and these increases correlated well with those of alanine aminotransferase (ALT), a well-known indicator

Abbreviations: ALT, alanine aminotransferase; CCl_4, carbon tetrachloride; DTNB, dithio-bis-nitrobenzoic acid; GPx, glutathione peroxidase; GR, glutathione reductase; i.p., intraperitoneally; Nrf2, nuclear factor erythroid 2-related factor 2; PBS, phosphate buffer saline; SOD, superoxide dismutase; TAA, thioacetamide; Trx, thioredoxin; TrxR, thioredoxin reductase

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for liver injury [15,16]. A number of other hepatic enzymes were, however, not increased to the same extent, thus suggesting unique kinetics or mechanisms in TrxR release from the liver.

2. Materials and methods

2.1. Chemicals and drugs

NADPH, DTNB, reduced glutathione, oxidized glutathione (GSSG), glutathione reductase (GR, from *Escherichia coli*), and rat TrxR1 were all obtained from Sigma (St. Louis, Missouri, USA). Auranofin was purchased from Santa Cruz Biotechnology, Inc. (California, USA). Other chemicals were of the highest grade available.

2.2. Animals

Healthy male Kunming-strain mice (20–22 g) and their diet were purchased from Shanghai SLAC Laboratory Animal Co. Ltd., China. The mice were housed in plastic cages in a room with controlled temperature (22 ± 1 °C) and humidity (50 ± 10%) and 12 h light/dark cycle. The mice were given food and water ad libitum. All protocols for the animal experiments complied with guidelines of the Anhui Agricultural University for care and use of laboratory animals.

2.3. Animal treatments

To first investigate serum TrxR levels upon TAA-triggered liver injury, ten mice were divided into two groups (5/group). The mice were intraperitoneally (i.p.) injected with saline as control or 200 mg/kg thioacetamide (TAA) dissolved in saline (10 mL/kg per injection), whereupon they were sacrificed 24 h later by cervical dislocation. Individual serum samples were obtained following the procedures described below. An aliquot of 0.3 mL serum from each mouse that received identical drug treatment was also used to pool the serum, which was subsequently denoted either “control serum” or “TAA serum” and utilized for the immunoblot analysis reported in Fig. 1 and validation of the DTNB-based assay of TrxR activity.

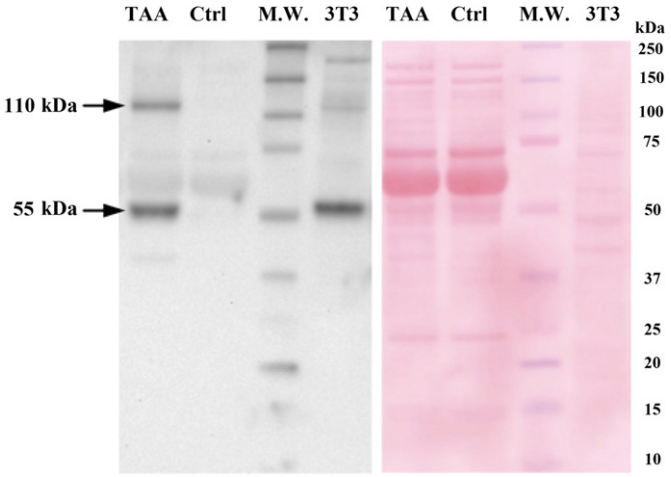

Fig. 1. TrxR protein levels in mouse serum are increased upon TAA treatment. The presence of TrxR protein in 0.5 μL serum samples of control (Ctrl) or TAA (200 mg/kg, sacrificed after 24 h) treated mice were compared with a whole cell lysate of mouse NIH 3T3 cells (3T3) as positive control using reducing SDS-PAGE and immunoblotting (left panel), with sample loading and molecular weight markers as indicated in the figure (M.W.; numbers to the right show M_r in kDa). The arrows indicate two bands that were clearly immunoreactive with a polyclonal anti-mouse TrxR1 antibody, with one band migrating slightly above 55 kDa, corresponding to the TrxR1 monomeric subunit, and one approximately at 110 kDa, most likely representing a covalently linked dimer of TrxR1. Total serum protein loading was visualized by Ponceau S staining as shown in the right panel. Both of the analyzed samples were pooled from serum of 5 mice handled under identical conditions and these pooled samples were also further analyzed in Fig. 2A–D.

