Guinea Pig Maximization Test for Trichloroethylene and Its Metabolites[1]

TANG XIAO-JIANG[#,+], LI LAI-YU[#], HUANG JIAN-XUN[#], AND DENG YING-YU[#]

[#] *Department of Toxicology, Guangdong Provincial Center for Occupational Disease Prevention and Treatment, Guangzhou 510300, China;* [+] *School of Life Science, Zhongshan University, Guangzhou 510275, China*

Objectives To study the contact allergenic activities of trichloroethylene (TCE) and its three metabolites trichloroacetic acid, trichloroethanol and chloral hydrate. **Methods** A modified guinea pig maximization test (GPMT) was adopted. The skin sensitization (edema and erythema) was observed in trichloroethylene, trichloroacetic acid, trichloroethanol, chloral hydrate and 2,4-dinitrochlorobenzene. **Results** The allergenic rate of TCE, trichloroacetic acid and 2,4-dinitrochlorobenzene was 71.4%, 58.3% and 100.0% respectively, and that of trichloroethanol and chloral hydrate was 0%. The mean response score of TCE, trichloroacetic acid and 2,4-dinitrochlorobenzene was 2.3, 1.1, 6.0 respectively. The histopathological analysis also showed an induction of allergenic transfomation in guinea pig skin by both TCE and trichloroacetic acid. **Conclusion** TCE appears to be a strong allergen while trichloroacetic acid a moderate one. On the other hand, both trichloroethanol and chloral hydrate are weak sensitization potentials. Immunologic reaction induced by TCE might be postulated as the pathological process of this illness. Consequently, it is suggested that in the mechanism of Occupational Dermatitis Medicamentose-Like (ODML) induced by TCE, the chemical itself might be the main cause of allergy. As one of its metabolic products, trichloroacetic acid might be a subordinate factor.

Key words: Trichloroethylene; Trichloroacetic acid; Trichloroethanol; Chloral hydrate; Guinea pig maximization test (GPMT)

INTRODUCTION

Trichloroethylene (TCE) has been used in industry as a degreasing agent, solvent and extraction agent for about a century. As early as in 1915, cases of TCE intoxication were reported. The damaged organs of TCE included central nerve system, peripheral nerve system, liver，kidney, heart and skin. Skin lesions of mucous membrane irritation, degrease and dermatitis were described in the past[1,2], but serious dermatitis such as Stevens-Johnson syndrome seemed to be very rare. After the first description[3], only 18 cases had been reported before 1987[4-7], but from 1988 to 1999, 68 cases of serious skin damage were found in Guangdong Province, China with 17 of them died[8-10]. In the previous study, the

[1] This work was an important item supported by Guangdong Provincial Committee of Science and Technology, China. (GCST 9622056-05)

Biographical note of the first author: TANG Xiao-Jiang(1967-), male, Ph. D. candidate of Zhongshan University, main research field is toxicology and pharmacology.

0895-3988/2002
CN 11-2816

authors had analyzed the distribution of occupational dermatitis caused by TCE in Guangdong in term of Occupational Dermatitis Medicamentose-Like (ODML) [11]. In this study, the authors found that ODML should be related to delayed hypersensitivity. To understand the mechanism of this serious dematitis induced by TCE, the contact allergenic activities of TCE and its three main metabolic products, trichloroacetic acid, trichloroethanol and chloral hydrate were studied by guinea pig maximization test (GPMT).

METHODS

Animals

Albino guinea pigs (FMMU strain) weighing 300-350 g were provided by the Medical Laboratory Animal Center of the First Military Medical University, China. They were housed in an animal room at temperature 23℃±1.5℃ and relative humidity 55%±10% with a 12 h day/night cycle. The animals were fed with a standard guinea pig diet, fresh vegetable and tap water. After one week observation they were randomly divided into 7 groups.

Test Materials

Trichloroethylene (AR), trichloroacetic acid (AR), trichloroethanol (AR), chloral hydrate (AR) and acetone (AR) were all purchased from Acros Organics (New Jersey, USA). 1,2-dinitrochlorobenzene (AR) was obtained from Tokyo Kasei Co., Ltd. (Tokyo , Japan). Sodium lauryl sulfate (SLS) (AR) was purchased from Sigma (Saint Louis Mo, USA). Freund's Complete Adjuvant (FCA) was provided by Disfco Laboratories (Detroit, Michigen, USA). All of the other test materials were not irritant and only had weak allergenicity in human being.

