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Viral mimic polyinosine-polycytidylic acid potentiates liver injury in trichloroethylene-sensitized mice – Viral-chemical interaction as a novel mechanism

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ABSTRACT

Occupational trichloroethylene (TCE) exposure can induce hypersensitivity dermatitis and severe liver injury. Recently, several clinical investigations indicate that viral infection, such as human herpesvirus-6, is associated with hepatic dysfunction in patients with TCE-related generalized skin disorders. However, whether viral infection potentiates TCE-induced liver injury remains unknown. This study aimed to explore the contribution of viral infection to the development of TCE-sensitization-induced liver injury in BALB/c mice. Female BALB/c mice were randomly assigned into four groups: solvent control group (n = 20), TCE group (n = 80), poly(I:C) group (n = 20) and combination of TCE and poly(I:C) (poly(I:C) + TCE) group (n = 80). Poly(I:C) (50 μg) was i.p. administrated. TCE and poly(I:C) + TCE groups were further divided into sensitization and non-sensitization subgroup. Complement 3 and C3a protein levels, and complement factors were measured. Combination treatment significantly enhanced TCE-induced liver injury, decreased complement 3, but increased C3a in serum and liver tissues in sensitization group. These changes were not correlated with the hepatic complement 3 transcription. Moreover, combination treatment specifically promoted complement factor B, but not factor D and factor H expressions. These data provide first evidence that poly(I:C) potentiates liver injury in BALB/c mouse model of TCE-sensitization. Upregulated C3a and factor B contributes to the poly(I:C) action in TCE-induced liver injury. This new mode of action may explain increased risk of chemical-sensitization induced tissue damage by viral infection.

1. Introduction

Trichloroethylene (TCE), a common industrial solvent, is used in large quantity in some developing countries. Increasing evidences from epidemiology and laboratory animal studies indicate that TCE is an immunotoxicant and is capable of disrupting human immune homeostasis, which results in autoimmune disease or even severe hypersensitive skin disorder (Chiu et al., 2013). It is well known that TCE-induced hypersensitivity is clinically characterized by serious generalized hypersensitivity dermatitis, often accompanied by lethal liver dysfunction, occurring among workers after a period of occupational TCE exposure (Kamijima et al., 2007; Xu et al., 2009). The prevalence of this illness is estimated to be as high as 13% and the morality is about 9–13% (Kamijima et al., 2007). For effective prevention and clinical management, this life-threatening syndrome has been classified as occupational disease and is termed as Occupational Dermatitis Medicamentosa-like of Trichloroethylene (ODMLT)(Kamijima et al., 2007).

Apart from the distinct cutaneous symptoms, epidemiological investigations show that the symptoms of infection such as fever and headache are frequently present in ODMLT patients at early stage (Xu et al., 2009). These clinical manifestations suggest that ODMLT may begin with infection. Interestingly, several studies have proposed that herpesvirus and cytomegalovirus are reactivated in a considerable proportion of the ODMLT cases with liver damage. Furthermore, the antibody titer against virus was significantly higher in the patients (Kamijima et al., 2013; Watanabe et al., 2010). Pathogenic invaders can

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act as danger signals by engaging pattern recognition receptors (PRRs) and activating innate immune system, which results in the expression of pro-inflammatory cytokines (Bowie and Unterholzner, 2008). There is also evidence that adapted immune response can be activated and amplified by priming T cells and inducing antigen specific responses (Bowie and Unterholzner, 2008), by which could play a crucial role in the pathology of ODMLT. Therefore it is possible that the original infection caused by virus may be involved in the initiation of the disease.

Many studies suggest that complement system plays an important role in ODMLT (Huang et al., 2014; Yue et al., 2007; Zhao et al., 2012). The complement cascade can be activated by classical, alternative and lectin pathways and all these routes lead to the cleavage of the central molecule, complement component 3 (C3), to C3a and C3b (Kolev et al., 2014). The C3 is mainly synthesized by liver, although it has been suggested that almost all cells in the body have the capacity to generate complement proteins in human (Kolev et al., 2014). It is known that the level of C3 dramatically decreases in serum during the early outset of disease and recovers to normal level after a period of hospitalization among ODMLT patients (Huang et al., 2014). Moreover, the decrease of C3 was significantly associated with the extent of liver injury induced by TCE (Huang et al., 2014). Considering the biochemical process of C3 activation, these C3 changes may be attributed to the balance between C3 cleavage and production. It is reported that increased breakdown of C3 can be detected in damaged liver tissue from TCE-sensitized mice with higher depositions of downstream complement fragments and the ultimate membrane attack complex (Wang et al., 2014; Zhang et al., 2013). In addition, elevated level of C3a-desArg in circulation is also observed with liver impairment in TCE-sensitized mice (Zhang et al., 2013). Furthermore, there is no clear evidence to suggest that hepatic C3 generation is suppressed among ODMLT cases. Therefore, we propose that excessive complement activation occurring during the progress of ODMLT, which may consume circulated C3 protein and cause declined serum C3 level, contributes to concurrent liver injury.