To investigate 1) the impact of TAA on hepatic TrxR and ALT activities at 12 h post treatment, and 2) the impact of TAA and carbon tetrachloride (CCl_4) on serum TrxR and ALT activities as well as other enzyme activities at 24 h post treatment, thirty mice were randomly divided into five groups (6/group). Mice were i.p. injected with saline, 200 mg/kg TAA or 200 μL/kg CCl_4 dissolved in peanut oil (10 mL/kg per injection). At 12 h, mice subjected to saline and TAA treatments were sacrificed to collect liver tissues. At 24 h, the remaining mice subjected to saline, TAA and CCl_4 treatments were sacrificed to collect serum samples.

To delineate TAA-triggered time-dependent changes of serum enzyme activities, thirty mice were randomly divided into five groups (6/group). Mice in group I were injected i.p. with saline as control and sacrificed at 24 h. Mice in groups II–V were i.p. injected with 200 mg/kg TAA and sacrificed at either 6, 12, 24 or 36 h to collect serum samples.

To analyze CCl_4-caused dose-dependent changes of serum enzyme activities, twenty-four mice were randomly divided into four groups (6/group). Mice in group I were i.p. injected with peanut oil as control whereas mice in groups II–IV were i.p. injected with 40, 200 and 1,000 μL/kg CCl_4, respectively. All mice were sacrificed at 24 h to collect serum.

To investigate changes of serum enzyme activities during chemically induced liver fibrosis, twenty-four mice were randomly divided into three groups (8/group). Mice in groups II and III were i.p. injected with 200 mg/kg TAA or 1,000 μL/kg CCl_4, twice weekly for a total of 6 weeks [17,18]. Four mice in group I were i.p. injected with saline as TAA control and another four mice in group I were i.p. injected with peanut oil as CCl_4 control. The enzyme levels in the two vehicle groups were not significantly different from each other and the values were thus merged as one control. All animals were sacrificed at 24 h after their last treatment to collect serum. Although the doses used here for CCl_4 were close to its generally accepted LD50 value no lethality was observed for the duration of the treatments, which may possibly be explained by the known variability between different mice in CCl_4 susceptibility [19].

2.4. Serum and tissue preparations

At the end of each set of experiments, mice were sacrificed by cervical dislocation. Peripheral blood from both ophthalmic veins was collected into Eppendorf tubes without anticoagulant. After standing for 30 min at room temperature to allow for complete coagulation, serum was obtained by centrifugation (9000 *g* at 4 °C for 10 min). All serum samples used in the present study were clear and yellow with hemoglobin levels being 0.35 ± 0.04 g/L as estimated from the absorbance at 540 nm, and thus lacked noticeable hemolysis. Whole livers were excised, rinsed in ice-cold saline and instantly homogenized in ice-cold 150 mM, pH 7.2 phosphate buffer saline (PBS) containing 1 mM EDTA-Na_2 (1:9, w/v) using a tissue homogenizer. After centrifugation (15,000 *g* at 4 °C for 15 min), the resultant supernatants were immediately used for the assessments of hepatic TrxR and ALT activities.

2.5. Immunoblotting

Serum samples, 0.5–1 μL, were diluted in deionized water, NuPAGE LDS sample buffer (Invitrogen) and 12 mM dithiothreitol to a final volume of 50 μL. Samples were then denatured by boiling in water for 5 min. Subsequently, 20 μL of the sample was loaded onto a 4–12% NuPAGE Bis-Tris SDS-PAGE gel (Invitrogen) and subjected to electrophoresis. Separated proteins were electroblotted to a nitrocellulose membrane and equal protein loading was confirmed by Ponceau S staining. Membranes were blocked for 1 h at room temperature using 5% non-fat dry milk dissolved in PBS containing 0.1% Tween 20 (PBS-T). Membranes were then probed for TrxR1 using a polyclonal antibody raised against the mouse TrxR1 protein (kindly

provided by Prof. Gary F. Merrill) diluted 1:4,000 in PBS-T solution containing 5% milk. After 3 hour incubation at room temperature the membranes were washed in PBS-T and incubated with a secondary antibody (diluted 1:2,000 in PBS-T) conjugated to horseradish peroxidase (Santa Cruz Biotechnology) for 1 h at room temperature. Immunoreactive proteins were visualized by enhanced chemiluminescence.

