

Role of blood transfusion in trichosanthin-induced anaphylaxis

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Received 6 April 2004; accepted 21 October 2004

Available online 9 December 2004

Abstract

Trichosanthin (TCS) is a type 1 ribosome inactivating protein extracted from Chinese medicinal herb. It possesses various biological functions such as abortifacient, anti-tumor and anti-viral activities. Clinical trial of this compound against human immunodeficiency virus (HIV) had been conducted. However, its use is limited by its high immunogenicity that elicits hypersensitivity reaction. This may lead to fatal anaphylactic response. The study described an approach of using blood transfusion to reduce TCS induced anaphylaxis in rats using a cross-circulation model. A TCS-sensitized Sprague Dawley rat was connected to a normal rat via the femoral vessels in a cross-circulation circuit before antigenic challenge. The donor rat served as a blood exchange basin to lower the level of the blood-borne components responsible for the anaphylactic reaction in the sensitized rat. Our results showed that cross-circulation shortened the duration of circulatory hypotension and reduced mortality of TCS induced anaphylaxis. The control group not undergoing cross-circulation had a mortality of 50% at 2 h post-TCS challenge and there was no mortality in the cross-circulation group. This demonstrated that prior blood transfusion can be one of the alternatives to reduce anaphylactic response of TCS.

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Keywords: Trichosanthin; Anaphylaxis; Immunogenicity; Blood transfusion; Ribosome inactivating protein

1. Introduction

Trichosanthin (TCS) is a type 1 ribosome-inactivating protein with a molecular weight of 27,000 Da. It is a single polypeptide chain extracted from the root tuber of the Chinese herbs *Trichosanthes kirilowii*. It possesses various biological functions and can be developed into a therapeutic agent. The potential areas can be in abortion, anti-tumor, protein synthesis inhibition, immunomodulation and antiviral (Yeung et al., 1987; Zheng et al., 2001; Wang et al., 2002, 2003). Phase II clinical use of TCS in the treatment of HIV had been conducted and one of the major adverse effects is antigenicity leading to anaphylactic reactions (Byers et al., 1994; Kahn et al., 1994). To make TCS a safer therapeutic agent, various methods including coupling to dextran (Chan et al., 1999; Ko et al., 1993), site-directed

polyethylene glycol modification (He et al., 1999; Wang et al., 2004) and mini-trichosanthin (Chan et al., 2000) had been constructed to reduce antigenicity.

TCS is able to elicit a primary antibody response with the production of TCS-specific antibodies. Subsequent re-exposure of TCS results in an exaggerated and fatal immune response (He et al., 1999). Most of the effector components produced during the sensitization phase of primary immune response are circulating factors and their blood levels are crucial for the severity of subsequent immune response. In our rat model, a TCS-sensitized rat is connected to a normal rat via the femoral vessels prior to receiving TCS challenge. The donor rat serves as a blood exchange basin in order to lower or remove the circulating factors in the sensitized rat.

The objective of this study is to determine whether prior blood transfusion is a potential therapeutics modality to reduce the anaphylactic response upon re-exposure of TCS.

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2. Materials and methods

2.1. Sensitization protocol

Animal studies are conducted in accordance with the guideline on the use of laboratory animals established by the animal ethics committee of the Chinese University of Hong Kong. Sprague-Dawley rats (female, 200–250 g) are purchased from the Laboratory Animal Service Center, the Chinese University of Hong Kong. All rats are caged in pathogen free environment. For sensitization, rats received intra-peritoneal injection of 100 µg/kg of TCS on days 1, 3 and 5. Trichosanthin was obtained from Shanghai Jinshan Pharmaceutical Co. Ltd., Shanghai, China.

2.2. Systemic anaphylaxis

On day 10, all rats will undergo pentobarbitone (5%) anesthesia according to body weight and were randomized into cross-circulation ($n = 8$) and non-cross-circulation group ($n = 8$).

2.2.1. Cross-circulation group

A cross-circulation circuit was created between a TCS-sensitized rat (recipient) and a normal donor rat with comparable weight, by connecting their femoral vessels using polyethylene tubing ($\varphi = 0.96$ mm). An extra left carotid artery cannulation of the recipient rat was created for blood pressure monitoring using Powerlab Chart 4.2 software on PC. Rats were given 60 U heparin for anticoagulation. The duration of cross-circulation lasted 60 min and the circuit was then disconnected. After a steady blood pressure maintained, each rat received 200 µg/kg TCS via femoral vein and the mean arterial blood pressure profile was recorded.

