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Dose-Response Relationships for Chemical Sensitization from TDI and DNCB

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Development of sensitivity as a result of exposure to chemicals is widely known. It has been reported about 10 % subjects become sensitized and suffer allergic asthma in some factories (Salvaggio 1982) and 60 % of all occupationally related illnesses are attributed to skin diseases, of these lesions, 25 to 30 % are traditionally cases of allergic sensitization (Adams 1990).

Although threshold limit values (TLVs) have been recommended to protect workers from a range of toxic effects of chemicals. For example, TLVs have been established to protect against carcinogenicity, neurotoxicity, primary irritation, and other toxic effects of chemicals. Rarely, however, has a TLV been proposed to protect against sensitization.

To propose a TLV which will protect against sensitization from a chemical, a dose-response relationship for the chemical between the exposure dose and the development of sensitizing potency is necessary. In the past, the dose-response relationship of chemical sensitization were not thoroughly recognized, and little information being emphasized on it. Whereas there have been appearing some reports on this topic in recent years, for example, the pulmonary sensitivity of toluene diisocyanate (TDI) indicated a dose-response relationship in an animal system (Karol 1983). The contact sensitivity of formaldehyde and nickel were also dose dependent, being elucidated in animal models (Lee et al. 1983; Rohold et al. 1991). Moreover, a relationship between the dose of antigen and the skin response in patch test reactions has been found (Eun and Marks 1990).

The present study was designed to obtain further information on the dose-response relationship for chemical sensitization in animal models and to justify

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the possibilities to set TLVs for chemical sensitizers against their allergic hazards.

MATERIALS AND METHODS

Hartley guinea pigs initially weighing from 250 to 350g were used for pulmonary sensitivity tests of TDI; BALB/c mice 8-10 weeks old were used for mouse ear swelling tests of dinitrochlorobenzene (DNCB). Both guinea pigs and mice were supplied by Hunan Medical University Animal Center. They were fed on pelleted diet and water ad libitum.

Seven groups of 7 guinea pigs were exposed to TDI (80:20 mixture of the 2.4- and 2.6-isomers, Nakarai Chemicals. LTD., Japan) atmospheres containing different concentrations via inhalation for 3hr a day on the first 5 consecutive days (Tab 1). All exposures were done in a wholebody animal chamber. TDI vapors were generated by blowing air through a sintered glass bubbler in a closed vessel chamber after dilution with clean, dry air to achieve appropriate concentrations which were measured by the method of Marcali (1957). In the 21st day from initial exposure, animals were challenged by TDI atmospheres for 30 min (Tab 1). And 30 min later, blood samples were drawn by cardiac puncture from animals to evaluate histamine contents of blood and mast cell degranulation indices (MCDI).

Table 1. Experimental conditions of TDI exposure groups; concentrations and times received by guinea pigs (Mean±SD)

Groups	No	Induction		Challenge	
		Con@	Times	Con@	Times
I	6	0.37±0.01	5d;3hr/d	0	1d;30min/d
II	7	0.37±0.01	5d;3hr/d	0.03±0.01	1d;30min/d
III	6	0.37±0.01	5d;3hr/d	0.11±0.02	1d;30min/d
IV	6	0.37±0.01	5d;3hr/d	0.37±0.01	1d;30min/d
V	7	0.11±0.02	5d;3hr/d	0.37±0.01	1d;30min/d
VI	6	0.03±0.01	5d;3hr/d	0.37±0.01	1d;30min/d
VII	7	0	5d;3hr/d	0.37±0.01	1d;30min/d

@Induction and challenge concentrations (ppm)

Histamine was measured spectrophotofluorimetrically following condensation with O-phthalaldehyde by the method of Hakanson et al (1972). MCDI was assessed as follows: a). TDI-protein conjugates were prepared by the method of Karol (1983). b). Mast cell suspension was prepared by the method of Lo and Yokoyama (1974). c). 0.1ml guinea pig serum, 0.1ml TDI-protein conjugates and

0.1ml mast cell suspension were mixed in a well of a microplate and incubated at 37°C for 30 min. One pipette drop of the mixture was placed on a slide covered with a glass coverslip stained with Neutral Red, and intact and degranulated mast cells were counted under microscopy. Mast cell degranulation index (MCDI) was obtained using the equation:

$$\frac{\text{No. of degranulated mast cells}}{\text{No. of intact mast cells + degranulated mast cells}} \times 100$$