2.6. Measurement of TrxR activity through NADPH dependent DTNB reduction

At the onset of this study, we found that the standard thioredoxin-linked insulin reduction assay for measurement of TrxR activity [2] became unreliable using serum samples, because of the interference of some unidentified serum components with either thioredoxin or insulin. We therefore instead utilized NADPH-dependent DTNB reduction that is inhibited by auranofin as a measure of TrxR activity, which was analyzed essentially according to the method of Smith and Levander [6] with some modifications, shortly summarized as follows. First a stock mixture (10 mM EDTA-Na_2, 5 mM DTNB, 240 μM NADPH, and 0.2 mg/mL bovine serum albumin in 100 mM, pH 7.0 PBS) was freshly prepared and kept at 37 °C. For analyses, 54 μL serum was mixed with 6 μL 1.47 mM auranofin solution containing 5% ethanol or 6 μL 5% ethanol, at 37 ºC for 10 min to prepare paired samples for the measurement of activity that was inhibited by auranofin. After adding 50 μL of both paired samples to a 96-well plate, the reactions were started by the addition of 250 μL stock mixture to each sample. Changes in absorbance at 412 nm over time were monitored for the first 3 min at 37 °C using a microplate reader. TrxR activity was calculated by subtracting the slope rate of the reaction with auranofin from the slope rate of the reaction without auranofin. As shown in the text, control experiments showed good linear correlation of activity with serum volume, little intra- or inter-assay variation, and no direct inhibition of TAA on the activity of pure TrxR1. One unit (U) of TrxR activity was subsequently defined as 1 μmol of NADPH oxidized/min, considering that one molecule of NADPH is required for reduction of one molecule of DTNB, resulting in two thio-bis-nitrobenzoic acid anions having an extinction coefficient at 412 nm of 13,600 M^{-1} cm^{-1}. The serum TrxR activity was expressed as U/mL.

2.7. Trx1 levels

Trx1 protein in serum was assessed using a mouse anti-Trx1 ELISA kit (Uscn Life Science Inc., Wuhan, China). The slope rates of TAA and control serum samples were prepared by serial dilutions, whereupon Trx1 protein levels were calculated according to the standard curve of Trx1 following the instructions of the manufacturer.

2.8. Measurement of ALT activity

ALT activities in serum were determined using a kit purchased from the Nanjing Jiancheng Bioengineering Institute. ALT activity was defined with Karmen units, with one Karmen unit being equivalent to 0.48 international units. One Karmen unit corresponds to 1 mL of serum that causes 0.001 decrease of NADH absorbance (in a 1-cm path length cuvette) at 340 nm per minute at 25 °C [20].

2.9. Assays of other enzymatic activities

Glutathione peroxidase (GPx) was measured following the method of Smith and Levander [6] with 300 μM of H_2O_2 as substrate, being coupled with GR and NADPH to reduce oxidized glutathione. GPx activity was calculated in terms of μmols of NADPH oxidized/min/mL serum (U/mL). GR activity was assessed by the method of Carlberg and Mannervik [21] with 800 μM of GSSG as substrate. GR activity was calculated in terms of μmols of NADPH oxidized/min/mL serum (U/mL). Superoxide dismutase (SOD) activity was estimated using xanthine/xanthine oxidase system to generate superoxide anions that promote nitroblue tetrazolium reduction [22]. One unit of SOD activity is defined as the amount of protein that inhibits the rate of nitroblue tetrazolium reduction by 50%, and serum SOD activity was expressed as U/mL.

2.10. Statistical analysis

Data are presented as mean ± SEM. The differences between groups were examined by Student's *t* test, and correlation was evaluated by Pearson's correlation coefficient. A *P* value of less than 0.05 was considered statistically significant.

3. Results

3.1. TrxR in serum of mice upon TAA treatment can be detected with immunoblotting

We first asked whether TrxR protein can be detected in serum of mice using Western blot analyses and whether it could be increased upon liver injury. These analyses showed that TrxR in serum of normal mice was below detection limits in this immunoblotting approach. However, with serum analyzed 24 h after a single dose of TAA (200 mg/kg) a protein was clearly detectable with anti-mouse TrxR1 antibodies, which agreed in size with monomeric and dimeric TrxR1 and closely resembled a corresponding analysis of a whole cell lysate of mouse NIH 3T3 cells (3T3), known to express low but detectable levels of TrxR1 protein [23] and thus here used as a positive control for the immunoblotting (Fig. 1). The results suggested that treatment of mice with the hepatotoxic compound TAA resulted in noticeable increases of TrxR1 in serum, wherefore we next analyzed the presence of TrxR in serum using quantitative enzyme activity-based methods.

