ROS-mediated inflammatory response in liver damage via regulating the Nrf2/HO-1/NLRP3 pathway in mice with trichloroethylene hypersensitivity syndrome

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ABSTRACT
Trichloroethylene hypersensitivity syndrome (THS), mainly caused by occupational exposure to trichloroethylene (TCE), can give rise to serious and fatal hepatic damage. To date, the precise mechanisms of hepatic damage in THS remain unclear. Recent studies showed that reactive oxygen species (ROS) play a core role in cell death and inflammatory response. Therefore, the present study sought to explore whether ROS-mediated inflammatory responses contribute to the hepatic damage in TCE sensitization. To this end, a mouse model of TCE sensitization was established; in some cases, hosts were pretreated with tempol, an ROS scavenger. The results showed that TCE sensitization caused hepatic pathological/functional changes, ROS generation, and oxidative stress, alterations of the anti-oxidant defense Nrf2/HO-1/NLRP3 pathway, and pro-inflammatory cytokine formation in the liver. ROS scavenging via pretreatment with tempol was found not only to inhibit the hepatic oxidative stress, but also to regulate Nrf2/HO-1/NLRP3 pathway activity. In all cases, tempol was able to mitigate the pathologic changes induced by TCE sensitization. In summary, the results here demonstrated a novel molecular mechanism wherein ROS-mediated inflammatory responses play a central role in TCE-induced liver damage. Therapies targeting ROS scavenging could help to protect against hepatic damage by regulating Nrf2/HO-1/NLRP3 pathway activities in TCE-sensitized hosts.

Introduction

Though trichloroethylene (TCE) has been widely used in industrial applications for decades, it is now considered a threat to public health, especially to workers routinely/repeatedly exposed to this agent (Lin et al. 2021; Liu et al. 2021). Trichloroethylene hypersensitivity syndrome (THS) is also referred to as occupational medicamentosa-like dermatitis due to TCE (OMDT) in China. THS is a severe mucocutaneous disease that often results in fatal outcomes mainly among TCE-exposed workers (Wang et al. 2019). Similar to severe cutaneous adverse reactions to drugs, THS is characterized by a series of clinical features, including fever, diffuse erythematous maculopapular rash, edema, lymphadenopathy, leukocytosis, multiple organ failure, etc. (Zhang et al. 2021).

Multiple organ failure, especially that of the liver, is a common reason for death among THS patients. Laboratory tests confirm that most THS patients often present with elevated levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activity, as well as of total bilirubin (TBIL) and total bile acid (TBA) (Jung et al. 2012). Ultrasonography often reveals abnormal echoes in the liver, hepatomegaly, and thickening of the gall bladder in THS patients (Pantucharaensri et al. 2004; Xu et al. 2009). In addition, animal studies have evaluated these induced liver histopathological changes in more detail, and have demonstrated increased incidences of cellular swelling, vacuolar degeneration, and inflammatory cell infiltration (Wang et al. 2015). It is thus clear that both the structure and function of the liver are compromised during TCE sensitization. Unfortunately, the complex mechanisms underlying all of these changes during THS still remain largely undefined.

Earlier studies in several laboratories provided indications that there was a generalized induction of oxidative stress during TCE sensitization. For example, proteome and mass spectrometry analyses identified differential expression of several oxidative stress-related proteins, such as plasma glutathione peroxidase (Hassan et al. 2016; Al-Griw et al. 2017). A previous study from this laboratory (Li et al. 2019) found that oxidative stress played a role in renal endothelial cell damage and aggravated renal impairment in TCE-sensitized mice. Another recent study by Banerjee et al. (2020) reported there were decreases in expression of anti-oxidant nuclear factor erythroid 2-related factor 2 (NRF2) in TCE-induced autoimmune liver damage. This would be in keeping with the fact that as a negative regulatory factor of oxidative stress, NRF2 can activate heme oxygenase-1 (HO-1) so as to provide a protective role against oxidative stress and inflammation (Luo et al. 2021).
Taken together, the above studies suggested that the balance between oxidative stress and the host anti-oxidant system might play an important role in TCE-induced inflammatory responses and subsequent tissue damage. Due to the potential role of reactive oxygen species (ROS) as key players in oxidative stress responses, changes in their presence have attracted increasing attention in the context of THS inflammatory responses, cell death, and tissue damage. Accordingly, the present study was undertaken to ascertain whether ROS in fact participate in TCE sensitization-induced hepatic damage. To further verify the potential key role of ROS in these pathologies, an ROS scavenger (i.e. Tempol) was employed to pre-treat THS mice in some of the studies below.