In our previous studies, we have established a TCE sensitization model using BALB/c mouse, which will be used to explore the mechanism of liver injury induced by TCE (Wang et al., 2014; Wang et al., 2015). This TCE sensitization-induced liver injury only occurs in TCE sensitization-positive group and is characterized by hepatocyte swelling, deposition of cytokines, activation of complement system, mild increments of ALT and AST, and recovery when TCE exposure is ceased (Wang et al., 2014; Zhang et al., 2013). This pathological feature is different from classic liver injury commonly induced by hepatotoxicants *via* direct chemical toxicity. In the present study, we hypothesize that the initial viral infection potentiates liver injury in TCE-sensitized BALB/c mice. Polyinosine-polycytidylic acid (polyI:C) is structurally similar to double-stranded RNA generated by virus and is widely used as a virus mimic in experimental studies (Cheng et al., 2009). Therefore, we employed polyI:C as a virus mimic to investigate whether it could exacerbate liver injury and facilitate C3 activation in TCE-sensitized BALB/c mice.

2. Material and methods

2.1. Treatments of animals

Female BALB/c mice (6–8 weeks) were purchased from the Experimental Animal Center of Anhui Province (Anhui, China) and maintained with a 12 h light/dark cycle in a controlled temperature (20–25 °C) and humidity (50 ± 5%) environment with free access to food and water. Animals were given one week adaptation before the treatment. The dorsal hair of the mice was shaved with an area about 4 cm^2 and remained bald throughout the experiment by shaving regularly. All experiments were performed in accordance with guidelines from Animal Care and Use Committee of Anhui Medical University.

Workers are commonly exposed to TCE through direct skin contact. This route of exposure was mimicked by cutaneous application of TCE on mice. In TCE treatment group, chemical sensitization was induced according to our previous protocol (Wang et al., 2014; Wang et al., 2015; Zhang et al., 2013). Briefly, 80 mice were randomly assigned into four subgroups (n = 20 for each subgroup) of different time points (day 20, 21, 22 and 26), and received the same treatment protocol as follows. On the first day, mice were intradermally injected with 100 µl mixture of 50% TCE and Freund complete adjuvant (Sigma Aldrich, St. Louis, MO, USA) with the volume ratio of 1:1 within the area of the naked back skin. The 50% TCE was prepared by mixing TCE, acetone and olive oil (all from Sigma Aldrich, St. Louis, MO, USA) with a volume ratio of 5:3:2. On day 4, 7 and 10, 100 µl 50% TCE was painted on the same area and the area was then covered with a filter paper, secured by a non-irritating tape. On day 17 and 19, 30% TCE (TCE: acetone: olive oil = 3:5:2) was applied as the first and second elicitation, respectively.

Viral infection is closely associated with TCE-induced hypersensitivity syndrome (Kamijima et al., 2013; Watanabe et al., 2010; Xu et al., 2009), and this infection was mimicked by poly(I:C) injection, which is widely used as a simulation of viral infection in animal model (Cheng et al., 2009). In poly(I:C) and TCE cotreatment group, another 80 mice were randomly assigned into four subgroups of different time points (n = 20 for each subgroup) followed by the same treatment. 50 µg polyI:C (Invivogen, San Diego, California, USA) was dissolved in 0.2 ml sterile saline and then an intraperitoneal injection was given three hours before the second challenge (Fig. 1). In order to ensure comparability, mice in TCE-treated group were also administrated with the same sterile saline as in other groups before the second challenge. In polyI:C control group, polyI:C and all the above solvents and saline were used. Solvent control group received only solvent and saline injection.

On day 20, 21, 22 and 26, animals were euthanized according to the subgroup assigned. The cutaneous response was scored before euthanasia according to a four-point scale: 0 (no reaction), 1 (scattered mild redness), 2 (moderate and diffuse redness) and 3 (intensive erythema and swelling). If the score number was no less than one, the corresponding mouse was categorized as sensitized. Otherwise, it would

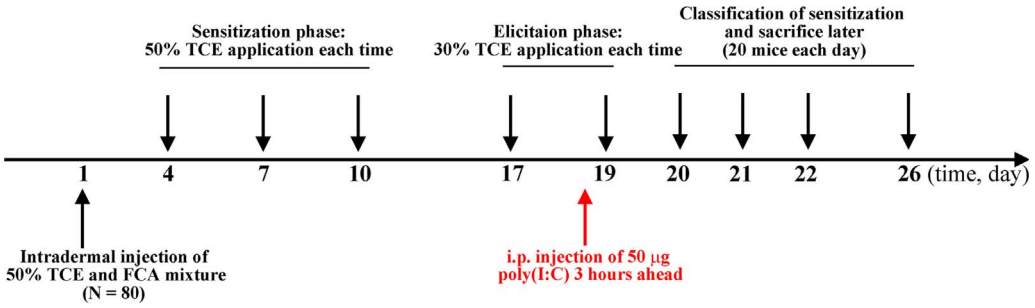

Fig. 1. Flow diagram of the treatment procedure. Initially, the hair on the back of female BALB/c mice was removed by shaving and then treated with intradermal injection of the mixture that consisted of 50 µl 50% TCE (TCE: acetone: olive oil = 5: 3: 2) and 50 µl Freund complete adjuvant on day one. Then mice were sensitized by applying 100 µl 50% TCE on the naked skin on day 4, 7 and 10. Subsequently the challenge phase was carried out by applying 100 µl 30% TCE (TCE: acetone: olive oil = 3: 5: 2) on the naked skin on day 17 and 19. During this stage, 50 µg poly(I:C) was i.p. injected 3 h prior to the second administration at day 19. Finally, mice were classified into sensitized and non-sensitized ones according to the score of cutaneous response, followed by euthanasia and sacrifice on day 20, 21, 22 and 26.