3.2. TrxR activity in mouse serum increases upon TAA treatment

We found that assessment of TrxR activity using a standard Trx1-linked insulin reduction assay was unreliable using serum samples because of yet unidentified interfering activities (not shown). However, measuring NADPH-dependent DTNB reduction that is inhibited by auranofin, which is another established assay for TrxR activity in biological samples [5], gave highly reliable activities using serum of TAA treated mice (Fig. 2A). We also confirmed that TAA itself did not directly inhibit pure TrxR1 using this assay (Fig. 2B). However, we found that TrxR activity in serum of healthy control mice was very low and close to detection limit (Fig. 2C), while the activity after TAA treatment was prominent, NADPH dependent, and with little standard deviation between six replicates (Fig. 2D). These measurements were performed in the same samples as had been analyzed with immunoblotting in Fig. 1 and thus showed that the appearance of the immunoreactive protein species in serum correlated with the appearance of enzymatic TrxR activity. We therefore considered the NADPH-dependent DTNB reduction that could be inhibited by auranofin as indeed being due to the presence of TrxR in the serum samples. The level of TrxR in serum was increased approximately 205-fold upon TAA treatment (Fig. 2C and 2D). In contrast, measuring the levels of Trx1 (the main substrate of TrxR1) in these serum samples, using ELISA, gave only a small 1.83-fold increase upon TAA treatment, from ≈103 ng/mL to 189 ng/mL (Fig. 2E and 2F). Thus, the very low basal levels of TrxR in serum and its large increase upon liver damage were not paralleled by Trx1.

3.3. The ALT and TrxR inductions in serum upon liver damage are larger than those of other liver enzymes

To analyze whether increases of either ALT or TrxR in serum after liver damage would be mirrored by similar increases in other liver