Materials and methods

Antibodies and reagents

Freund’s complete adjuvant (FCA), trichloroethylene (TCE), and Tempol (2,2,6,6-tetra-methylpiperidine) were purchased from Sigma (St. Louis, MO). AST, ALT, malondialdehyde (MDA), and reactive oxygen species (ROS) test kits were obtained from the Nanjing Jiancheng Bioengineering Institute (Nanjing, China). General-purpose streptavidin-peroxidase detection kits and H&E staining kits were purchased from ZSJQ-BIO (Beijing, China). PCR primers were purchased from Shenggong Biotech (Shanghai, China). The phosphatase inhibitor cocktail tablets were obtained from Roche (Shanghai). Total RNA extraction reagent (TRizol) and RevertAid First Strand CDNA Synthesis kits were purchased from Thermo Scientific (Santa Clara, CA). Nuclear protein extraction kits were purchased from BestBio (Shanghai, China). All antibodies used in this study are listed in Table 1.

Animal model and experimental design

Forty BALB/c mice (female, 6–8-week-of-age, 18–22 g) were purchased from the Experimental Animal Center of Anhui Medical University. Upon arrival, the mice were housed in pathogen-free facilities maintained at 24°C with 50% relative humidity and a 12-h light-dark cycle. All mice had ad libitum access to standard rodent chow and filtered tap water. After allowing for 1 week to acclimate, the mice were allocated randomly into groups (n/group indicated in Table 2) to establish the model of TCE sensitization as described earlier in Wang et al. (2015) (Figure 1). In brief, the mice were intradermally injected with 100 µl of a 1:1 solution of 50% TCE (diluted with a mixture of acetone and olive oil [3:2 v/v]) and Freund’s complete adjuvant (FCA) on Day 1. Then, 100 µl of a 50% TCE solution (diluted with a mixture of acetone and olive oil [3:2 v/v]) was applied to a shaved area on the back of each mouse on Days 4, 7, and 10 (sensitization stage). On Days 17 and 19 (challenge stage), 100 µl of a 30% TCE solution (diluted with acetone:olive oil [5:2 v/v]) was applied. For subsets of mice, a single intraperitoneal injection with Tempol (100 mg/kg) was performed 2 h prior to the Days 17 and 19 of TCE treatment; counterpart controls received only a saline injection.

Allergy scores were calculated on Day 20 based on visual observations of the severity of erythema and blisters on the backs of the mice. Scoring was based on no reaction = 0; scattered mild erythema = 1; moderate and diffuse erythema = 2; and intensive erythema with blisters = 3. On Day 22 of the study, all mice were euthanized by asphyxiation with CO2, and biosamples, including blood and liver, were immediately collected at necropsy. Serum was prepared and then measured for AST and ALT.

Table 1. Antibodies used in the study.

| Antibody                                | Catalog #          | Company                      |
|-----------------------------------------|--------------------|------------------------------|
| Rabbit mAb to mouse Nrf2                | AF0639             | Affinity (Cincinnati, OH)    |
| Rabbit mAb to mouse HO-1                | AF5393             | Affinity                     |
| Rabbit pAb to mouse NLRP3               | ab214185           | Abcam (Cambridge, UK)        |
| Rabbit pAb to mouse IL-1β               | ab9722             | Abcam                        |
| Mouse mAb to mouse Lamin A/C            | 4777S              | CST (Boston, MA)             |
| Rabbit mAb to mouse GAPDH               | ab181602           | Abcam                        |
| Rabbit Anti-Mouse IgG                   | E-AB-1033          | ElabScience (Wuhan, China)   |
| Goat Anti-Rabbit IgG                    | E-AB-1034          | ElabScience                  |

mAb: monoclonal. pAb: polyclonal.

