Evaluation of Skin Irritants Caused by Organic Solvents by Means of the Mouse Ear Thickness Measurement Method

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Skin exposure to organic solvents can cause several problems. For example, it can cause irritant contact dermatitis, allergic contact dermatitis and scleroderma. The most common problem is the irritant contact dermatitis seen in industrial workers exposed to organic solvents. Organic solvents have two major factors that cause skin damage: one is defatting and the other is irritation. Besides lipid extraction, evaluation of skin irritation by organic solvents is very important when predicting skin damage. Skin irritation is visualized as erythema and edema, the result of a local inflammatory process. The mouse ear edema model has been previously utilized in a number of investigations such as mechanistic and quantitative studies of chemical irritation. Our purpose in this study was to evaluate the skin irritation caused by major organic solvents by using the mouse ear model so that organic solvents with minimal irritating properties or none at all can be selected for use in industries.

Materials and Methods

Animals and test materials. Female BALB/c mice weighing 20–24 g (Saitama Experimental Animals Supply Co. Ltd. Saitama Japan) were used for the studies. Organic solvents used in the studies included acetone, ethanol, n-hexane, methyl ethyl ketone (MEK), toluene, trichloroethylene, 1,1,1,-trichloroethane and m-xylene (Wako Pure Chemical, Osaka, Japan).

Application of test agent and ear measurements. In order to use unanesthetized mice, a specially designed restrainer was used to restrict mouse movement (Fig. 1). Organic solvents were applied by micro-pipette to the skin of the front and back of the ear (total 80 µl). Mouse ear thickness measurements (METM) were taken prior to application and several times after application. Three alternating measurements were made at an adjacent site using a pair of calipers with a pressure gage at 0 N (The lever of the calipers was slowly closed until the lever of the micrometer came in contact with the surface of the ear. This was judged visually and the pressure gage was confirmed to show 0 N) to avoid too much pressure.

Statistical Analysis. Triplicate measurements of ear thickness were averaged. To adjust for nontreatment-related differences, the time 0 value was subtracted from the postexposure values. ANOVA and Fisher’s PLSD were applied to the adjusted measurements comparing the response of control and treated mice at the various checking times after application.

Results

Dose-response for toluene irritancy

Concentrations of toluene (3, 30, 100%) diluted by ethanol were applied to the right ear. The ethanol vehicle was also applied to the left ear of individual mice. The resulting tissue swelling was measured several times after application (Fig. 2). At 3% and at 30%, toluene produced negligible swelling at all time point but, at 100% toluene produced significant ear swelling at 0.5, 1, 2, 6 and 24 h after application compared to the ethanol-treated control (P<0.01). These results indicated that the irritancy of toluene is dose dependent.

Comparison of irritancy of 8 organic solvents

METM was taken at 0.5, 1, 2, 6, 24, 48 and 72 h after application. Figure 3 shows the course of ear swelling up to 24 h after application. There was not any significant difference between the 8 organic solvents at the 48 and the 72 h check points after application (data not shown). Toluene (100%) and m-xylene produced much more ear swelling than ethanol at the 0.5, 1, 2, 6 and 24 h check points after application (P<0.01). Trichloroethylene, n-
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Fig. 2. Dose-response for toluene irritancy. Data represent the mean and standard error (SE) of values for mice treated with various doses of toluene. Asterisks (***) indicate that the values are statistically different (p<0.01) from ethanol-treated controls. The number of mice was 8 in each group.

Fig. 3. Time course of increase in ear thickness caused by 8 organic solvents. Data are the mean and standard error (SE) of values for mice treated with 8 organic solvents including ethanol as the control. The numbers (n) of mice used were as follows: acetone (n=5), ethanol (n=5), n-hexane (n=5), methyl ethyl ketone (MEK) (n=5), toluene (n=8), trichloroethylene (n=5), 1,1,1-trichloroethane (n=5) and m-xylene (n=8), respectively. Asterisks (**, p<0.01) (*) (p<0.05) indicate that values are statistically different from ethanol-treated controls at 0.5, 1, 2, 6 and 24 h after application.

hexane, 1,1,1-trichloroethane and MEK also produced more ear swelling than ethanol at several time points after application. Acetone produced negligible swelling compared to ethanol during the experiment.

Discussion

In this study, we selected 8 solvents (including ethanol) which could be classified into 5 groups (aromatic
hydrocarbon (AH), chloric aliphatic hydrocarbon (CAH), ketone, alcohol and others) according to chemical structure. AH (such as toluene, m-xylene) and CAH (such as trichloroethylene and 1,1,1-trichloroethane) produced ear swelling. In the ketone group, acetone as well as ethanol produced negligible swelling, while MEK produced ear swelling but the degree was less than in the AH, CAH and n-hexane groups. The alcohol and ketone groups with a polar molecule and a high dielectric constant have a tendency to cause less ear swelling than non-polar organic solvents. The present study confirmed the results of previous studies5) in other animal species on organic solvent-induced cutaneous irritation. Previous studies of skin irritancy reaction in guinea pigs and rabbits5) and also the histopathological study in the guinea pig12) showed that a skin lesion which is exposed to organic solvents is more severe in more lipophilic solvents, and less severe in more water-soluble solvents. We previously reported the result of measurement of plasma extravasation induced by organic solvents in the abdominal skin of hairless rats, indicating that toluene, m-xylene and cyclohexane induced plasma extravasation, but acetone did not induce plasma extravasation14). Moloney and Teal10) have demonstrated that the severity of the cumulative irritant reaction elicited by mineral oil fraction in mouse ear skin is dependent on the hydrocarbon chain length. These experiments indicate that the chemical structure may have some affect on skin irritancy but the mechanism is still not clear. In addition, the duration of chemical contact determined by the evaporation of organic solvents may affect ear swelling. In this study, the slower evaporating organic solvents, such as toluene and m-xylene, produced predominant ear thickness, whereas more rapid evaporating organic solvents, such as acetone, produced negligible swelling. This dose-response study indicated that even toluene, the most irritating solvent in this study, did not produce signs of skin irritancy at concentrations under 30%. It is therefore important to take into consideration the concentration.

In conclusion, we can predict to some degree the skin irritation caused by organic solvents by using mouse ear thickness measurement methods.

References