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Characterization of Liver Injury Associated with Hypersensitive Skin Reactions Induced by Trichloroethylene in the Guinea Pig Maximization Test

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Abstract: Characterization of Liver Injury Associated with Hypersensitive Skin Reactions Induced by Trichloroethylene in the Guinea Pig Maximization Test: Xiaojiang TANG, *et al*. Guangdong Poison Control Center, China— Trichloroethylene (TCE) can induce non-dose-related hepatitis, possibly classified as delayed-type hypersensitivity (immune-mediated hepatitis), as well as dose-related toxic liver injury. However, the difference in pathophysiology between the two kinds of hepatitis remains unknown. This study aimed to characterize the liver injury associated with hypersensitive skin reactions induced by TCE in guinea pigs. As a model of dose-related acute toxic liver injury, the animals were treated with intradermal injection (*ii*) (0, 167, 500, 1500 or 4500 mg/kg of TCE) or dermal patch (*dp*) (0 or 900 mg/kg of TCE). The guinea pig maximization test (GPMT) was also carried out as a model of immune-mediated liver injury, in which the total TCE dosage was below 340 mg/kg. In the group of TCE 4500 mg/kg (*ii*), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) increased (p<0.01), while total protein and globulin decreased (p<0.05). Evident fatty degeneration, hepatic sinusoid dilation and inflammatory cell infiltration were observed. No significant change was found in animals treated with TCE of doses below 500 mg/kg (*ii*) or 900 mg/kg (*dp*). In the GPMT, sensitization rates of TCE-induced dermal allergy were 66%. ALT, AST, lactate dehydrogenase and the relative liver weight increased significantly (p<0.05) while albumin, IgA and γ-glutamyl transpeptidase decreased significantly (p<0.05). Lesions of ballooning changes were observed in liver pathology. Thus, TCE could cause both acute-type toxic liver injury and immune-mediated liver injury, the so-called delayed-type hypersensitivity at doses below the dosage for toxic liver injury. Interestingly, the histopathological features were quite different: fatty degeneration was most prominent in the former, and ballooning in the latter.
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Trichloroethylene (TCE) has been used in industry as a degreasing solvent and extraction agent for about a century. Intoxication cases of TCE have been reported since as early as 1915, showing organ damage involving the central nervous system, peripheral nervous system, liver, kidney, heart and skin. As for skin lesions, mucous membrane irritation, defatting skin and irritant dermatitis have been described[1, 2], but the severe generalized skin disorders accompanying hepatitis which resemble drug hypersensitivities have become the main clinical issue in the past 20 yr in Asia, especially in Guangdong, China, because of rapidly increasing numbers of patients and their serious consquences[3]. Up to now, more than 240 cases with 31 fatalities have been reported by the Guangdong Poison Control Center, China. These disorders have been grouped under "Occupational medicamentosa-like dermatitis (OMLD) induced by TCE" in the Chinese National Diagnostic Criteria[4].

OMLD induced by TCE is completely different from TCE-induced irritant contact dermatitis in terms of unclear dose-response relationship, period of exposure

before disease onset, generalized rash, fever, lymphadenopathy, and recurrence just after minimal re-exposure[5–7]. The patients typically show rash on the extremities, face, neck or trunk with or without fever 2 wk to 2 months after commencement of occupational TCE exposure[3]. The mortality is 9–13%, which is close to the corresponding figures observed for drug-induced generalized skin disorders[3]. Liver failure is one of the principal causes of mortality[8–12]. Xia *et al*. found that 93.3% (167/179) of OMLD patients induced by TCE in China had suffered from hepatitis[4, 13]. It has also been reported that the hepatitis was non-viral and apparently different in its clinical course from the usual TCE-induced hepatitis, which occurs without showing rash at high concentrations in direct relation to P450-derived metabolites[14, 15].

Thus, TCE can induce two pathophysiologically-different types of hepatitis. The first one is the well-known dose-dependent toxic liver injury and the second one is atypical non-dose-related, immunogically-induced liver injury. We previously reported that TCE had a strong sensitization potential related to delayed-type hypersensitivity in the guinea pig maximization test (GPMT) for TCE and its metabolites, and that its sensitization rate was 71.4%[16]. Unfortunately, the histopathological changes of liver were not investigated in that study.

