EXPERIMENTAL STUDIES ON TDI DERMATITIS IN MICE

Kazumasa YASUDA, Goro NOZAWA, Tomoko GOTO,
Norihiko SASAKI and Sumiko ISHIZU

*Department of Public Health,
Tokyo Women's Medical College, Tokyo 162 Japan*

Accepted January 13, 1980

Abstract.....Allergic contact dermatitis occurred with 2, 4-toluene diisocyanate (TDI) in BALB/c mice.

The results were obtained by the measurement of the ear thickness after challenged to the ear with 1% TDI in olive oil in mice previously sensitized with 5% TDI.

The ear swelling rate at 48 hr postchallenge in sensitized mice was more than twice of that in unsensitized mice.

In BALB/c-nu/nu (nude) mice, delayed type hypersensitivity could not be induced even when a 30% TDI solution was applied.

These results suggest that T cells may play an essential role in contact sensitivity to TDI in BALB/c mice.

Key words : contact dermatitis, 2, 4-toluene diisocyanate, mice.

INTRODUCTION

Isocyanates are widely used in various industries as raw materials for producing such things as soft and hard urethane foam, synthetic rubber, adhesives and paints. Among many forms —TDI (toluene diisocyanate), MDI (diphenylmethane diisocyanate), NDI (naphthylene diisocyanate), HDI (hexamethylene diisocyanate) and other derivatives —TDI is the most well known. This compound is highly irritable to the respiratory organs, eye and skin. Since some people suffer from frequent asthmatic coughs after their second exposure to TDI, it has been considered a sensitizing agent (Silver, 1963 ; Peters, 1970).

Earlier fundamental studies concerned with the effects of TDI in vivo were conducted only to observe the lethal dose and primary irritation to the eye and skin (Swensson et al., 1955 ; Duncan et al., 1962 ; Iino, 1963).

Subsequently, Zapp et al. (1957) studied the toxic effects of cutaneous, oral and inhalant applications on animals and reported that contact sensitivity to TDI was observed only in guinea pigs. However, such asthmatic symptoms caused by TDI in humans could not be induced in any kind of experimental animals even after repeated inhalation of TDI vapor.

Similar inhalation tests with TDI in various species were conducted by a few researchers. However, they reported only changes due to the primary irritation (Niemenhis,

1965).

Scheel et al. (1965) studied the toxicity of this compound from the immunological aspect in rabbits. Although they detected the specific antibody of TDI in the blood, they found hypersensitive reaction on neither the skin nor the respiratory system.

In 1967, Stevens reported on the screening of contact sensitivity of various chemical substances used in industrial fields in guinea pigs by means of the ear-flank test. Consequently, he revealed that TDI and MDI were identified as having contact sensitivity among five isocyanates examined in his study. In this method, the test substance was firstly applied on the ears of guinea pigs daily for 3 days or longer. It was applied again on the shaved flank as a challenge after a week, and reddening on the flank was determined qualitatively 24hr after challenge. He executed the test with more than 100 chemicals and concluded that 45 chemicals were sensitive and 58 not.

The ear-flank test is said to have been developed by Turk and Stone (1973) as a technique to check contact sensitivity of chemicals in animals. It was further carried out and propagated by Davies (1964). The method of the flank-ear route is used by others. Similar results are obtained with either method, but with this route it is possible to measure quantitatively the swelling of the ear with a thickness gauge.

Recently, this method using picryl chloride as a sensitizer has frequently been employed in the study of cellular immunity in mice.

As mice proved to be suited in immunological research, the authors have chosen mice to study TDI dermatitis. The present report confirms contact sensitivity to TDI by the back-ear route in hairy mice. The same attempt was also made in nude mice.

METHODS

Animals

Male BALB/c (hairy), 10 weeks old, and male BALB/c-nu/nu (nude) mice, 8 weeks old, were used. The mice were divided into four groups: group A—28 hairy mice sensitized with TDI; group B—28 unsensitized hairy mice, the control for group A; group C—10 nude mice sensitized with TDI; group D—10 unsensitized nude mice, serving as the control for group C. Each animal was kept in a separate cage. In case of nude mice each cage was covered with a filter cap. The room temperature was adjusted to $22 \pm 1^\circ$ for the hairy mice and $25 \pm 1^\circ$ for the nude mice, while the humidity was $55 \pm 5\%$ for both cases of mice.