Seven groups of 8 mice were exposed topically to 200ul of various concentrations of DNCB (Sigma) in acetone on the shaved abdomen on the first three consecutive days. On day 21, 20ul DNCB in acetone was applied to the left ear (test ear) and 200ul acetone was applied to the right ear (control ear) (Tab 2). The thickness of each ear was measured at 24 and 48hr after the challenge using a dial thickness gauge (Ozski MFG, Tokyo, Japan). Ear swelling rate (ESR) was expressed as follows:

$$\frac{\text{Thickness of test ear - thickness of control ear}}{\text{Thickness of control ear}} \times 100$$

Table 2. Experimental conditions of DNCB exposure groups; concentrations and times received by mice

Groups	No	Induction		Challenge	
		Con@	Times	Con@	Times
I	8	1.00	3d;200ul/d	0	1d;20ul/d
II	8	1.00	3d;200ul/d	0.05	1d:20ul/d
III	8	1.00	3d;200ul/d	0.20	1d;20ul/d
IV	8	1.00	3d;200ul/d	1.00	1d;20ul/d
V	8	0.20	3d;200ul/d	1.00	1d;20ul/d
VI	8	0.05	3d;200ul/d	1.00	1d;20ul/d
VII	8	0	3d;200ul/d	1.00;	1d;20ul/d

@Induction and challenge concentrations (g/100 ml)

Animal whose ESR exceeded the mean value+2SD of the control group was considered as positive animal.

RESULTS AND DISCUSSION

Affects of TDI concentration at induction on histamine content and MCDI (Tab.3). As shown in Table 3, group IV-VII were exposed to different TDI induction concentrations. No elevation of histamine content and MCDI was observed in guinea pigs exposed to 0.03 ppm TDI

at induction (Group VI). However, when it reached to 0.11 ppm or 0.37 ppm (Group V and VI), the histamine content and MCDI displayed markedly higher values than the control (Group VII). Histamine contents and MCDI were clearly TDI concentration-dependent at induction.

Affects of TDI concentration at challenge on histamine content and MCDI (Tab.4). Group I-IV were exposed to 0, 0.03, 0.1, 0.37 ppm TDI at challenge respectively. No remarkable effect was seen upon 0.03 ppm challenge to histamine and MCDI (Group II). By contrast, when it was elevated to 0.11 ppm or 0.37 ppm (Group III and IV), histamine content and MCDI were apparently higher than that of Group I, which indicated that TDI concentration at challenge also had important affects on the immediate hypersensitivity of TDI.

Table 3. Effect of TDI concentration at induction on histamine content and MCDI (Mean±SD)

Groups	No	Con@		Histamine (ng/ml)	MCDI (%)
		Ind	Cha		
IV	5	0.37	0.37	945.3±123.6**	55.9±8.7**
V	7	0.11	0.37	849.6±584.7*	52.9±4.9*
VI	6	0.03	0.37	337.3±124.8	41.3±6.3
VII	6	0	0.37	232.8±88.8	40.3±6.4

@Induction and challenge concentrations (ppm).
*p<0.05, **p<0.01, compared with group VII (t-test)

Table 4. Effect of TDI concentration at challenge on histamine content and MCDI (Mean±SD)

Groups	No	Con@		Histamine (ng/ml)	MCDI (%)
		Ind	Cha		
I	5	0.37	0	364.8±90.1	41.0±7.2
II	7	0.37	0.03	306.2±76.2	50.7±7.3
III	6	0.37	0.11	819.2±120.8**	54.5±7.4**
IV	5	0.37	0.37	945.3±120.8**	55.9±8.7**

@Induction and challenge concentrations (ppm)
**, compared with group I (t-test)

Affects of DNCB concentration at induction on ESR and percentage of positive mice. Table 5 indicated that DNCB concentration at induction had positive correlation with both ESR and percentage of positive mice. Group IV had the highest concentration at induction (1.0 %), the ESR and positive mice were maximal; Group V had a lower concentration (0.2 %), these two indices were also a

little lower. Further, Group VI exposed to the lowest DNCB concentration at induction (0.05 %) displayed no significant ear swelling and positive mice.

Affects of DNCB concentration at challenge on ESR and percentage of positive mice were shown in Table 6. It presents that DNCB concentration at challenge also played significant roles in the contact sensitivity of DNCB. Following the increment of challenge concentration, the ESR and positive mice were also increased. At 0.05 % group, the ESR and positive mice were 9.0 % and 33.3 %, respectively. At 0.2 % group, they were increased to 18.8 % and 50.0 %, and when the concentration at challenge was up to 1.0 %, both the ESR and percentage of positive mice reached to maximum.