In the present study, we repeated the above-mentioned GPMT together with an acute toxicity study and characterized the liver injury associated with the hypersensitive skin reactions induced by TCE. This is the first report showing TCE can induce immune-mediated liver injury with ballooning changes in liver cells at doses below the dosage causing toxic liver injury.

Materials and Methods

Animals

Insensitized albino female guinea-pigs (FMMU strain) weighing 300–350 g were provided by the Medical Laboratory Animal Center of the South China Medical University located in Guangzhou, China (No. 2004A061). Since the susceptibility is not different between genders, and females are easier to treat, only females were used in the present study. The animals were housed in an animal room kept at a temperature of 23 ± 1.5°C and relative humidity of 55 ± 10% with a 12 h day/night cycle. The animals received care in compliance with the Guide for Care and Use of Laboratory Animal Research, Department of Toxicology, Guangdong Poison Control Center, P.R. China. All animals were fed with a standard guinea pig diet, fresh vegetables and tap water.

Chemicals

Freund's complete adjuvant (FCA) was provided by Difco Laboratories (Detroit, MI, USA). TCE (99.9% pure) and olive oil (vehicle for TCE) were purchased from Acros Organics (Morris Plains, NJ, USA). 1,2-Dinitrochlorobenzene (DNCB), which was used as a positive control substance inducing dermal sensitization, was obtained from Tokyo Kasei Co., Ltd. (Tokyo, Japan). Sodium lauryl sulfate was purchased from Sigma (Saint Louis, MO, USA). All of the other test materials were not irritants. Filter papers impregnated with the test material and mounted on Leucoflex purchased from Beiersdorff AG (Hamburg, Germany) were used for the topical induction patch test.

Acute toxic liver injury induced by intradermal injection (ii) of TCE

FMMU guinea pigs were randomly divided into 5 groups (5 guinea pigs per group) and treated with intradermal injection (*ii*) of TCE of 0, 167, 500, 1,500 or 4,500 mg/kg. Guinea pigs were sacrificed at 48 h after the injection. Serum alanine transaminase (ALT), aspartate aminotransferase (AST), total protein (TP), albumin (ALB) and globulin (GLB) were analyzed with a CL-8000 Clinical Chemistry Analyzer (Shimadzu Co., Japan). Liver pathology was observed with an Axioskop 40 microscope (Carl Zeiss, Germany).

Acute toxic liver injury induced by TCE dermal patch (dp)

Twelve FMMU guinea pigs were randomly divided into 2 groups and occlusively patched with TCE of 0 or 900 mg/kg. Guinea pigs were sacrificed 48 h after the patching. Serum ALT, AST, TP, ALB and GLB as well as liver pathology were analyzed as described above.

Immunological liver injury induced by GPMT

GPMT was performed as described by Magnusson and Kligman with some modifications[17]. Seventy FUMM guinea pigs were randomly divided into 7 groups (10 animals per group); one control group, one positive control group (DNCB group), and five TCE groups which were then combined into one group with 50 animals. Prior to the GPMT, the maximal tolerated concentrations of TCE in olive oil (weight/weight) were determined as described previously[16]. The test concentrations for intradermal and topical inductions, and the challenge of TCE, were selected as 10%, 20% and 10%, respectively. These induction concentrations induced moderate erythema, but the challenge topical concentration did not induce any abnormal skin reactions.

At the beginning of the first induction stage of GPMT, dorsal skin in the scapular region was shaved, followed by the 3 GPMT steps. Twenty-four hours after hair shaving, 3 pairs of the following 0.1 m*l* intradermal injections were performed on each animal: (1) an emulsified mixture of FCA; (2) a suspension of test agent dissolved in vehicle: TCE (10%) or DNCB (0.1%) in olive