2.2.2. Non-cross-circulation group

All rats received 200 µg/kg TCS via femoral vein without cross-circulation. Blood pressure profile was recorded.

2.2.3. Sham group

Sham rats (normal saline sensitization) underwent the same protocol as control. Mortality was monitored for 2 h after TCS challenge for all groups. Wounds were closed with silk and all rats will put in cage with neck extension under light warming for recovery.

2.3. Semi-quantification of immunoglobulin G

Rat sera were collected on days 10, 20, 30 and 60 after sensitization using the same TCS sensitization protocol. Blood samples were collected and incubated at 37 °C for 60 min, centrifuge at $10,000 \times g$ for 5 min. The sera collected were stored at –20 °C for later analysis.

2.4. Enzyme-linked immunosorbent assay (ELISA) for TCS-specific IgG

ELISA plates (96-well Immuno-plate, Maxisorp, Nunc) were coated with 100 µl of TCS (5 µg/ml) in 0.05 M sodium carbonate–bicarbonate buffer (pH 9.6) overnight at 4 °C. Plates were rinsed with washing buffer (PBS containing 0.05% Tween 20) three times and followed by incubation for 1 h at 37 °C with 200 µl blocking buffer (PBS containing 0.05% Tween 20 and 1% BSA). Rat sera sample 100 µl/well were added in duplicate and incubated at 37 °C for 1 h. Plates were washed three times using automatic plate washer (µ-Quant, USA). Polygonal rabbit anti-rat IgG-HRP 100 µl/well (1:2000; DAKO, Denmark) was added to each well. After incubation for 1 h at 37 °C, 100 µl freshly prepared substrate solution (*o*-phenylenediamine dihydrochloride, 1 mg/ml) was added to each well and incubated for 30 min at 37 °C. Fifty microliters of 2.5 M H₂SO₄ was added to each well to stop the reaction. Optical density was measured using an automatic ELISA plate reader (µ-Quant, USA) at 490 nm.

2.5. Statistics

Two-way ANOVA followed by Scheffe test was used for our statistical analysis. A *P*-value of <0.05 is considered statistically significant. All data are expressed as means $\pm$ S.E.M.

3. Results

3.1. Immunogenicity of TCS

TCS-specific IgG produced after sensitization process was measured by ELISA and the serum IgG level was shown (Fig. 1). There was a gradual increase of IgG from day 10 onward and peaked around day 30 after sensitization. Afterward, there was a decline of antibody level as shown on day 60. This demonstrated TCS that was able to elicit a primary antibody response without adjuvant.

3.2. Kinetics of hypotensive response

The hypotensive response after TCS challenge was shown in Fig. 2. A steady blood pressure was maintained before any TCS challenge. There was no difference in the starting control blood pressure between the cross-circulation and the non-cross-circulation group ($P > 0.05$). There was an initial rapid drop of mean arterial blood pressure to 50 ± 2.3 and 47 ± 3.4 mmHg in the cross-circulation and non-cross-circulation group, respectively ($P > 0.05$) 2 min after TCS challenge. After 50 min, the mean blood pressure of the cross-circulation group was 104 ± 4.1 and 78 ± 6.8 mmHg for the non-cross-circulation group ($P < 0.05$). The dropped blood pressure of the cross-circulation group was able to return normal within 1 h, whereas the other group did not. This sug-