Body weight determination

The hairy mice (groups A and B) were weighed every other day during application and on the day of autopsy. The nude mice (groups C and D) were weighed only on the day of autopsy.

Sensitization

TDI was prepared in olive oil to give 1%, 5% and 30% solutions just before the experiment. For sensitization, 5% and 30% solutions were used for the hairy and the nude mice, respectively. With a 1 ml syringe used for the tuberculin test, about $30 \mu\text{l}$ of TDI

TDI dermatitis in mice

solution was applied in drop on the skin of the back, from which the hair was previously depilated by hand. This application was conducted once a day for 5 days.

The control mice (groups B and D) received only the equivalent amount of olive oil.

Challenge and Quantification

The thickness of the ear was measured with a Type G-1 dial thickness gauge (Ozaki Seisakusho, Co.) on the 4th day after the last sensitizing application. All the groups were then challenged with a solution of 1% TDI applied on both sides of the ear using a drawing brush. The ear was measured again at 24 and 48 hr postchallenge.

The thickness of the ear was determined for both ears and the results were expressed as the mean value. The significance of the difference between the mean values for the control and the test group was evaluated using Student's t test.

Histopathological Studies

After completion of the ear measurement, the animals were sacrificed under pentobarbital anesthesia by cutting the aorta and autopsied as soon after death as possible.

The thymus (hairy mice only), spleen, liver and other organs were removed, weighed, observed macroscopically and fixed in formalin. Thereafter, these organs were stained with hematoxylin-eosin and examined histopathologically.

RESULTS

1. General Observations and Body Weight

a) Hairy mice

When 0.03 ml of 5% TDI solution was applied to the skin of their depilated backs, the mice in group A ran around in the cages. The animals seemed to be rather excited due to the primary irritation but calmed down within a short period. No other behavioral change was observed.

Their body weights began to decrease 3 or 4 days after application by several percent. This decrease continued progressively even after cessation of sensitization, and after cessation restoration of growth was not observed. Accordingly, there was a significant difference in weight between group A and B ($p < 0.05$) on and after the 5th day of sensitization.

b) Nude mice

Since the nude mice did not show any sign of primary irritation on the skin with a solution of 5% TDI in the preliminary experiment, a 30% solution was used as a sensitizer for group C. However, there was no notable change in general observations.

Only when measured on the day of autopsy, their body weights showed slight reduction (about 1 g), but, this decrease was not significantly different from the weight of group D.

2. Skin Change

a) Hairy mice

From the 3rd day of sensitizing application of a solution of 5% TDI in olive oil on the depilated backs of mice in group A, signs of primary irritant contact dermatitis were

observed on the depilated surrounding area such as reddening, erosion, depilation and inhibition of hair growth. This dermatitis seemed to continue even after completion of 5 daily applications.

Photo 1 shows a histopathological picture of the skin on the 6th day. Compared with that of group B in Photo 2, growth of epidermis was remarkable. The necrogenic response was marked on the skin and thus hair follicles had disappeared. In addition, some crusting, proliferation of fibroblasts in the papillary layer, infiltration of polymorphonuclear leukocytes and intercellular edema were also observed.

b) Nude mice

The skin of mice in group C showed only a slight reddening as compared with that of mice in group A in spite of the application of a 30% TDI solution. Only slight desquamation occurred after the fifth sensitizing application as shown in Photo 3. These small changes disappeared after a few days.

From the gross features stated above, it was concluded that no abnormality was induced histopathologically.