oil, or vehicle only for the control group; and (3) a suspension of TCE, DNCB or vehicle only in FCA. In the second induction stage 7 d after the first injections, the interscapular region was again shaved, and sodium lauryl sulfate (10% weight/weight) was applied on the skin where non-irritant olive oil and FCA had been injected. On the next day, 0.2 m*l* of a test material (TCE 20% or DNCB 1%) was occlusively patched on the same region for 48 h. Control animals were applied with vehicle (olive oil) only. At the challenge stage, 21 d after the initial intradermal injection, all animals were challenged by topical application of the test agents, TCE 10% or DNCB 0.1%. One hundred microliters of the respective test agents in vehicle was applied to the shaved areas of the guinea pigs by the closed patch test method, and left for 24 h. Patch test responses were read 24 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; and 3, intensive erythema and swelling. Scores of redness and swelling were calculated according to this scale, and values of 1 and over were regarded as positive. Skin reactions of sham-treated control animals were read as a blind reading. The responses were read again 48 h after removing the patches.

The important statistic in these tests 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[17]. The mean response score was calculated by the following formula: Mean score=(Score of redness + Score of swelling) / Number of animals in the group.

On the 22nd d, animals treated with TCE were divided into dermatitis positive (+) and negative (–) groups according to their dermal allergenic reactions. All of the guinea pigs were sacrificed on the 23rd d. ALT, AST, alkaline phosphatase (ALP), L-γ-glutamyl transpeptidase (GGT), TP, ALB, total bilirubin (T-BIL), blood urea nitrogen (BUN), creatinine (CRE), lactate dehydrogenase (LDH), C-reactive protein (CRP), glucose (GLU), complement 3 (C3), complement 4 (C4), IgA, IgG, IgM, IgE, ceruloplasmin (CRE), transferrin (TF), pre albumin (PA) and GLB were analyzed with a CL-8000 Clinical Chemistry Analyzer. A small liver section (2 mm × 2 mm) was rapidly sliced and fixed in glutaraldehyde (4%, 4°C), then processed by the conventional histopathological method and observed with a transmission electron microscope (TEM). Heart, liver, spleen, lungs and kidneys were weighed, and the relative organ weights were calculated. Skin of the test and control sites as well as of the liver were taken and fixed in 10% neutral phosphate-buffered formalin, embedded in glycol methacrylate and polyethlene glycol, cut into 3-μm sections and stained with May-Grunwald-Giemsa. Light microscopic assessment was performed with an Axioskop 40 microscope.

The TCE dosages above the GPMT were estimated as follows. In the first induction stage, the dosage was estimated to be 24 mg [0.1 m*l*/point ii × 0.8 g/m*l* (specific gravity of TCE in olive oil) × 10% (TCE concentration) × 3 (2 points of 10% TCE and 2 points of 5% TCE resulting from dilution with the same volume of FCA)]. In the second induction stage, TCE dosage of topical induction was estimated to be 54 mg [0.3 g patch/animal × 20% (TCE concentration) × 0.9 (specific gravity)]. In the challenge stage, the TCE dosage of the challenge patch was estimated to be 24 mg [0.3 g patch/animal] × 10% (TCE concentration) × 0.8 (specific gravity)]. Given the above estimated amount and the lowest body weight (300 g at the beginning of the first induction stage) of the tested guinea pigs, total TCE dosage from the induction through the challenge stage per animal was below 340 [(24+54+24)/0.3] mg/kg. Since the body weight would have increased by 50% after 21 d, the actual dosage would have been far under 340 mg/kg.

Statistical analysis

Student *t* test was used to compare arithmetic means between groups. When the treatment had more than two levels, one-way ANOVA was used followed by *post hoc* Tukey's multiple comparison method if the variances showed homogeneity, or by Tamhane's method if not, or by Dunnett's method if several groups were compared to the same control group. Differences in *p* values less than 0.05 were regarded as significant. The Pearson chi-square test was used to compare the frequency of animals showing liver injury between dermatitis (+) and (–) groups.