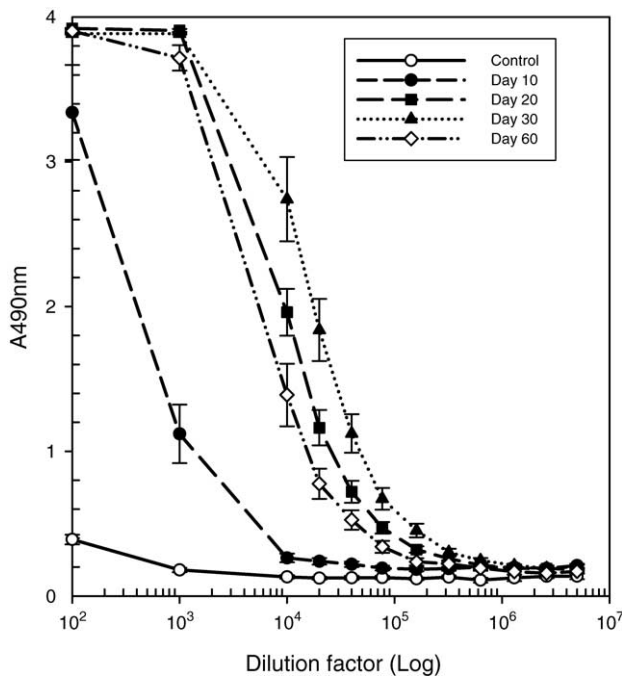


Fig. 1. Dilution curves of rat sera TCS-specific IgG on days 10, 20, 30 and 60. TCS 100 $\mu\text{g}/\text{kg}$ was injected intraperitoneally on days 1, 3, and 5 to rats. Data are expressed as means $\pm$ S.E.M. ($n = 8$).

gested that anaphylactic response might have an initial rapid phase followed by a persistent phase and the blood level of circulating factors that were being induced or sensitized, were contributing the observed difference. There was no hypoten-

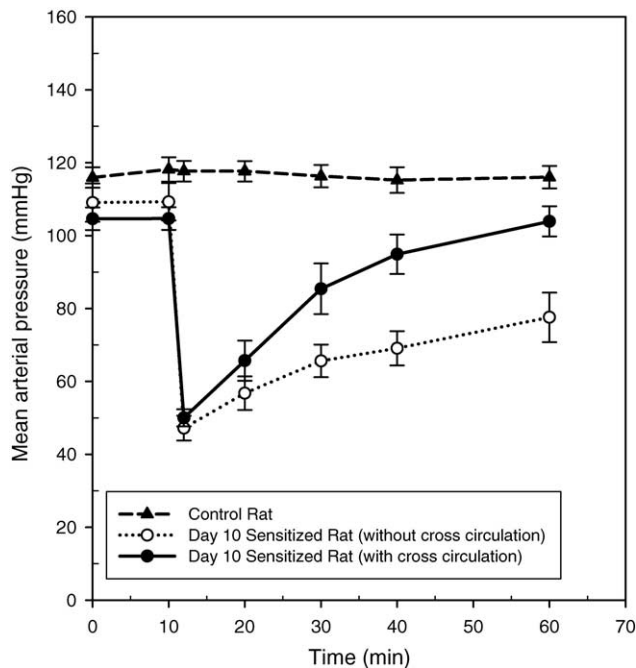


Fig. 2. Effect of TCS challenge on mean arterial blood pressure in: ($\blacktriangle$) sham rat; ($\circ$) sensitized rat without cross-circulation; ($\bullet$) sensitized rat with cross-circulation. Data are expressed as means $\pm$ S.E.M. ($n = 8$).

sive effect in the sham group (no previous TCS sensitization) upon TCS challenge.

3.3. Mortality

There was no mortality in the sham and cross-circulation group till 48 h. In the non-cross-circulation group, four rats died within 2 h post-TCS challenge. One more rat died at 24 h (Fig. 3) and the remaining rats recovered and survived at 48 h.

4. Discussion

Clinical trials had been carried out to treat AIDS patient by using TCS (Byers et al., 1994) and the high immunogenicity was one of the drawbacks. In many occasions, the protocol for treatment of severe viral infection requires multiple consecutive use of drug and the side effects are encountered as a result of aggressive treatment. Anaphylaxis varies from mild response to fatal anaphylactic shock, and this is the outcome of re-exposure of an antigen to previously sensitized animals. At the turn of the last century, Richet and Portier already noticed that repeated injection of previously well-tolerated dose of a foreign protein manifested as untoward effects including systemic shock and fatal outcome (Cohen and Zelaya-Quesada, 2002). In our study, we used systemic blood pressure profile as an indicator of systemic shock.