Table 2. Mouse groups and sensitization rates.

| Groups                        | n  | Score = 0 | 1  | 2  | 3  | Sensitization rate (%) |
|-------------------------------|----|-----------|----|----|----|------------------------|
| Blank control                 | 5  | 0         | 0  | 0  | 0  | 0                      |
| Vehicle control               | 5  | 0         | 0  | 0  | 0  | 0                      |
| TCE treatment                 | 15 | 9         | 4  | 1  | 1  | 40.00                  |
| TCE positive (TCE +)          | 6  | –         | 4  | 1  | 1  | –                      |
| TCE negative (TCE –)          | 9  | 9         | –  | –  | –  | –                      |
| TCE + tempol treatment        | 15 | 10        | 4  | 1  | 0  | 33.33                  |
| TCE + tempol +                | 5  | –         | 4  | 1  | 0  | –                      |
| TCE + tempol –                | 10 | 10        | –  | –  | –  | –                      |

Figure 1. Flow chart explaining methodology used for generating BALB/c mouse model of TCE sensitization.
ALT activities in the test kits according to manufacturer instructions. The liver samples were processed for use in the various assays outlined below.

All protocols were approved under the Animal Care and Use Committee of Anhui Medical University (No. LLSC 20180310). All experiments were performed in accordance with ARRIVE guidelines and the U.S. Public Health Service Policy on Humane Care and Use of Laboratory Animals.

**Histopathologic analysis**

A random section of each liver was isolated and placed into paraffin wax using standard protocols and then processed with an RM2255 tissue slicing system (Leica, Waltham, MA) to yield 5-μm sections. For histopathologic analyses, the sections from each liver were placed on slides and then de-waxed and re-hydrated. The sections were then stained using hematoxylin and eosin. Changes in the liver samples were evaluated using a BX53 light microscope (Olympus, Tokyo, Japan) at 400× magnification. All evaluations were performed in a blinded manner by a certified histologist. A minimum of 5 sections/mouse were evaluated.

**Immunohistochemistry (IHC)**

From the above block of fixed tissue samples, liver sections were de-paraffinized and re-hydrated with xylene and an ethanol gradient. Endogenous peroxidases in each section were then blocked by coating the slides with a 3% H2O2 solution for 30 min. The sections were then coated with citrate solution and heated for 15 min in a microwave to permit epitope retrieval. While the sections were cooling, each slide was coated for 30 min with phosphate-buffered saline (PBS, pH 7.4) containing 10% normal goat serum (ZSJQ-BIO, Beijing, China) to block nonspecific antigens. Thereafter, each slide received a solution of PBS containing rabbit antibody to mouse IL-1β (diluted at 1:200) and was then incubated overnight at 4°C. After gentle rinsing with PBS, each slide was coated with a solution of PBS containing biotin-conjugated goat anti-rabbit IgG (diluted at 1:200) for 30 min at 37°C. Each slide was then gently rinsed with PBS and coated with PBS containing horseradish peroxidase (HRP)-conjugated streptavidin (diluted at 1:1000). After incubation at room temperature for 15 min, the slides were rinsed with PBS and then coated with a solution of 0.01% 3,3’-diaminobenzidine (DAB) and hematoxylin for 1–3 min. After a final rinsing with PBS, each stained section was examined using a BX53 microscope. Five slides/mouse were evaluated for each cytokine.

**ROS level measures**

Samples of the liver tissues (≈50 mg/mouse) were minced and filtered to obtain single-cell suspensions. A solution containing 20 mM DCFH-DA (Abcam, Cambridge, UK) was added to each cell suspension, and the mixtures were incubated for 30 min at 37°C in the dark. Each sample was then centrifuged at 1000 × g for 5 min; the pelleted cells were then gently washed with/re-suspended in PBS; an aliquot was removed, stained with trypan blue, and then counted in a hemocytometer. Aliquots containing 10^6 cells were then transferred into 96-well plates (in triplicate), and fluoresce intensity (IF) in each was measured in a Varioskan LUX microplate reader (ThermoFisher Scientific, Waltham, MA) using 488 nm excitation and 525 nm emission wavelengths.