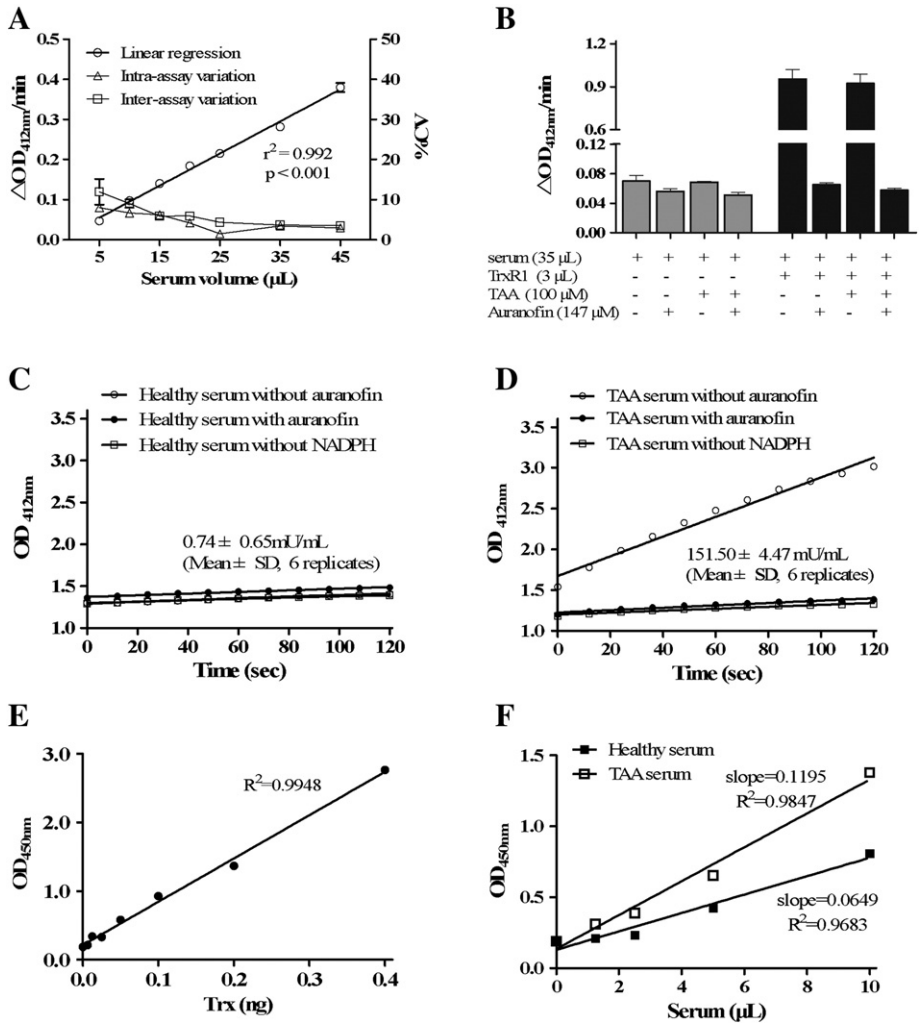

Fig. 2. TrxR activities and Trx1 protein levels in mouse serum increase upon treatment with TAA. (A) Linear correlation of TrxR activity measurements with serum volume, using pooled serum of TAA treated mice and the auranofin-inhibited NADPH-dependent DTNB reduction activity assay. The graph also displays the low intra- and inter-assay variation of the assay (average %CV, $n = 3$, righ y-axis). (B) Lack of inhibition of TrxR in serum or pure TrxR1 by TAA, using additions to the NADPH-dependent DTNB reduction assay as indicated. (C) Reaction kinetics curve of DTNB reduction with 50 μL healthy serum. (D) Reaction kinetics curve of DTNB reduction with 50 μL TAA serum. (E and F) The presence of Trx1 protein in serum was assessed using a mouse anti-Trx1 ELISA kit. (E) The standard curve of Trx1 protein. (F) The slopes of linearly increased Trx1 ELISA signal using different amounts of TAA and control serum samples.

enzymes, we treated mice with either TAA or CCl_4 and after 24 h measured the levels of ALT and TrxR in serum, as well as those of GPx, GR and SOD. This showed that all of these liver enzymes increased in serum upon liver damage, but the increases were by far the largest for ALT and TrxR (Fig. 3A).

3.4. Increased TrxR and ALT levels in serum upon TAA treatment correlate with decreased levels in liver tissue

With TAA being a liver damaging agent, we reasoned that the increase of TrxR in serum upon TAA treatment might relate to release of the enzyme from liver, in analogy to serum ALT that serves as an indicator for liver damage. Indeed, 12 h after TAA treatment (200 mg/kg), we found a significant reduction of TrxR as well as ALT activities in liver extracts (Fig. 3B), thus enforcing the notion that the increased levels of TrxR found in serum derived from the liver injury.

3.5. Close linear correlation between TrxR and ALT in serum

With the TrxR and ALT levels showing major increases in serum upon liver damage we next wished to analyze time- and dose-dependent increases upon treatment with TAA and to compare the responses of TrxR in serum with those of ALT. Mangipudy et al. showed that a 12-fold dose range of TAA (50–600 mg/kg) failed to elicit a typical dose–response relationship as indicated by plasma ALT activity, but time-dependent alterations of plasma ALT activity were evident [24–26], therefore, we only investigated the time course of TAA. However, we found that CCl_4 could trigger liver injury in a salient dose-associated fashion. Using the single dose TAA-induced acute liver injury model, we found that serum levels peaked at 24 h for both ALT (Fig. 4A) and TrxR (Fig. 4B). For both enzymes the levels were significantly increased compared to control at 12, 24 and 36 h after treatment, but not as early as 6 h (Fig. 4A and 4B). There was furthermore a strong linear correlation between ALT and TrxR levels, when analyzing all individual samples from the experiment (Fig. 4C). Upon CCl_4 treatment, increases of ALT in serum (Fig. 4D) were again mirrored by TrxR (Fig. 4E) and the levels of the two enzymes in serum showed a close linear correlation also in this model of hepatotoxicity (Fig. 4F).

Repeated treatments of higher doses of either CCl_4 or TAA induce liver fibrosis. We therefore also analyzed ALT or TrxR in serum as indicators in this alternative model for liver damage. Again we found similar profiles in the increase of serum ALT (Fig. 4G) and TrxR (Fig. 4H), with the two indicators displaying close linear correlation (Fig. 4I).