The concentrations of sensitized effector components for anaphylaxis are crucial (Galli, 1987; Galli and Kitamura, 1987; Kim and Lee, 1999a). It was known that TCS is a strong allergen (Cai et al., 2002) and we demonstrated that

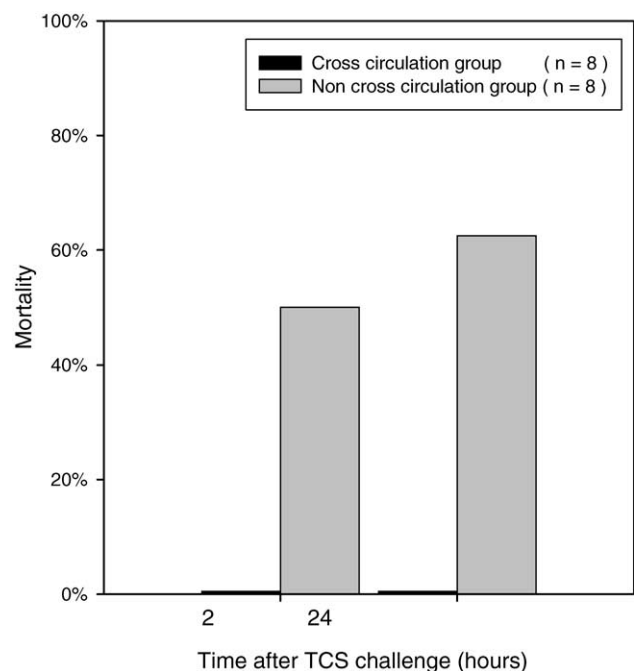


Fig. 3. Mortality of rat in cross-circulation and non-cross-circulation group at 2 and 24 h post-TCS challenge.

TCS without adding adjuvant was able to stimulate the production of TCS-specific antibodies.

There are many ways to prevent anaphylaxis in animals. This can be achieved by using drugs. For example, sodium salicylate and succinic acid are showed to inhibit compound 48/80 induce anaphylaxis in mice (Kim et al., 1999b, 1999c). In rat models, corticosterone was able to inhibit dextran-induced shock in rats (Kogure et al., 1986) and chlorpheniramine and cimetidine were able to inhibit the hypotensive response induced by antigen-mediated anaphylaxis (Guo et al., 2001). In addition, the allergen can be modified to reduce antigenicity. For example, the antigenicity of trichosanthin can be significantly reduced by masking its antigenic site by dextran. This study explored yet another alternative of reducing antigenicity in animals. This is by way of blood replacement so as to reduce the antibody titer or any blood borne factor contributing to anaphylaxis.

In our study, both the cross-circulation group and the non-cross-circulation group showed a rapid initial drop in blood pressure after TCS challenge. However, the recovery kinetics was different in both groups. Blood pressure returned to normal after 50 min in the cross-circulation group; whereas the non-cross-circulation group stayed in prolonged period of hypotension. This may be due to the difference in circulating level of effector components responsible for anaphylactic reaction. Moreover, the longer the duration stayed in hypotension, irreversible organ damage may occur as a result of prolonged hypoperfusion and hence, mortality.

In summary, results from this study demonstrated that antigenicity can be reduced when a sensitized rat is cross-circulating with a normal one. This implied that prior blood transfusion can minimize anaphylactic response upon multiple administered of trichosanthin.

Acknowledgements

The authors wish to thank Mr. Lee Wing Kei Lee, Miss Lam Ming Wai and Mr. Huang Hai for their kind technical assistance.