Tichloroethylene, trichloroacetic acid, trichloroethanol, chloral hydrate and 2,4-dinitrochlorobenzene, were used as test agents for induction. FCA and olive oil were used as control agents. The number of guinea pigs for each group was 10-14. In each series, the animals were shamly-treated controls to obtain a blind reading of challenge reactions. Filter paper impregnated with the test material and mounted on Leucoflex (Beiersdorff AG) was used for the topical induction patch test.

Primary Irritation Studies

Before the starting of challenge phases, primary irritant studies had been performed by 3-4 fresh animals. An area of 4cm×6 cm on the back of animals were clipped free of hair. 24 h later, the intact animals were selected in the test. According to the previous descriptions[12,13], the moderate irritant concentration for intrademal and topical induction, and the non-irritant concentration for challenge were established in guinea pigs. The tested agents included TCE, trichloroacetic acid, trichloroethanol, chloral hydrate, 2,4-dinitrochlorobenzene, FCA and olive oil. Trichloroacetic acid, chloral hydrate and FCA were dissolved in distilled water and 2,4-dinitrochlorobenzene was dissolved in acetone first, then mixed with olive oil. Both TCE and trichloroethanol were dissolved in olive oil respectively. If the test agent was irritant, its concentation was reduced to a potential just causing mild to moderate inflammation. If it was not irritant, the area was pretreated with *w* (SLS)=10 % in petrolatum 24 h before the topical induction exposure. The concentrations of test agents used for induction were adjusted to the highest ones able to be well tolerated systematically.

Guniea Pig Maximixation Test (GPMT)

GPMT was performed as described by Magnusson & Kligman (1969) with some modifications. For induction, dorsal skin in the scapular region was shaved. 24 h later, 3 pairs of 0.1 mL intradermal injections were performed: (i) an emulsified mixture of FCA; (ii) a suspension of test agent in olive oil or in distilled water; (iii) a suspension of test agent in FCA. 7 days after injection, the interscapular region was again shaved, and treated with *w* (SLS)=10 % in the groups into which olive oil and FCA (non-irritant) were injected. 8 days later, 0.2 mL of test agent preparation was occlusively patched on the same region for 48 h. Control animals were supplied with vehicles only.

21 days after the initial intradermal injection, all animals were challenged by topical application of test agent. 0.1 mL test agent in vehicles was applied to the shaved areas of the guinea pigs by closed patch test method, and left for 24 h. Patch test responses were read 24 h and 48 h after removing the patches. Allergenic reactions observed in animal groups were graded as follows: 0, no reaction; 1, scattered mild redness; 2, moderate and diffuse redness; 3, intensive erythema and swelling.

The important statistics in these tests, however, was not the intensity but the frequency of sensitization. Based upon the percentage of animals sensitized, the grading of allergenicity was calculated. The allergenic potency of the tested agents was classified according to Magnusson and Kligman[12].

Skin of the test site and the controls was taken by punch biopsies, then fixed in 10% neutral phosphate-buffered formalin solution *w* (HCHO)=10 %, embedded in glycol methacrylate and polyethlene glycol, cut into 3 μm sections and stained with May-Grunwald-Giemsa. Light microscopic assessment was performed with a 1 000× oil immersion lens.

RESULTS

The test concentrations were selected by their irritant potency. Except olive oil and FCA, the other agents just induced moderate erythma at two induction concentrations, but the challenge topical concentration did not induce any abnormal appearance. The maximal toler-

TABLE 1

Maximal Tolerated Concentrations *w* (agent) (%) for Intradermal, Topical Induction and Challenge

Agent	Number of Animals(*n*)	Induction		Challenge
		Intradermal Concentration	Topical Concentration	Topical Concentration
TCE	4	10.0	20.0	10.0
Trichloroacetic Acid	4	0.5	5.0	2.0
Trichloroethanol	4	2.5	20.0	10.0
Chloral Hydrate	4	1.0	5.0	2.0
1,2-dinitrochlorobenzene	4	0.5	3.0	1.5
Olive Oil	3	100.0	100.0	100.0
FCA	3	100.0	100.0	100.0

able concentrations [e.g. *w* (TCE)=5 % in olive oil or the former %, weight/weight) for intradermal , topical induction and challenge were listed in Table 1.