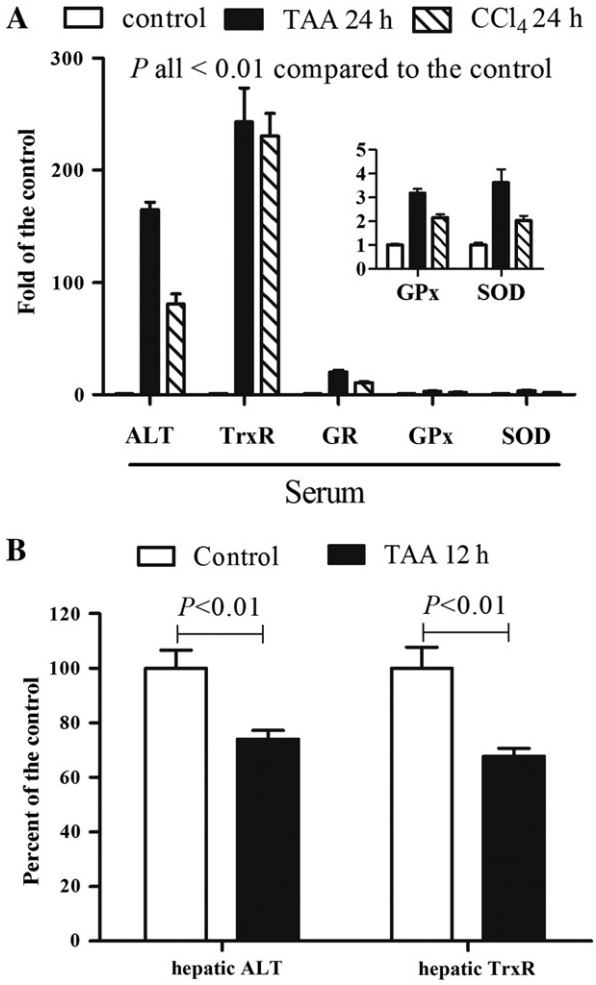

Fig. 3. Serum and hepatic enzyme activities. Mice were i.p. injected with saline, 200 mg/kg TAA or 200 μL/kg CCl_4, and then were sacrificed at 12 or 24 h. (A) Serum enzyme activities. Basal activities of ALT, TrxR, GR, GPx and SOD were 51.0 ± 9.2 U/mL, 0.58 ± 0.33 mU/mL, 74.8 ± 5.7 mU/mL, 897.8 ± 51.4 mU/mL, and 569.5 ± 58.5 U/mL, respectively. (B) Hepatic ALT and TrxR activities. Basal activities of ALT and TrxR were 31,206 ± 1826 U/g liver and 933.3 ± 64.7 mU/g liver, respectively. Data are presented as mean ± SEM (n = 6).

4. Discussion

In this study we found that TrxR levels in serum were greatly increased upon treatment of mice with either of two hepatotoxic compounds, TAA or CCl_4, which are known to provoke acute hepatotoxicity with centrilobular necrosis [27,28]. It was clear that TrxR showed closely similar behavior to ALT with a striking positive correlation in all the experimental models used herein, including the time course of TAA, dose effect of CCl_4, and TAA- or CCl_4-induced liver fibrosis. The findings suggest that ALT and TrxR are released from the liver with similar kinetics and, furthermore, have similar stabilities in serum.

During TAA-induced liver injury, ALT is believed to be released from hepatocytes into the serum, resulting in the increase of serum ALT activity, although it may be released also from other cells and several other biomarkers for liver damage have therefore been proposed; still ALT is yet the golden standard biomarker in serum for liver damage [15,29]. Considering ALT as a reference biomarker, the present results suggest that hepatic TrxR release and increase of TrxR in serum follow similar mechanisms, although TrxR, just like ALT, is also found in tissues other than liver. Hepatocytes contain cytosolic TrxR1 [30] and mitochondrial TrxR2 [31]. Hepatic TrxR1 constitutes at least 75% of the total hepatic TrxR activity [30]. The measured TrxR activity in serum upon chemically induced liver injury as found here most likely included the TrxR1 isoform, because it could be detected using an anti-TrxR1 antibody and is the major TrxR species in hepatocytes. However, it cannot be disregarded that also TrxR2 isoform may be released into serum upon liver damage. With regards to the amount of TrxR1 in liver it can be calculated to be around 44 μg/g crude rat liver extract, or about 18 μg/g bovine liver extract, as extrapolated from published activities measured during early purification schemes [10,14]. In the present study, hepatic and serum TrxR activities of healthy mice were 933.3 mU/g liver and 0.58 mU/mL serum, respectively, which should be equivalent to 28 μg/g liver and 18 ng/mL serum, if extrapolated from the standard curves prepared with purified rat TrxR1 and the commercial protocol (Sigma T9698) utilized herein. Thus, there are significant amounts of TrxR1 in liver that may be released to serum. If the liver of the mouse is expected to weigh 2 g and provided that the 32.2% loss of hepatic TrxR1 activity at 12 h after TAA treatment that we detected would all be released into serum and remain stable, the TrxR1 serum activity would be calculated to approach about 400 mU/mL serum (assuming a total serum volume of 1.5 mL). The highest serum TrxR activity that we observed at 24 h post TAA treatment was 141 mU/mL, thereby being well within the theoretical maximal levels that would be possible to achieve, and the lower value might also suggest that some extracellular TrxR degradation occurs. Removal of the protein from serum by time is furthermore supported by the fact that the serum TrxR activities were lower at 24 to 36 h post TAA treatment, similar to the profiles of ALT in serum.