Results

Acute toxic liver injury induced by TCE (ii)

The animals treated with TCE 4,500 mg/kg (*ii*) showed extensive flare on the back and hypopraxia 1 h later. Compared with the control group, ALT and AST increased ($p<0.01$), TP and GLB decreased ($p<0.05$) in the 4,500 mg/kg group, and AST increased ($p<0.05$) in the 1,500 mg/kg group (Table 1). No significant differences were found in the other 2 TCE-treated groups. Pathologically, evident fatty degeneration, hepatic sinusoid dilation and inflammatory cell infiltration were observed in the animals of the 4,500 mg/kg group (Fig. 1). The hepatic sinus around the central veins was enlarged in an animal of the 1,500 mg/kg group. No significant histopathological changes were found in the groups of TCE 500, 167 or 0 mg/kg (*ii*).

Acute toxic liver injury induced by TCE (dp)

Compared with the control group, no significant difference was found in the analyzed indexes in animals treated with TCE 900 mg/kg (*dp*).

Table 1. Serum ALT, AST, TP, ALB and GLB in guinea pigs intradermally injected (*ii*) with TCE

TCE (mg/kg)	Number of Animals (n)	Body weight (g)	ALT (IU/*l*)	AST (IU/*l*)	TP (g/*l*)	ALB (g/*l*)	GLB (g/*l*)
0	5	323.5 ± 15.3	84.0 ± 23.8	202.8 ± 55.8	57.8 ± 5.6	30.3 ± 1.4	27.5 ± 6.1
167	5	322.0 ± 16.5	84.5 ± 21.4	185.0 ± 40.1	58.2 ± 4.3	32.0 ± 2.7	26.1 ± 4.3
500	5	322.4 ± 24.6	106.5 ± 47.7	174.3 ± 28.3	56.3 ± 4.1	30.8 ± 0.9	25.5 ± 3.5
1,500	5	332.8 ± 25.8	95.0 ± 38.7	291.0 ± 48.2*	57.5 ± 5.7	31.8 ± 5.0	25.7 ± 0.9
4,500	5	334.5 ± 16.3	315.0 ± 158.8**	1135.8 ± 579.3**	49.9 ± 1.2*	28.0 ± 1.8	21.9 ± 1.7*

Values are expressed as mean ± SD. * $p<0.05$, ** $p<0.01$, compared with control group (0 mg/kg).

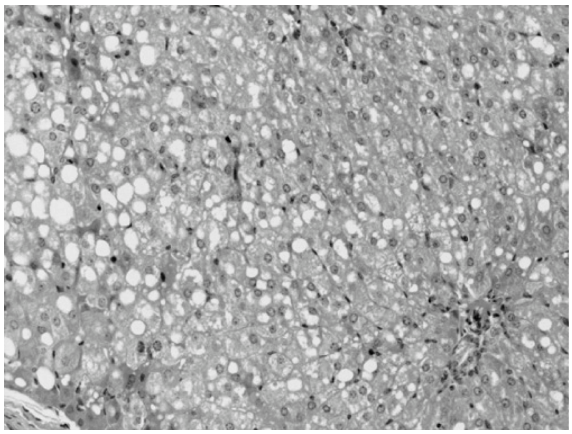

Fig. 1. Fatty degeneration in a liver of a guinea pig intradermally injected (*ii*) with trichloroethylene (TCE) 4,500 mg/kg. The animal was sacrificed 48 h after injection. Representative photomicrograph (× 200, H.E.) of a liver tissue from the guinea pig whose ALT and AST values were 553 IU/*l* and 1,952 IU/*l*, respectively.

Sensitization rates of GPMT

The sensitization rates and scores of guinea pigs in the GPMT challenged by TCE or DNCB are shown in Table 2. The sensitization rate in the TCE challenge group was 66%, and was 100% in the DNCB challenge group. There were 4 animals showing swelling as well as redness on the skin induced by the TCE challenge (Fig. 2 A).

Serum and organs of guinea-pigs challenged by TCE in the GPMT

On the 22nd d of the GPMT, the animals treated with TCE were divided into a sensitization-positive group [Dermatitis (+)] and -negative group [Dermatitis (–)] according to their dermal allergenic reactions. The two groups respectively contained 33 and 17 animals. Serum analytes and relative organ weights in guinea pigs challenged by TCE or DNCB in the GPMT are listed in Tables 3 and 4, respectively. Compared with the control group, ALT, AST, LDH and relative liver weight increased significantly ($p<0.01$), while GGT, ALB ($p<0.01$) and IgA ($p<0.05$) decreased significantly in the TCE dermatitis (+) group. The other parameters did not change significantly ($p>0.05$). On the other hand, in the TCE dermatitis (–) group, except for ALB decreasing significantly ($p<0.05$), none of the other 25 parameters changed significantly. The DNCB group, a dermatitis-positive control for the GPMT, showed that except for decreased ALB ($p<0.05$) and increased relative kidney weight ($p<0.01$), all of the other 24 indexes did not change significantly.