The sensitization rates and scores of GPMT with TCE and its metabolites were shown in Table 2. The mean response score was caculated by the following formula: Mean score = (Score of redness + Score of swelling)/ Number of animals in the group.

TABLE 2

Sensitization Rates and Scores of GPMT With TCE and Its Metabolites

Agent	Number of Animals(*n*)	Number of Positive(*n*)[a]	Sensitization Rate(%)	Classification	Score of Redness[b]	Score of Swelling[b]	Mean Score
TCE	14	10	71.4	Strong	22	10	2.3
Trichloroacetic Acid	12	7	58.3	Moderate	13	0	1.1
Trichloroethanol	11	0	0.0	Weak	0	0	0.0
Chloral Hydrate	14	0	0.0	Weak	0	0	0.0
1,2-dinitrochloro Benzene	12	12	100.0	Extreme	36	36	6.0
Olive Oil	10	0	0.0	Weak	0	0	0.0
FCA	10	0	0.0	Weak	0	0	0.0

[a] The positive animals were the same at 24 h and 48 h after removing the patches.
[b] The scores were read at 24 h after removing the patches.

Histopathologic findings: In the positive group (2,4-dinitrochlorobenzene), the challenge affected skin showed inflammatory cell infiltration in the cornification, corium and granular cell layers. Granular cells were slightly swollen. No abnormality was found in basal cells. The capillary vessle expanding and edema with the mononuclear infiltration were found in corium. In the groups of TCE and trichloroacetic acid, the stickle cell layer became thicker evidently, and the stickle cells swelled. The hypersensitive reactions were obvious in both groups. On the other hand, no abnormality was found in the control groups.

DISCUSSION

The GPMT method seemed to be an excellent system for evaluating skin sensitization in guinea pigs. It has been widely used for evaluation of chemical- or cosmetics-induced allergic contact dermatitis involving delayed type hypersensitivity[14-16]. In some countries such as China, it has become a national standard test method[17].

So far as the authors know, there is no GPMT which was done with TCE and its metabolic products. According to the authors previous study[11], TCE could induce serious ODML. By the end of 1998, 17 articles had been published. Although 63 cases of ODML were reported, only 4 were tested by patch-test with TCE and its metabolites. In 1983, Conde-Salazar *et al.* reported a 25-year-old woman who had worked in a Spain factory with TCE contact developed subcorneal pustular eruption and erythema[5]. Although routine laboratory tests were negative, yet a red, scaly reaction was found in standard patch tests with *w* (TCE)=5 % (in olive oil), while 20 controls were negative. In 1984 in Singapore, Phoon *et al.* reported 5 cases of Stevens-Johnson syndrome who had been occupationally

exposed to TCE[6]. A patch test later with *w* (TCE)=5 % (in olive oil) was performed for one of them, but the result was negative. In 1988, Nakayama *et al*. bulletined a printer who developed exfoliative dermatitis after occupational exposure to TCE[7]. Positive patch test reactions to *w* (TCE)=25 % and 10 % (in olive oil), and to one of its metabolites, *w* (trichloroethanol)=5 %, 0.05% and 0.005% (in water) were observed. But in cases of lower concentration of *w* (TCE)=5 % (in olive oil) and *w* (trichloroacetic acid)=5 % (in water), the patch tests were nagative. In 1997 in Thailand, Chittasobhaktra *et al*. described a female patient with generalized dermatitis secondary to TCE exposure[18]. The diagnosis was confirmed by positive skin patch testing with *w* (TCE)=50% solution.

In order to explore the mechanism of ODML induced by TCE, GPMT was used to study the contact allergenic activity of TCE and its three main metabolic products trichloroacetic acid, trichloroethanol, chloral hydrate. As shown in the results, TCE appeared to be a strong sensitization potential and its sensitizaion rate was 71.4%. The sensitizaion rate of trichloroacetic acid was 58.3%. The histopathological analysis also showed both chemicals induced allergenic transfomation in guinea pig skin. On the other hand, trichloroethanol and chloral hydrate showed themselves a weak sensitization potential, and their sensitizaion rates were 0. Comparing the scores of TCE and trichloroacetic acid, TCE induced swelling (10 scores) but trichloroacetic acid did not.