Photo. 1 Part of skin from a mouse in group A treated with a 5% TDI solution

The disappearance of the hair follicles and the sebaceous glands, and increase of connective tissues were observed. HE stain; $\times 100$

TDI dermatitis in mice

Photo. 2 Part of skin from a mouse in group B HE stain; $\times 100$

Photo. 3 Part of skin from a nude mouse in group C treated with a 30% TDI solution
No change was observed. HE stain; $\times 200$

3. Ear Swelling

a) Hairy mice

As mentioned above, a solution of 5% TDI in olive oil was applied on the depilated skin of group A for 5 consecutive days and then left without any treatment for the next 3 days. On the fourth day after the fifth sensitizing application, a solution of 1% TDI in olive oil was applied to the ears for challenge.

The ear of group B to which TDI was applied on the ear only showed a slight reddening and swelling 24hr after challenge. The ear thickness increased from 24.3×10^{-2} mm before challenge to 27.5×10^{-2} mm in group B receiving challenge. The increased percentage of ear thickness was 13% but not statistically a significant increase (Table 1, Fig. 1).

Table 1. Contact sensitivity to TDI

	Ear thickness before challenge ($\times 10^{-2}$ mm)	Ear thickness at 48hr after challenge ($\times 10^{-2}$ mm)
Group A (TDI-sensitized hairy mice) n=28	25.1 ± 1.67	56.7 ± 6.01 *
Group B (unsensitized hairy mice) n=28	24.3 ± 0.88	27.5 ± 1.98
Group C (TDI-sensitized nude mice) n=10	25.8 ± 1.80	26.1 ± 1.9
Group D (unsensitized nude mice) n=10	26.4 ± 1.29	26.9 ± 1.91

*Significantly different ($p < 0.001$)

Fig. 1 Comparison of increment in ear thickness at 48hr postchallenge. Only group A showed significant ear swelling at 48hr after challenge.

TDI dermatitis in mice

Photo. 4 Part of an ear from a mouse in group A with a 5% TDI solution showing a pronounced swelling. Edema and cellular infiltration could be observed. HE stain; $\times 100$

Photo. 5 Part of an ear from a mouse in group B HE stain; $\times 100$

The ears of group A began to redden and swell drastically following the challenge. The ear thickness increased from 25.1×10^{-2} mm to 56.7×10^{-2} mm. This increase rate was 126%, a statistically significant difference.

The histological evaluation of the ear in group A showed edema in the subcutaneous tissue associated with remarked vasodilatation as shown in Photo 4 and 5. Also, contact dermatitis characterized by infiltration of leukocytes and dilatation of hair follicles was observed.

b) Nude mice

In sharp contrast to group A in which remarked changes were observed as stated above, group C only suffered from a temporary change of slight primary irritation on the skin to which was applied a highly concentrated solution as great as 30%. Moreover, these slight changes soon returned to normal. After challenge of 1% TDI, no change was observed on the ear.

Neither group C nor group D showed any change such as reddening or swelling of the ear upon challenge as shown in Table 1 and Fig. 1.

4. Macroscopic Observation and Weight Variation in Major Internal Organs

For the purpose of this experiment, special attention was paid to the thymus, spleen and liver. At autopsy, the following tissues were also taken for macroscopic examination: heart, lung, gastro-intestinal tract, kidney and testis. However, these tissues were within the normal range.

Accordingly, microscopic findings and variations of the weight of the thymus, spleen and liver are mentioned in this section.

a) Hairy mice

At macroscopic examination, treatment-related pathological changes were notably observed in the thymus. All animals showed atrophy of the thymus. The weight decreased from 41.2 ± 5.5 mg in group B to 22.9 ± 4.0 mg in group A being reduced by nearly half.

In addition, it was observed that the weight of the spleen (140.5 ± 9.3 mg) increased in group A as compared with group B (110.8 ± 9.7 mg).

These variations in the thymus and spleen weights were significant by statistical analysis ($p < 0.05$).

As for the liver, there was no special change in gross observation, while the differences in weight between group A and group B being 1.20 ± 0.13 g and 1.22 ± 0.12 g, respectively, were not significant.

b) Nude mice

Since there is no thymus in nude mice, the other organs were investigated with the same observations as in the hairy mice.

The weight change of the spleen showed a significant increase ($p < 0.05$) in group C, being 151.5 ± 35.1 mg, as compared with group D which was 111.4 ± 14.3 mg, but changes in

TDI dermatitis in mice

pathologic observation were not observed macroscopically.