References

- Byers, V.S., Levin, A.S., Malvino, A., Waites, L., Robins, R.A., Baldwin, R.W., 1994. A phase II study of effect of addition of trichosanthin to zidovudine in patients with HIV disease and failing antiretroviral agents. *AIDS Res. Hum. Retroviruses* 10, 413–420.
- Cai, X., Yao, G., Xu, G., Yang, C., Xu, H., Lin, Y., Yu, J., Sun, B., 2002. Identification of the amino acid residues in trichosanthin crucial for IgE response. *Biochem. Biophys. Res. Commun.* 297, 510–516.
- Chan, S.H., Shaw, P.C., Mulot, S.F., Xu, L.H., Chan, W.L., Tam, S.C., Wong, K.B., 2000. Engineering of a mini-trichosanthin that has lower antigenicity by deleting its C-terminal amino acid residues. *Biochem. Biophys. Res. Commun.* 270, 279–285.
- Chan, W.L., Shaw, P.C., Li, X.B., Xu, Q.F., He, X.H., Tam, S.C., 1999. Lowering of trichosanthin immunogenicity by site-specific coupling to dextran. *Biochem. Pharmacol.* 57, 927–934.
- Cohen, S.G., Zelaya-Quesada, M., 2002. Portier, Richet, and the discovery of anaphylaxis: a centennial. *J. Allergy Clin. Immunol.* 110, 331–336.
- Galli, S.J., 1987. New approaches for the analysis of mast cell maturation, heterogeneity, and function. *Fed. Proc.* 46, 1906–1914.
- Galli, S.J., Kitamura, Y., 1987. Genetically mast-cell-deficient W/W^v and Sl/Sl^d mice. Their value for the analysis of the roles of mast cells in biologic responses in vivo. *Am. J. Pathol.* 127, 191–198.
- Guo, Y., Hedqvist, P., Gustafsson, L.E., 2001. Absence of mast cell involvement in active systemic anaphylaxis in rats. *Eur. J. Pharmacol.* 430, 305–310.
- He, X.H., Shaw, P.C., Tam, S.C., 1999. Reducing the immunogenicity and improving the in vivo activity of trichosanthin by site-directed pegylation. *Life Sci.* 65, 355–368.
- Kahn, J.O., Gorelick, K.J., Gatti, G., Arri, C.J., Lifson, J.D., Gamber-toglio, J.G., Bostrom, A., Williams, R., 1994. Safety, activity, and pharmacokinetics of GLQ223 in patients with AIDS and AIDS-related complex. *Antimicrob. Agents Chemother.* 38, 260–267.
- Kim, H.M., Lee, Y.M., 1999a. Role of TGF- β 1 on the IgE-dependent anaphylaxis reaction. *J. Immunol.* 162, 4960–4965.
- Kim, H.M., Shin, H.Y., Choo, Y.K., Park, J.K., 1999b. Inhibition of mast cell-dependent anaphylaxis by sodium salicylate. *Immunology* 96, 551–556.
- Kim, H.M., Jung, H.S., Shin, H.Y., Jung, K.Y., 1999c. Inhibition of mast cell-dependent anaphylaxis by succinic acid. *Pharmacol. Toxicol.* 84, 154–158.
- Ko, W.H., Yeung, H.W., Tam, S.C., 1993. The biological activities of trichosanthin after coupling to dextran. *Gen. Pharmacol.* 24, 757–762.
- Kogure, K., Ishizaki, M., Nemoto, M., Nakamura, T., Suzuki, M., 1986. Antishock effects of corticosterone on dextran-induced shock in rats. *Am. J. Physiol.* 251, E569–E575.
- Wang, J.H., Nie, H.L., Tam, S.C., Huang, H., Zheng, Y.T., 2002. Anti-HIV-1 property of trichosanthin correlates with its ribosome inactivating activity. *FEBS Lett.* 531, 295–298.
- Wang, J.H., Nie, H.L., Huang, H., Tam, S.C., Zheng, Y.T., 2003. Independence of anti-HIV-1 activity from ribosome-inactivating activity of trichosanthin. *Biochem. Biophys. Res. Commun.* 302 (1), 89–94.
- Wang, J.H., Tam, S.C., Huang, H., Ouyang, D.Y., Wang, Y.Y., Zheng, Y.T., 2004. Site-directed PEGylation of trichosanthin retained its anti-HIV activity with reduced potency in vitro. *Biochem. Biophys. Res. Commun.* 317 (4), 965–971.
- Yeung, H.W., Poon, S.P., Ng, T.B., Li, W.W., 1987. Isolation and characterization of an immunosuppressive protein from *Trichosanthes kirilowii* root tubers. *Immunopharmacol. Immunotoxicol.* 9, 25–46.
- Zheng, Y.T., Chan, W.L., Chan, P., Huang, H., Tam, S.C., 2001. Enhancement of the anti-herpetic effect of trichosanthin by acyclovir and interferon. *FEBS Lett.* 496 (2–3), 139–142.