In view of antioxidant enzyme release from hepatocytes during acute liver injury, it is important to note that the serum levels of GPx, GR and SOD also increased. However, the increase in serum TrxR activity was far more exacerbated. It should be noted that Trx1, the main endogenous substrate of TrxR1, did not show the same elevation in serum. Because both Trx1 and TrxR1 are abundant in liver [10,14], the preferential increase of TrxR1 levels in serum over Trx1 may reflect different serum half-lives between the two proteins. Interestingly, the intracellular Trx1/TrxR1 system in liver is involved in protection against TAA-triggered hepatotoxicity, at least as illustrated by the fact that transgenic overproduction of Trx1 protects against TAA-induced liver fibrosis [32]. Furthermore, as one out of only nine genes from a total of 9936 transcripts in a microarray-based study, *Txnrd1* encoding TrxR1 was found to be consistently upregulated upon TAA-induced liver damage in rat [33]. Also, overexpression of Trx1 using adenoviral transfer in mice protects against CCl_4-induced liver damage [34]. It thus seems as if the Trx system is involved in protecting the liver from hepatotoxic agents, which furthermore agrees with the fact that TrxR1 is an important target of nuclear factor erythroid 2-related factor 2 (Nrf2) induction; the well-characterized transcriptional inducer of protective enzymes in the liver [35]. In this context it is interesting to note that also the converse applies, i.e. an excessive Nrf2 induction is triggered upon conditional deletion of TrxR1 in hepatocytes of adult mice [36], leading to improved resistance to acetaminophen hepatotoxicity [37,38]. It thereby seems as if TrxR1 is intimately linked to protection of the liver against toxic insults, but in a rather complex manner. Its release to serum adds to this complexity. Because expression of TrxR1 in liver is extensively induced by hepatotoxic agents, including TAA and CCl_4 [39], this may perhaps help to explain why its increase in serum shows such high amplitude upon liver damage provoked by these agents, i.e., its production in hepatocytes may initially be extensively induced upon toxic challenge, followed by its release to serum.

Elevated serum Trx1 levels have been observed in many diseases, including cancer [40], diabetes [41], rheumatoid arthritis [42], chronic kidney disease [43], acute lung injury [44], asthma [45], sepsis [46], acute pancreatitis [47], severe burn injury [48], acquired immunodeficiency syndrome [49], heart disease [50], unstable angina [51], coronary disease [52], and various types of liver diseases [12,13,40], such as hepatitis C virus infection, nonalcoholic steatohepatitis, alcoholic steatohepatitis, and hepatocellular carcinoma. Recently, serum Trx1 level has been suggested to be a clinical biomarker for detecting malignant pleural mesothelioma [53]. In sharp contrast, the possible presence