Correlation between dermatitis and liver injury in guinea pigs in GPMT

In the results of the control group, the 95% confidence interval of ALT and AST in the guinea pigs were 49.6–108.4 U/*l* and 69.6–320.4 U/*l*, respectively. Accordingly, 88.2% (15/17) of animals in the TCE dermatitis (–) group were below the upper limits of both ALT and AST, while 90.9% (30/33) of animals in the TCE dermatitis (+) group were over the upper limits of either ALT or AST (Table 5). The Pearson chi-square test showed the dermatitis was related to liver injury ($p<0.01$).

Histopathological findings of skin in GPMT

In the positive control group (DNCB), the challenge-affected skin showed inflammatory cell infiltration in the corneum and granular cell layers. Granular cells were slightly swollen. No abnormality was found in basal cells. Capillary vessel expansion and edema with mononuclear infiltration were found in the corneum. In the group with TCE dermatitis (+), the challenge-affected skin showed falling-off of keratoderma, evidently thinner epidermis, and detachment, necrosis or loss of some epidermis cells. Inflammatory cell infiltration was observed in the corneum and subcutaneous layer. Dermal collagen showed denaturalization and swelling (Fig. 2 B). On the other hand, no abnormalities were found in the control groups as well as in the TCE dermatitis (–) group.

Histopathological findings of liver in GPMT

In the TCE dermatitis (+) group, the hepatocytes

Table 2. Sensitization rates and scores of guinea pigs after GPMT challenged with trichloroethylene (TCE)

Group	Number of animals (*n*)	Number positive (*n*)[a]	Sensitization rate (%)	Classification[b]	Score of redness per animal[c]	Score of swelling per animal[c]	Mean score[d]
Control	10	0	0.0	Weak	0	0	0.0
DNCB	10	10	100.0	Extreme	3.0	3.0	6.0
TCE	50	33	66.0	Strong	1.4	0.2	1.7

[a] Positive animals were the same 24 h and 48 h after removing patches. [b] Classification was based on sensitization severity grading (Magnusson and Kligman 1969). [c] Scores were read 24 h after removing patches. [d] Mean score=(Score of redness + Score of swelling) / Number of animals in group.