Table 5. Effect of DNCB concentration at induction on ear swelling rate and percentage of positive mice (Mean±SD)

Groups	No	Con@		ESR (%)	Positive mice (%)
		Ind	Cha		
IV	7	1.00	1.00	23.6±18.4**	71.4**
V	7	0.20	1.00	16.9±11.6*	57.1**
VI	5	0.05	1.00	2.5±4.8	0
VII	7	0	1.00	3.9±5.9	0

@Induction and challenge concentrations (g/100ml)
*$p<0.05$, **$p<0.01$, compared with group VII (t-test)

Table 6. Effect of DNCB concentration at challenge on ear swelling rates and percentage of positive mice (Mean±SD)

Groups	No	Con@		ESR (%)	Positive mice (%)
		Ind	Cha		
I	5	1.00	0	0	0
II	5	1.00	0.05	9.0±7.5**	33.3**
III	6	1.00	0.20	18.8±17.3**	50.0**
IV	7	1.00	1.00	23.6±18.4**	71.4**

@Induction and challenge concentrations (g/100ml)
**$p<0.01$, compared with group I (t-test)

The dose-response concept is the core in toxicological evaluation and the most important basis for setting hygienic criteria for chemicals to protect the exposed subjects. It was generally accepted in the past that the allergic effects of chemicals did not show dose-response pattern as the ordinary toxicities of them

(Doull and Klaassen 1980). Whereas there have been appearing many reports that were not consistent with the point of view above. For example, Karol (1983) reported that no specific antibodies were detected in any of the serum samples from animals exposed to 0.12 ppm; however, some antibody production was noted in about 55 % of animals exposed to 0.36 ppm. Animals exposed to higher TDI concentration produced higher titers of antibody and higher percentage of animals which developed TDI-specific antibodies in serum. Also, pulmonary sensitivity was influenced by exposure concentrations of TDI. None of the animals exposed to 0.12 ppm TDI displayed pulmonary sensitivity, By contrast, several animals exposed to TDI above 0.36 ppm experienced pulmonary sensitivity. In some cases, respiratory rates increased by 80 to 90 %.

Though histamine content and MCDI are two sensitive indices to explore immediate-onset hypersensitivity (Simon et al. 1977; Sydbom 1979; Girsh et al. 1982; Gomez 1986), they have not been used to assess the dose-response relationship of chemical sensitization. Then, we tried to make a further exploration to the dose-response relationship of TDI induced pulmonary sensitivity using these two indices. A strong correlation was observed between TDI concentration at induction or challenge and histamine content and MCDI. Histamine and MCDI in test group did not show any difference with those in control group in the case that both induction and challenge concentration of TDI did not reach a certain level (0.03 ppm). Guinea pigs displayed increased histamine content and MCDI with increased concentration of TDI, plateaued at higher concentrations. The results are consistent with those cited previously.

There have been several animal models proposed to assess the contact sensitivity of chemicals. For example, Buehler Test (Buehler 1965) and the Guinea Maximization Test (Magnusson and Kligman 1969) have been widely used. In recent years, the mouse ear swelling test has been considered a possible tool to detect contact sensitivity of simple chemicals (Gad et al. 1986; Thorne et al. 1987). It dose not require use of adjuvants, intradermal injections, occlusive patches, or other procedures used in the models described above. In addition, it is more objective and markedly less expensive. The current study investigated the dose-response relationship of DNCB induced contact sensitivity utilizing the mouse ear swelling test. It was verified that the ear swelling rates and the percentage of positive mice were apparently affected by both the induction and challenge doses of DNCB topically applied. The results suggested that the contact sensitivity of chemicals was also also

dose-dependent.

TDI and DNCB are typical pulmonary and contact sensitizers. We used these two chemicals as representatives to study the dose-response relationship of chemical sensitization. By summarizing the results of our study and the other reports before, we are convinced that there does exist dose-response relationship between the exposure dose of chemical sensitizer and the prevalence and severity of the sensitivity caused by them. The recognition of dose-response relationship for chemical sensitization can be expected to provide the necessary guidance for setting appropriate TLVs for chemicals in workplaces to protect against sensitization of workers, which has also been acknowledged by other reports (Karol 1983; Thorne et al. 1986).

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