As to the macroscopy and weight of the liver, they were within the normal range.

5. Histopathological Findings in Internal Organs

The macroscopic and weight changes in the various organs and tissues were mentioned above. The findings of some organs are briefly explained histopathologically below.

a) Thymus

No special histological change was noted on the cortex or medulla of any lobule in the hairy mice. Compared with group B, group A showed reduction of lymphocytes. This feature was found in all of 10 mice randomly selected from 28 mice of group A and only 3 out of 10 in group B.

b) Spleen

Although an increase in weight was clearly noted in group A, no change was observed histologically. This increase, of statistical significance, was not considered an important finding in view of the absence of pathological changes and was considered to be probably due to hyperemia, which caused dilatation of the venous sinuses.

c) Bone marrow

Polymorphonuclear leukocytes seemed to be somewhat increased in the bone marrow of group A. However, as the main purpose of this study was to detect TDI contact sensitivity, blood cell counting was not performed and the mechanism of the feature stated above remains to be studied in future.

DISCUSSION

TDI is widely known to irritate respiratory mucosa and cause symptoms similar to bronchial asthma. It has been considered as a sensitizer in industrial chemicals.

In spite of this fact, there has been only a few reports of allergic contact dermatitis caused by isocyanates. Fisher (1968) gave the reason for its rare occurrence, stating that since TDI is very highly irritable to the eye and respiratory organs, strict protection are widely adapted in the industrial fields, thus minimizing exposures to the skin. He also suggested that TDI itself may only be a weak sensitizer to the skin.

Nomura (1974) stated that respiratory mucosa was strongly irritated by TDI, causing asthmatic symptoms in many cases to persons exposed to this substance, but that the number of cases of dermatitis was comparatively less. Nishiura et al. (1964) and Tanaka et al. (1953) reported cases of workers who had contact with TDI or other isocyanates and developed dermatitis, but their cases were found to be of primary irritation.

Recently, however, Hino and Harada (1978) reported some cases of allergic contact dermatitis due to TDI, MDI and hydrogenated MDI among workers at urethane resin plants. They also experienced cases of contact dermatitis suspected to have been caused by alpha-naphthylisocyanate. Furthermore, Rothe (1976) reported 20 cases of occupational dermatoses in workers at the plants handling isocyanates (MDI, TDI, IPDI, etc.).

Although Fisher (1968) stated TDI may not be so dangerous, it cannot be denied from

above considerations that it is not only a sensitizer to the respiratory system, but also a skin sensitizer.

In general, mice, unlike guinea pigs, are known to be one of the difficult species to develop contact sensitivity. However, with TDI in the back-ear route method, allergic contact dermatitis occurred in BALB/c strain (Ishizu et al., 1978 and Nozawa et al., 1979). The ears of mice previously sensitized with TDI swelled after challenge with a low concentrated TDI solution, while unsensitized mice gave almost no reaction. This dermatitis was considered to be of delayed hypersensitivity.

Marked edema and dilatation of capillaries were the notable histopathological changes observed locally in the TDI challenged area of the ear. Polymorphonuclear and mononuclear cells infiltrated the area, with the former seeming to predominate. This predominance of polymorphocytes indicates that TDI dermatitis may be somewhat different from the typical delayed hypersensitivity. However, this feature is probably related to the invasive route of antigen or the timing of reaction. Thus, it can essentially be understood as allergic contact dermatitis.

At present, the onset mechanism of this contact dermatitis is considered mainly to involve T cells and macrophages. The finding which TDI allergic dermatitis did not occur in the athymic nude mice even sensitized with a highly concentrated solution suggests that T lymphocytes play the main role in causing the dermatitis. By means of cell transfer (Tanaka and Yamazaki, 1979), immunologic studies are in progress to elucidate the onset mechanism in the cellular level.

SUMMARY

Contact dermatitis occurred in BALB/c mice sensitized with 5% TDI in olive oil on the skin of the back for 5 consecutive days. Four days after the last application on their backs, the mice were challenged on both ears with 1% TDI in olive oil.