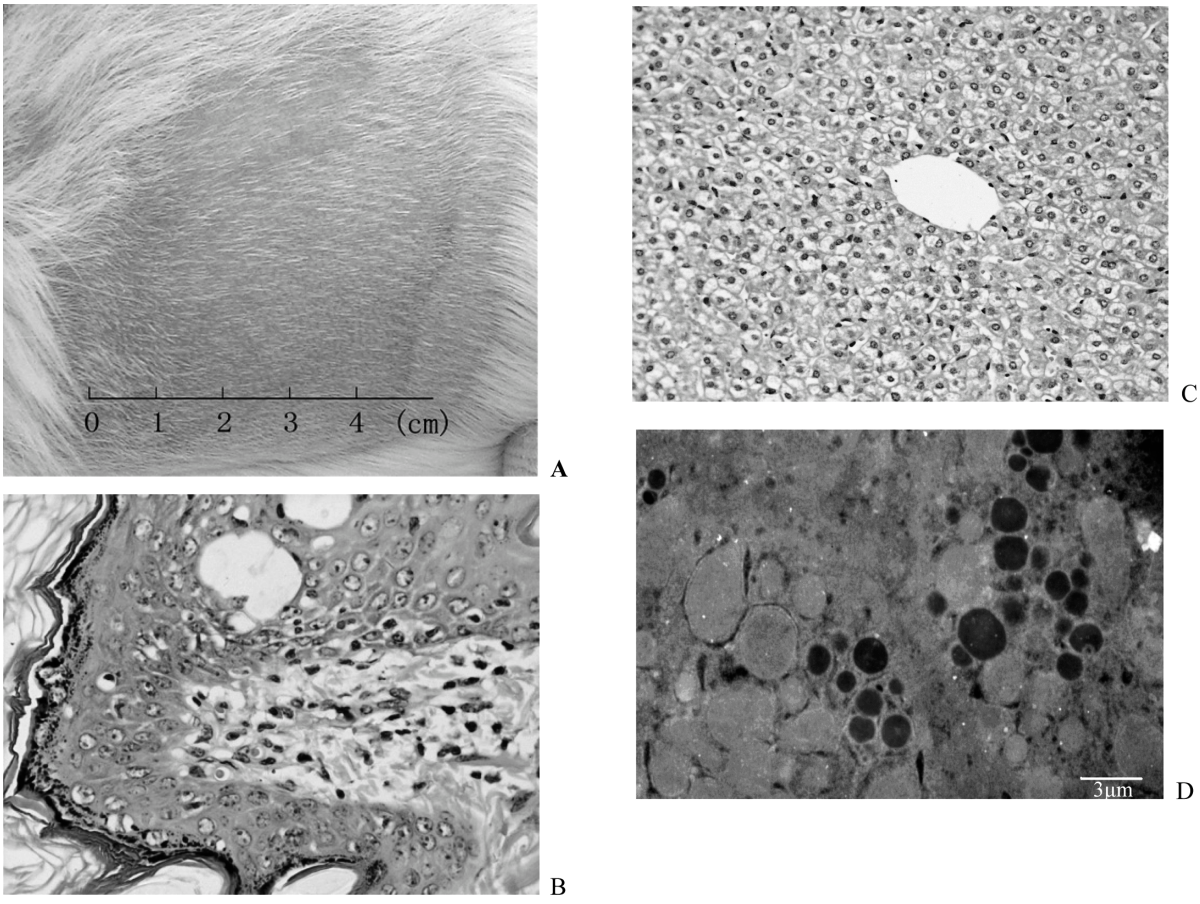

Fig. 2. Typical skin damage and typical liver injury of a dermatitis-positive (+) guinea pig after GPMT challenged with trichloroethylene (TCE). The photo of guinea pig shows typical skin erythema and swelling (A). Representative photomicrograph (× 200, H.E.) of skin tissue from a guinea pig showing pathological falling-off of keratoderma, inflammatory cells infiltration in the corneum and subcutaneous layer (B). Representative photomicrograph (× 200, H.E.) of liver tissue from a guinea pig showing histopathological ballooning changes (C). Typical ultrastructural changes (transmission electron microscopy) of hepatocytes suffused with ballooning changes as well as many large and small vacuoles in the liver (D). Serum ALT and AST were 243 IU/*l* and 690 IU/*l*, respectively.

showed diffuse ballooning changes without lymphocyte infiltration and necrotic hepatocytes. The cell membrane as well as the cell nucleus was clear, but a significant swollen cloudy and granular appearance was observed in hepatocyte cytoplasma (Fig. 2 C). Ultrastructural findings observed with TEM revealed anomalous but not round hepatocyte nucleoli, enlargement of nuclear pores an increased number of rough endoplasmic reticulum,

Table 3. Serum parameters on guinea pigs after GPMT challenge with trichloroethylene (TCE)