**qRT-PCR**

From a 50 mg piece of isolated tissue, total liver mRNA was extracted using TRIzol reagent, and cDNA was synthesized using a RevertAid First Strand cDNA Synthesis Kit according to manufacturer instructions. The resulting cDNA was then amplified using a SYBR Light Cycler 480 system with the following programs: 95°C for 5 min; 95°C for 15 s 60°C for 15 s, and 72°C for 20 s (45 cycles); 72°C for 10 min. In all cases, the relative expression of the mRNA of interest was analyzed using the 2^−ΔΔCt method, and outcomes were normalized to GAPDH expression (housekeeping gene). The primer sequences used for the qRT-PCR are listed in Table 3.

**Western blotting**

Mouse liver samples (50 mg/mouse) were extracted on ice for 20 min using RIPA lysis buffer (containing protease inhibitor PMSF and phosphatase inhibitor cocktail). The concentration of total protein in each extract was evaluated via a BCA protein assay kit (Pierce, Rockford, IL). An aliquot containing 100 μg protein was then placed into the well of a 15% SDS-PAGE gel, and proteins were resolved. The materials were then electrotransferred to a PVDF membrane that in turn was blocked for 2 h at room temperature in a solution of TBS (Tris-buffered saline, pH 7.4) containing 5% nonfat milk. A solution of TBS containing rabbit primary antibody against Nr2 (diluted 1:1000), HO-1 (diluted 1:1000), NLRP3 (diluted 1:1000), or Lamin A/C (diluted 1:1000), in each case along with antibody against GAPDH (diluted 1:10000), was then applied, and the membrane was incubated at 4°C overnight. Dedicated membranes were generated for each primary antibody to avoid issues of antigen loss that are associated with repeated stripping/probing of a single membrane. After gentle rinsing with TBS, the membrane was placed in TBS containing HRP-conjugated anti-rabbit IgG antibody for 2 h at room temperature. After a final TBS rinsing, enhanced chemiluminescence reagent (ECL, ThermoFisher Scientific) was applied, and band detection/intensity measurements were performed in a ChemiScope 6000 imager (Clinx, Shanghai, China). All data are reported in terms of band density after normalization to the level of GAPDH present in the same membrane.

**Statistical analysis**

All data are expressed as the means ± SD. The difference between means of two groups or among various groups was evaluated for significance using a Student’s t test or a one-way analysis of variance (ANOVA). A p value < 0.05 was accepted as significant. All statistical analyses were performed using Prism software (v.8.0, GraphPad, San Diego, CA).

| Gene     | F/R       | Primer sequence                  |
|----------|-----------|----------------------------------|
| Nr2      | Forward   | 5'-CCATTACGGGAGCCACCGG-3'        |
| HO-1     | Reverse   | 5'-TGGATTACGCCGATAGACG-3'        |
| NLRP3    | Forward   | 5'-CAGAAGGGCTAAACGGCC-3'         |
| GAPDH    | Forward   | 5'-CCCAGCTTAGTTGCTACAGGT-3'      |
| Lamin A/C| Reverse   | 5'-ATGGGCTTCCCGTGATGAC-3'        |
Results

Mouse groups and sensitization rates

Based on the scores of the skin sensitization, mice from the TCE treatment group were subsequently divided into TCE sensitization-positive group (TCE⁺) and TCE sensitization-negative group (TCE⁻). Similarly, mice from the TCE + tempol treatment group were subsequently divided into TCE sensitization-positive with tempol pretreatment group (TCE⁺ tempol⁻) and TCE sensitization-negative with tempol pretreatment group (TCE⁺ tempol⁺). The results indicated that among the non-blank/non-vehicle groups, the overall sensitization rate in this study was 36.67%. The details about each group are listed in Table 2.