Ear swelling of these sensitized mice was more than twice of that compared with unsensitized mice.

Upon autopsy, significant atrophy was observed in the thymus and while, the spleen was quite enlarged in the sensitized mice.

In BALB/c-nu/nu (nude mice), severe contact dermatitis seen in the hairy mice did not develop, and only slight reddening and desquamation occurred even when 30% TDI in olive oil was applied on their backs. After challenge to the ears, no swelling was seen in this strain.

These results suggest that thymus-derived T lymphocytes may play an essential role in contact sensitivity to TDI in BALB/c mice.

REFERENCES

Charles, J. et al. (1976) : Hypersensitivity pneumonitis after exposure to isocyanates. *Thorax*, **31**,

TDI dermatitis in mice

127—136.

- Duncan, B. et al. (1962): Toluene diisocyanates inhalation toxicity: Pathology and mortality. *Industrial Hygiene Journal*, Nov.-Dec. 447—456.
- Duprat, P. et al. (1976): Pouvoir irritant et allergisant de deux isocyanates toluéne diisocyanate (T. D. I.), Diphénylméthane diisocyanate (M. D. I.). *European Journal of Toxicology*, **9** (1), 41—53.
- Fisher, A. A. (1968): Contact dermatitis. pp. 134—135. Lea & Febiger, Philadelphia.
- Hino, H. and Harada S. (1978): Allergic contact dermatitis due to isocyanate monomers. *Chiryo* (suppl.) **60** (8), 24—28.
- Iino, F. (1963): Occupational health in the polyurethane foam industry. *J. Scien. Labour (Rōdō Kagaku)*, **39** (7), 356—365.
- Ishizu, S. et al. (1978): Studies on the effects of TDI on the animals. from Proceedings of the 5th Meeting for the Study of Toxic Effect. *J. Toxicol. Scien.*, **3** (3), 254.
- Niemenhis, R. et al. (1965): Toxicity of chronic low level exposure to toluene diisocyanate in animals. *Industrial Hygiene Journal*, Mar.-Apr., 143—149.
- Nishiura, T. and Tani, T. (1964): On the Skin manifestation caused by Toluylene-diisocyanate (TDI). *Hifu to Hinyō*, **26** 56—63.
- Nomura, S. (1974): Occupational Dermatoses. *Rōdō Kagaku Sōsho*, **34**, Institute of Science of Labour, Tokyo, Japan.
- Nozawa, G. et al. (1979): Studies on the effects of TDI on the animals. *Jap. J. Hyg.*, **34** (1), 178.
- Peters, J. M. (1970): Studies of isocyanate toxicity. *Proc. Roy. Soc. med.*, **63**, 14—17.
- Rothe, V. A., (1976): Zur frage arbeitsbedingter hautschädigungen durch polyurethanchemikalien. *Berufsdermatosen*, **24** (1), 7—24.
- Scheel, L. D. et al. (1964): Immunochemical aspects of toluene diisocyanate (TDI) toxicity. *Industrial Hygiene Journal*, Mar.-Apr., 179—184.
- Silver, H. M. (1963): Toluene diisocyanate asthma. *Archives of Internal Medicine*, **112**, (Sep.), 401—404.
- Stevens, M. A. (1967): Use of albino guinea-pig to detect the skin-sensitizing ability of chemicals. *Brit. J. Indust. Med.*, **24**, 189—202.
- Swensson, A. et al. (1955): Injury to the respiratory tract by isocyanates used in making lacquers. *Brit. J. Indust. Med.*, **12**, 50—53.
- Tanaka, K. et al. (1979): A trial of contact dermatitis due to tolylene diisocyanate (TDI) in mice. *Jap. J. Hyg.*, **34** (1), 177.
- Tanaka, T. et al. (1953): Dermatitis caused by a bonding chemical for rubber. *J. Scien. Labour (Rōdō Kagaku)*, **29**, 126—130.
- Zapp, J. A., et al. (1957): Hazards of isocyanates in polyurethane foam plastic production. *A. M. A. Archives of Industrial Health*, **15**, 324—330.