parameters	Control	TCE dermatitis (–)	TCE dermatitis (+)	DNCB
Number of Animals (n)	10	17	33	10
ALT (U/*l*)	79 ± 15	74 ± 14	123 ± 40**##	88 ± 21
AST (U/*l*)	195 ± 64	195 ± 63	623 ± 300**##	295 ± 134
ALP (U/*l*)	295 ± 66	225 ± 61	282 ± 104	212 ± 65
GGT (U/*l*)	52.1 ± 13.0	51.4 ± 15.7	40.8 ± 11.6**##	52.4 ± 13.3
LDH (IU/*l*)	860 ± 261	819 ± 206	1596 ± 848**	1143 ± 464
TP (g/*l*)	54.02 ± 3.95	52.69 ± 3.80	53.78 ± 3.58	54.57 ± 2.23
ALB (g/*l*)	36.40 ± 1.76	34.72 ± 1.70*	34.34 ± 1.55**	34.39 ± 1.26*
GLB (g/*l*)	17.40 ± 3.51	17.96 ± 2.47	19.45 ± 3.89	20.18 ± 2.25
TF (g/*l*)	0.13 ± 0.01	0.14 ± 0.05	0.14 ± 0.02	0.12 ± 0.01
CER (mg/*l*)	41.56 ± 11.51	43.80 ± 5.79	39.20 ± 17.01	51.75 ± 19.53
PA (mg/*l*)	5.64 ± 4.47	4.51 ± 2.25	4.99 ± 4.71	5.23 ± 4.17
GLU (mmol/*l*)	2.78 ± 1.21	3.01 ± 1.15	2.87 ± 1.08	3.29 ± 1.32
TBIL (μmol/*l*)	5.18 ± 1.92	5.03 ± 1.88	8.15 ± 4.69	7.25 ± 3.03
BUN (mmol/*l*)	10.30 ± 1.33	10.23 ± 1.01	9.72 ± 1.28	11.46 ± 2.87
CRE (μmol/*l*)	54.09 ± 5.47	48.06 ± 5.60	53.42 ± 7.17	50.16 ± 2.77
CRP (mg/*l*)	4.22 ± 0.69	4.05 ± 0.63	5.98 ± 3.31	4.89 ± 1.12
C3 (mg/*l*)	0.32 ± 0.08	0.28 ± 0.03	0.29 ± 0.08	0.39 ± 0.14
C4 (mg/*l*)	0.05 ± 0.02	0.05 ± 0.01	0.06 ± 0.03	0.06 ± 0.02
IgA (g/*l*)	0.15 ± 0.03	0.15 ± 0.01	0.12 ± 0.04*	0.16 ± 0.04
IgM (g/*l*)	0.11 ± 0.05	0.12 ± 0.01	0.13 ± 0.04	0.15 ± 0.04
IgE (IU/m*l*)	8.24 ± 7.87	9.46 ± 9.28	10.05 ± 7.07	4.96 ± 5.48

Values are expressed as mean ± SD. * $p<0.05$, ** $p<0.01$, compared with control; # $p<0.05$, ## $p<0.01$, compared with TCE dermatitis (–) group. TCE dermatitis (–): Negative dermal reaction after challenge application with TCE. TCE dermatitis (+): Positive dermal reaction after challenge application with TCE. DNCB: DNCB-treated group as positive control.

Table 4. Relative organ weight (%) of guinea pigs after GPMT challenge with TCE

Group	Number of animals (n)	Body weight (g)	Relative organ weight (%)[a]				
			Liver	Spleen	Heart	Lung	Kidney
Control	10	414.2 ± 40.1	3.36 ± 0.29	0.27 ± 0.12	0.45 ± 0.19	0.87 ± 0.14	1.05 ± 0.19
TCE dermatitis (–)	17	396.8 ± 35.5	3.65 ± 0.35	0.29 ± 0.14	0.38 ± 0.05	0.82 ± 0.12	1.02 ± 0.11
TCE dermatitis (+)	33	408.7 ± 43.1	3.98 ± 0.46**#	0.24 ± 0.12	0.46 ± 0.15	1.04 ± 0.36	1.13 ± 0.11
DNCB	10	378.5 ± 42.7	3.54 ± 0.49	0.29 ± 0.12	0.48 ± 0.09	1.06 ± 0.34	1.30 ± 0.32**##

Values are expressed as mean ± SD. * $p<0.05$, ** $p<0.01$, compared with control; # $p<0.05$, ## $p<0.01$, compared with TCE dermatitis (–) group. TCE dermatitis (–): Negative dermal reaction after challenge application with TCE. TCE dermatitis (+): Positive dermal reaction after challenge application with TCE. DNCB: DNCB-treated group as positive control.