TCE sensitization caused liver damage in mice

Based on the histopathologic analyses, hepatocytic vacuolar degeneration and edema were often evident in the livers of the TCE-sensitized mice (Figure 2). No abnormal liver structures or hepatocyte morphology were found in mice samples from the blank, vehicle, or relevant TCE⁻ groups. In conjunction with this damage, the serum analyses revealed that levels of both ALT and AST were significantly increased in TCE⁺ mice compared to TCE⁻ mice and controls (Figure 3). Furthermore, the increases in ALT and AST levels were consistent with moderate histopathological injury in TCE⁺ mice. No significant differences in serum ALT and AST were noted among the blank, vehicle control and TCE⁻ groups.

Generation of ROS in TCE-induced liver damage

ROS generation was assessed to estimate the oxidative stress status in the mouse livers (Figure 4). The results indicated that the generation of ROS in TCE⁺ mice was significantly higher than that in TCE⁻ mice. ROS levels in livers from the blank control, vehicle control, and TCE⁻ mice did not significantly differ. Because it is a stable metabolite of lipid peroxidation products, liver levels of malondialdehyde (MDA) were also evaluated. As with the ROS, the data showed that MDA levels were up-regulated in TCE⁺ mice compared with levels in the livers of any of the other mice (the latter three groups did not significantly differ).

Inhibition of anti-oxidant system in TCE-induced liver damage

As each plays a role in cellular resistance to oxidative stress, expression of both Nrf2 and HO-1 were evaluated to assess the anti-oxidant status in the host livers (Figure 5). Based on the qRT-PCR data, the levels of both Nrf2 and HO-1 mRNA were down-regulated in TCE⁺ mice compared to those in TCE⁻ mice. No statistical differences in expression of Nrf2 and HO-1 mRNA were noted among the liver samples from the blank control, vehicle control, and TCE⁻ mice.

ROS scavenging regulated Nrf2/HO-1/NLRP pathway in TCE-induced liver damage

Because of its potential significant role in the TCE-induced inflammatory responses, the status of the Nrf2/HO-1/NLRP3...
The pathway was evaluated in the mouse livers. It was first confirmed that tempol pretreatment could inhibit generation of ROS in these hosts (Figure 6). Thereafter, expressions of Nrf2, HO-1, and NLRP3 proteins in each sample were evaluated. The results indicated that the levels of both Nrf2 and downstream HO-1 were lower in TCE⁺ mice than those in samples from the other groups. However, the expressions of Nrf2 and HO-1 were higher in the TCE−/R7050⁻ mice than in samples from the TCE⁺ mice (p < 0.05). In contrast, the levels of NLRP3 protein were higher in TCE⁺ mice than in samples from any other group (Figure 6).
Figure 6. ROS scavenging effects on Nrf2/HO-1/NLRP pathway during TCE-induced liver damage. (A) Tempol pretreatment decreased ROS generation. (B) Representative immunoblot of central proteins of the Nrf2/HO-1/NLRP3 pathway. (C–E) Relative expression of Nrf2, HO-1, and NLRP3 normalized to Lamin A/C. *p < 0.05 vs. vehicle control; **p < 0.05 vs. relevant TCE/C0 group; ***p < 0.05 vs. TCE group.

Figure 7. ROS scavenging effects on IL-1β-induced inflammatory responses in mouse liver. (A–F) IHC analyses of IL-1β expression in (A) Blank control. (B) Vehicle control. (C) TCE− group. (D) TCE+ group. (E) TCE + tempol− group. (F) TCE + tempol+ group. (G) Representative immuno-blot of IL-1β expression. (H) Relative IL-1β expression normalized to GAPDH. *p < 0.05 vs. vehicle control; **p < 0.05 vs. relevant TCE− group; ***p < 0.05 vs. TCE− group.
in expression of IL-1β/C0 responses in the mouse livers, expression of IL-1β was evaluated using IHC analyses. The data here showed there was an increase in the levels of IL-1β in samples from the TCE+ mice compared to that in livers of any other test groups (Figure 7). This increase in expression was reduced in the mice from the TCE+ + tempol+ group. Western blots confirmed that IL-1β expression was significantly higher in the livers of the TCE+ mice than in livers from any other group. As above, compared to TCE+ mice, IL-1β expression was lower in TCE+ + tempol+ mouse livers. In both the IHC and Western blot studies, IL-1β levels in the TCE+ + tempol+ mice were still greater than those in any of the other (blank control, vehicle control, TCE-, and TCE+ + tempol−) mice. Among those groups, the data failed to reveal any significant differences in hepatic IL-1β expression.