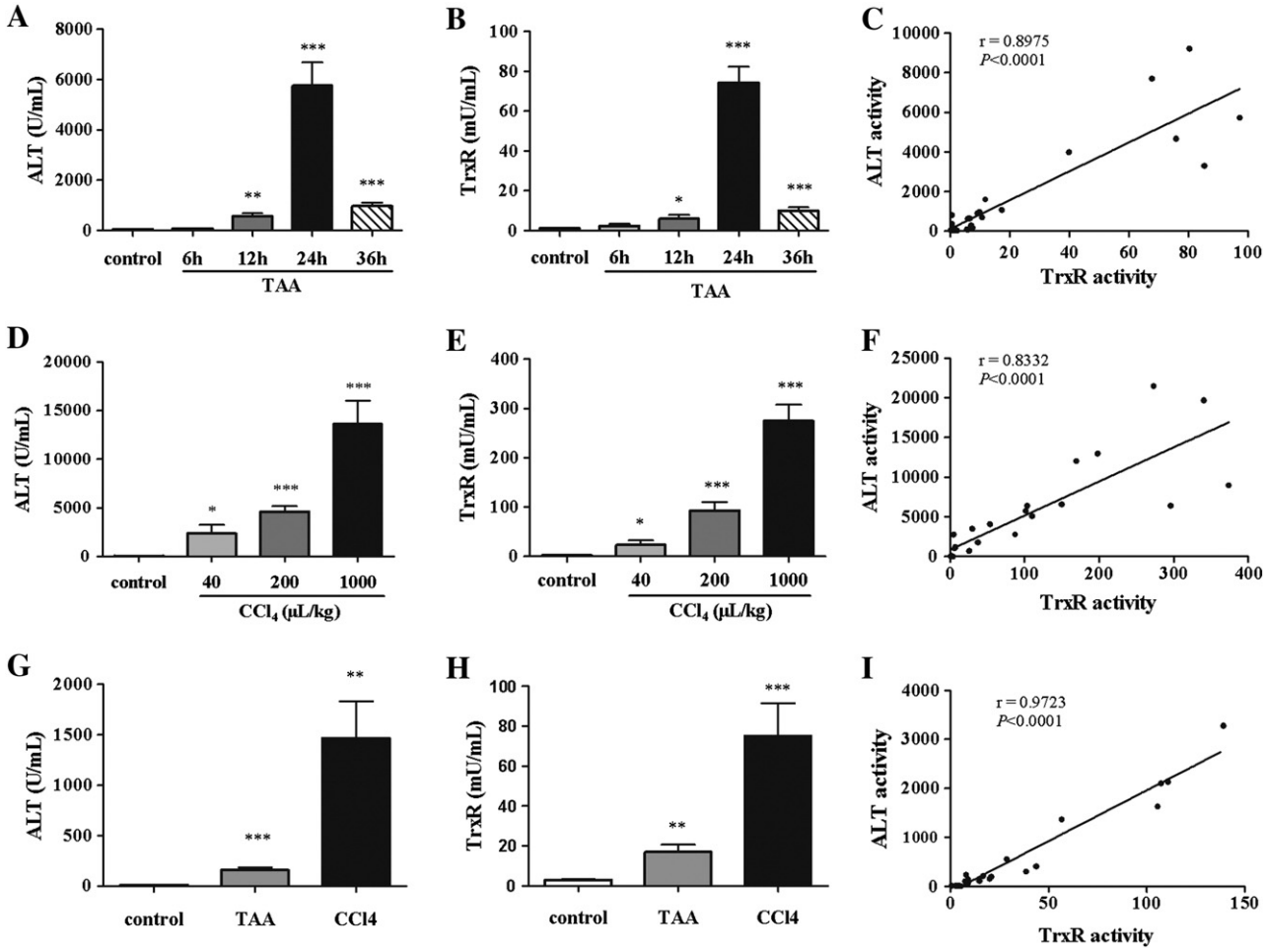

Fig. 4. TrxR activities in serum mirror closely the ALT activities in serum upon chemically induced liver damage. Time-dependent alterations of ALT (A) and TrxR (B) in serum of mice treated with TAA (200 mg/kg, sacrificed after 6, 12, 24 and 36 h, respectively), and their correlation (C). Dose-dependent elevations of ALT (D) and TrxR (E) in serum of mice treated with CCl_4 (40, 200 and 1,000 μL/kg, respectively, sacrificed after 24 h), and their correlation (F). Hepatic fibrosis associated changes of ALT (G) and TrxR (H) in serum of mice treated with hepatotoxic agents twice weekly for a total of 6 weeks (200 mg/kg TAA or 1,000 μL/kg CCl_4, respectively, sacrificed at 24 h after the last dose), and their correlation (I). Data are presented as mean ± SEM (n = 6 in A, B, D and E; n = 8 in G and H). Compared to the control, $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

of TrxR1 in serum has remained much less elucidated. Herein, we have provided clear evidence that a robust elevation of serum TrxR1 levels occurs in chemically triggered liver injury models.

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