[a] (organ weight/body weight) × 100

Table 5. Relationship between dermatitis and liver injury in the animals after GPMT challenge with TCE

TCE dermatitis	ALT or AST		Total
	Over upper limit*	Below upper limit*	
Negative (–)	2	15	17
Positive (+)	30	3	33

*Upper limits of ALT and AST are 108.4 U/*l* and 320.4 U/*l*, respectively. Pearson chi-square value=30.504, contingency coefficient=0.616, $p<0.001$.

reduced numbers of mitochondria, and the disappearance of mitochondrial cristae. Hepatocytes were suffused with ballooning changes as well as many large and small vacuoles (Fig. 2 D).

Discussion

The present study characterized the liver injury associated with delayed hypersensitivity skin reactions induced by TCE in the GPMT model, a model for type IV allergy[18, 19]. TCE induced immune-mediated hepatic injury in the animals showing allergic contact dermatitis, as well as acute toxic liver injury due to direct toxic action of TCE induced by a single intradermal injection. The following four lines of evidence support the notion that TCE may induce delayed-type hypersensitivity like OMCD in human.

First, the dermal sensitization rate was 66% in the present GPMT, which was consistent with our previous report[16]. On the other hand, IgE, the main antibody induced by type I allergens, was not significantly different between the groups of TCE dermatitis (+) and dermatitis (–), suggesting TCE may not induce type I allergy. Since the GPMT is a method for predicting delayed-type hypersensitivity, but not type I allergy, the finding is not inconsistent with our supposition.

Second, significant increases in ALT, AST, GGT, LDH and relative liver weights in the group of TCE dermatitis (+), but not in the dermatitis (–) group, clearly indicate that only the former group suffered from hepatic injury. This finding is consistent with findings that patients with OMLD due to TCE suffered hepatic injuries but unaffected persons under the same exposure did not (unpublished observation). It also suggests that the hepatic injury is different from the TCE-induced acute hepatitis observed at relatively high TCE dosages.

Third, the liver injury seen in the TCE dermatitis (+) group could not have been caused by FCA, which is widely used and considered to be the most effective adjuvant. FCA is composed of inactivated and dried mycobacteria, usually Mycobacterium tuberculosis, which may induce delayed-type hypersensitivity reactions including dermatitis and liver injury when injected more than twice with antigen[20]. However, the guinea pigs were treated with FCA only once in the current experiment, and in addition, no hepatic injuries were observed in the other three groups treated with FCA in the induction stage (the control, TCE dermatitis (–) and DNCB groups). These facts again support the non-involvement of FCA in the hepatic injury.

Fourth, total dosages of TCE in the GPMT model were estimated to be 340 mg/kg at most, well below 500 mg/kg, a level at which intradermal TCE injection did not induce any significant differences in histopathological findings as well as ALT and AST activity. Liver injuries resulting from the intradermal injection were observed at 1,500 mg/kg or over. Thus, hepatic injuries detected in the TCE dermatitis (+) group seem to have been caused at a dose lower than that inducing toxic liver injury. Interestingly, the histopathological phenotypes in the liver induced by intradermal injection of TCE 4,500 mg/kg were characterized by the fatty degeneration, hepatic sinusoid dilation and inflammatory cell infiltration, whereas the hepatotoxicity occurring with GPMT-induced dermatitis was characterized by diffuse ballooning changes without lymphocyte infiltration or necrotic cells. However, it should be borne in mind that a above difference might have just reflected the different degree of hepatocellular injury since animals with different treatments showed different levels of ALT and AST.

The immunological mechanism of TCE-induced liver injury in the GPMT remains unclear. Though the possible role of trichloroacetylated protein acting as an antigen is suggested by analogy with the hepatitis induced by 2,2-dichloro-1,1,1-trifluoroethane[21]. The reason why TCE, but not DNCB used as a positive control for GPMT, induced hepatic damage should be further investigated.

In conclusion, the present study clearly shows that TCE can induce dermatitis with hepatic injury by the mechanism of delayed-type hypersensitivity in guinea pigs. The GPMT, adopted as one of the national standardized test methods in some countries including China[22], established as a TCE-induced liver injury model. The mechanism by which hepatitis is induced in patients with OMLD may be disruption of the immune system through TCE exposure.

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