Therefore, it is suggested that in the mechanism of ODML induced by TCE, the chemical itself is a main cause of allergy. As one of its metabolic products, trichloroacetic acid may be a subordinate factor. TCE-induced immunologic reaction may be postulated as the pathological process of ODML.

AKNOWLEDGEMENT

The authors thank professor Zhou Jiong-Liang for his help with modification of the manuscript.

REFERENCES

1. Smith, G.F. (1966). Trichloroethylene: A Review. *Int. J. Industry Med.* **23,** 249-262.
2. McCunney, R.J. (1998). Diverse manifestations of trichloroethylene. *Br. J. Ind. Med.***45**, 122-126.
3. Schwartz, L., Tulipan, L., and Birmingham, D. (1947). *Occupational Diseases of the Skin* (3rd ed). Lea & Febiger, Philadelphia.
4. Bauer, M., and Rabens, S.F. (1974). Gutaneous manifestation of trichloroethylene toxicity. *Arch Dermatol.* **110**, 886-890.
5. Conde, S.L., Guimaraens, D., Romeron, L.V., and Sanchez, Y.E. (1983). Subcorneal pustular eruption and erythema from occupational exposure to trichloroethylene. *Contact Dermatitis*. **9,** 235-237.
6. Phoon, W.H., Magdalene, O.Y., Chan, V.S., Rajan, K.J., Thirumoorthy, T., and Goh, C.L. (1984). Stevens-Johnson Syndrome associated with occupational exposure to trichloroethylene. *Contact Dermatitis*. **10**, 270-276.
7. Nakayama, H., Kobayashi, M., Takahashi, M., Ageishi, Y., and Takano, T. (1988). Generalized eruption with severe liver dysfunction associated with occupational exposure to trichloroethylene. *Contact Dermatitis*. **19**, 48-51.
8. Li, L.Y., Chen, B.J., Huang, X.Q., Lin, B.J., and Huang, L.A. (1998). The distribution and analysis of occupational dermatitis caused by trichloroethylene in Guangdong Province. *Chinese Journal of Industrial Medicine* **11,** 349-351. (in Chinese)
9. Kuang, S.R., Kong, L.Z., Liu, H.F., Xia, L.H., Zhu, G.H., Liang, W.H., Yang, A.C., and Liang, H. (1999). Analysis on 19 cases of occupational dermatitis medicamentose-like induced by trichloroethylene. *China Occupational Medicine* **26** (4), 27-28. (in Chinese)
10. Li, J.T. (1999). Dermatitis after trichloroethylene occupational exposure: 6 cases report. *China Occupational Medicine* **26** (5), 31-32. (in Chinese)
11. Tang, X.J., Li, L.Y., and Chen, B.J. (2000). A review of researches on occupational dermatitis medicamentose-like. *Chinese Journal of Industrial Hygiene and Occupational Diseases* **18,** 111-113. (in Chinese)
12. Magnusson, B., and Kligman, A.M. (1969). The identification of contact allergens by animal assay. The guinea

pig maximization test. *The Journal of Investigative Dermatology* **52,** 268-276.
13. Momma, J., Kitajima, S., and Inoue, T. (1998). The guinea-pig skin sensitization test revisited: an evaluation formula to predict possible sensitization levels for eight chemicals used in household products. *Toxicology* **126**, 75-82.
14. Anderson, C., Sundberg, K., and Groth, O. (1986). Animal model for assessment of skin irritancy. *Contact Dermatitis* **15**, 143-151.
15. Basketter, D.A. and Chamberlain, M. (1995). Validation of skin sensitization assay. *Food Chem. Toxicol*. **33**, 1057-1059.
16. Kligman, A.M. and Basketter, D.A. (1995). A critical commentary and updating of guinea pig maximization test. *Contact Dermatitis* **32**, 129-134.
17. National Standard of People' s Republic of China (1987). *The Pocedures and Methods of Safety Evalution for Cosmetics* (GB 7919-87). (in Chinese)
18. Chittasobhaktra, T., Wannanukul, W., Wattanakrai, P., Pramoolsinap, C., Sohonslitdsuk, A., Nitiyanant, P. (1997). Fever, skin rash, jaundice and lymphadenopathy after trichloroethylene exposure: a case report. *J. Med. Assoc. Thai*. **80,** Suppl 1: *s*144-148.

(Received November 21, 2001 *Accepted February* 8, 2002*)*