To ascertain whether blocking of ROS formation could mitigate the pathological changes and alterations in liver function due to TCE, livers from the TCE+ and TCE+ + tempol+ mice were re-analyzed. These evaluations showed that compared to in livers of TCE− mice, the levels of hepatic pathological changes were reduced in the TCE+ + tempol+ hosts and, further, that the earlier-noted elevations in both serum ALT and AST were mitigated (Figure 8). Therefore, the data here suggest that ROS scavenging (via tempol) could play a protective role against TCE-induced inflammatory damage in the liver.

**Figure 8.** ROS scavenging impacts on extent of liver damage in mice. (A, B) Pathological changes in livers of TCE+ and TCE+ + tempol+ mice. Black arrows indicate hepatocytic vacuolar degeneration and edema. (C, D) Liver function in TCE+ and TCE+ + tempol+ mice. *p < 0.05 vs. TCE+ group.

**ROS scavenging blocked IL-1β formation in TCE-treated mice**

To obtain a general indication of any ongoing inflammatory responses in the mouse livers, expression of IL-1β was evaluated using IHC analyses. The data here showed there was an increase in expression of IL-1β in samples from the TCE− mice compared to that in livers of any other test groups (Figure 7). This increase in expression was reduced in the mice from the TCE+ + tempol+ group. Western blots confirmed that IL-1β expression was significantly higher in the livers of the TCE+ mice than in livers from any other group. As above, compared to TCE+ mice, IL-1β expression was lower in TCE+ + tempol+ mouse livers. In both the IHC and Western blot studies, IL-1β levels in the TCE+ + tempol+ mice were still greater than those in any of the other (blank control, vehicle control, TCE−, and TCE+ + tempol−) mice. Among those groups, the data failed to reveal any significant differences in hepatic IL-1β expression.

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**Discussion**

To ascertain if there were any critical roles for oxidative stress-mediated inflammatory responses in THS-associated pathologies, the present study examined the potential role(s) of ROS generation in the development of liver pathologies associated with THS. A mouse model of TCE sensitization was established (as well as a parallel model that was pretreated with the ROS-reducing agent Tempol) to permit those determinations to be made. In these various mice, ROS generation and anti-oxidant defenses in the liver of TCE-sensitized mice were examined (all in the context of levels of histopathological changes that occurred concurrently). The potential role of the Nrf2/HO-1/NLRP3 pathway in the induction of any TCE-related liver damage, as well as during any ameliorative treatments, was also evaluated in these hosts.

Several studies have reported that excessive ROS generation caused local inflammation and tissue injury (Sho and Xu 2019; Sul and Ra 2021). TCE exposure as an inducer of intracellular ROS formation has also been well documented in vitro (e.g. see Toyooka et al. 2018). A previous study (see Li et al. 2019) showed a significant increase of oxidative stress-related components, such as MDA, nitric oxide (NO), and superoxide dismutase (SOD), during TCE sensitization-induced skin and renal damage. However, this evidence was not sufficient to characterize ROS as the critical factors associated with all TCE sensitization-related liver pathologies. Thus, it remains to be determined which (if any particular) ROS might be the primary damaging agent, as well as whether the oxidative stress their increased presence can induce liver damage during TCE sensitization. The present study reported that both liver ROS and MDA production were markedly up-regulated in TCE-sensitized mice; this strongly suggests to us that states of oxidative stress (due to excessive ROS generation) were likely to have been evolved in these hosts after their repeated TCE exposures.

On the other hand, there are inherent protective mechanisms against over-formation of ROS in toxicant-exposed hosts. For example, the anti-oxidant defense Nrf2/HO-1 pathway plays a role in the strict management of ROS generation; accordingly, the activity of this pathway was assessed here. A recent study found that an increase in cellular ROS production in nigericin-stimulated keratinocytes could inhibit the Nrf2/HO-1 pathway and pretreatment with 1,25-(OH)2-Vitamin D3 could suppress ROS production and re-activate this pathway in vitro (Nakajo et al. 2021). Similarly, another study also confirmed that a reduction activity of Nrf2/HO-1 pathway was associated with pro-inflammatory signaling. From these studies, it was recently suggested that anti-oxidants targeting Nrf2 activation might be potential therapeutics for the treatment of many inflammation-based pathologies, including TCE-mediated autoimmunity (Banerjee et al. 2020).

Inflammation is a major process during the pathology of TCE sensitization-induced liver damage (Wang et al. 2019). A previous study (Zhang et al. 2021) demonstrated that the levels of pro-inflammatory cytokines, such as tumor necrosis factor (TNF)-α, IL-6, IL-1β, etc., were highly elevated in the livers of TCE-sensitized mice. Kupffer cell polarization to the M1 type could be one potential reason for the accumulation of pro-inflammatory cytokines. Another study (Zhang et al. 2013) also found that levels of members of the complement cascade (i.e. anaphyla-toxin C3a and terminal complement complex C5b-9) were elevated in livers of TCE-sensitized mice, and that these factors might also contribute to increases in local inflammatory responses. The study confirmed that TCE sensitization alone led
to increases in hepatic IL-1β expression and that these effects were mitigated, albeit only slightly, by Tempol pretreatment of the hosts.

Recent studies suggested a link between oxidative stress and inflammation, i.e. this stress could also amplify the inflammatory response (Simpson and Oliver 2020). In this type of pathologic loop, ROS can trigger activation of the NLRP3 inflammasome, causing increased secretion of inflammatory IL-1β (Qiu et al. 2019). The present study, by evaluating the larger Nr2/HO-1/ NLRP3 pathway, provided further evidence of the role that ROS play in TCE-induced liver damage. The role of this pathway in the long-term pathology induced by TCE was demonstrated by showing how the Nr2/HO-1/NLRP3 pathway was strictly regulated and the production of downstream pro-inflammatory IL-1β.

Previous studies indicated that activation of CD4 + T-lymphocytes might also be considered critical to the development of THS (Pan et al. 2019). Still other studies suggested that some additional novel mechanisms, such as complement activation, viral re-activation, and Kupffer cell activation, might also be involved in the inflammatory responses during THS liver damage (Watanabe et al. 2010; Zhang et al. 2013, 2021). The present study reports in addition to all those novel mechanisms, increased ROS formation might play a key role in TCE sensitization-induced liver damage. This viewpoint was bolstered by the finding regarding tempol pretreatment and reductions (albeit not complete reversal) of the liver injury in the THS mice.

Although these studies clearly demonstrated a role for ROS generation in TCE-induced liver damage in mice, this mouse model could not completely recapitulate hepatitis in humans. In part, this was due to an inability to perform comparative analyses due to a lack of histopathological studies of human livers during clinical screenings for THS. Further, while the BALB/c mouse model used has been repeatedly used in TCE skin sensitization studies to model human hosts, for now the BALB/c mouse itself might not as-yet be the best system to directly compare/contrast the mechanisms involved in hepatic damage that occurs in human THS patients. Continuing studies in the laboratories here will better define the relevance of this mouse model to the human condition.

The authors are careful to note for the readers that although it was shown that scavenging could largely inhibit ROS generation during TCE sensitization, and thereby play a protective role against hepatic damage, only a single anti-oxidant was tested. At present, Tempol and N-acetyl-l-cysteine (NAC) are widely used as anti-oxidants in vitro and in vivo studies (Hermes et al. 2019). Hu et al. (2021) treated diabetic mice with Tempol and NAC and found that this treatment resulted in similar degrees of reduction in ROS levels and modification of bone endothelial cell injury. Thus, it will be important in future studies here to ascertain whether NAC (and even other anti-oxidants) could serve as useful agents to mitigate ROS formation and thus liver damage in hosts with repeated exposures to TCE.

Disclosure statement

No potential conflict of interest was reported by the author(s). The authors alone are responsible for the content of this manuscript.

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