〈Original〉

Effects of Toluene Inhalation on Vasomotion and Lymph Circulation in Mice

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Effects of toluene as a major component of thinner on microcirculatory system were studied for a purpose of showing an unknown side of harmful actions caused by volatile solvents. Mice were exposed to 1000 ppm toluene at intervals of 30 seconds after each inhalation for 90 seconds, repeating it 4 or 8 cycles, and vasomotion of small arteries and arterioles in the skin as well as lymph circulation in the tail was observed intravital-microscopically. The following results were obtained: 1) Effects of toluene on vasomotion differed between small arteries and arterioles, being accelerated by the 4 cycles inhalation and inhibited by the 8 cycles inhalation in the arteries, while not accelerated by the 4 cycles inhalation and more stronger inhibited by the 8 cycles inhalation in the arterioles. 2) A definite trend of lymph circulation being inhibited by toluene inhalation was not noted, but travel rate of the fluorescein isothiocyanate-dextran was fluctuated in the 8 cycles inhalation.

Key Words Toluene, Thinner, Vasomotion, Lymph circulation, Microcirculation

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Introduction

According to the criminal white paper in Japan nearly 10,000 youngsters per annum in recent years were arrested by abuses of volatile solvents, which is one of the serious social problems. This study was performed for the purpose of showing an unknown side of harmful actions caused by toluene and of supplying new materials for health education to prevent youngsters from misuse of the volatile solvents.

General anesthetics such as pentobarbital, urethane, etc., are known to reduce the contractile activity of small arteries and arterioles, which makes vasomotion disappear[1,2]. Depressant effects on central nervous system same as that of these anesthetics are took notice of toluene[3], therefore, it is supposed that vasomotion is inhibited or disappeared by inhalation of toluene. The spontaneous time-dependent contraction and relaxation of small arteries, arterioles, and in some instances venules (termed vasomotion) has been observed since the inception of in vivo microvascular studies[4,5]. Vasomotion plays an important role in regulating blood pressure, forming lymph, reducing edema and improving the distribution of microvascular blood flow[6,7,8]. In the present study effects of toluene as a major component of thinner on vasomotion of skin microvasculature and lymph circulation in the tail of mice were observed intravital-microscopically.

Materials and Methods

Animals: Male BALB/C mice of 6 weeks old were raised with free intake of water and food during the test period. Three days before the experiment dorsal skinfold chambers were implanted into the back of mice for intravital-microscopic observations according to a previously described procedure[9]. Two symmetrical titanium frames (workshop, Department of Radiation Oncology, Harvard Medical School, Boston, USA, weight 3.2g), which are mirror images of each other were implanted so as to sandwich the extended double layer of skin. Following implantation of the transparent access chamber, animals were allowed to recover for 72h from microsurgery and anesthesia. Preparations fulfilling the criteria of intact microcirculation[10] were utilized for the experiments.

Toluene Inhalation: The animals inhaled toluene vapor of 1000 ppm (3750 mg/m^3: 10 times greater than the level permitted by Japan Society for Occupational Health) prepared by a self-made toluene vapor generator (Fig. 1) for 90 seconds at intervals of 30 seconds each, in 4 cycles (x4) or 8 cycles (x8) repeatedly. This is to follow the custom of the volatile solvent abusers inhaling that intermittently. Toluene special grade (Wako Pure Chemical Industries, Ltd., Osaka) was used. For

determination of toluene concentrations, a Kitagawa model gas detector (SA, Komyo-rikagaku-kogyo Ltd., Tokyo) was employed. Before, immediately and 30 minutes after the inhalation, vasomotions were observed with the intravital-microscope. Referring to Fig. 1, flow rate of the air pump was fixed at 600 m*l*/min, water bath temperature was kept at 45℃.

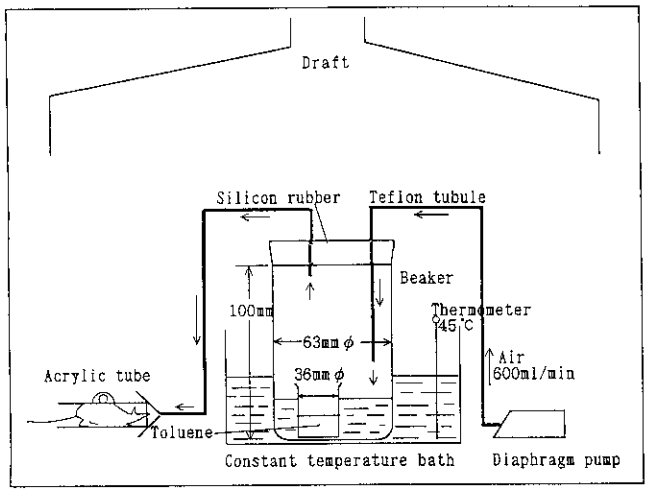

Fig. 1 Schematic diagram of 1000 ppm toluene vapor generator

Vasomotor Observations : Vascular systems branched in order from the same mean artery, namely, small arteries (A2 : twice branched, 40-70μm) and arterioles (A3 : thrice branched, 15-40μm) were selected previously, and at the time of observation fixed as above each vasomotion was recorded into a videocassette recorder (SVO-9600, Sony, Tokyo) for 2.5 minutes through a video camera (CCD Camera model KP-M1, Hitachi Denshi, Ltd., Tokyo) set to a microscope (x20 long working distance objective, NA 0.40, CDPlan, Olympus, Tokyo, x2.5 camera lens). The observations were made with illumination passed through the green-blue filter. This provides us with a dark and high-contrast outline of the column of blood. The x4 and x8 inhalations were carried out by using the same individual at an interval long enough for recovering from the effects of the former inhalation. Room temperature was controlled to be at 23.5±0.5℃ during the observation. Playing back a video tape, changes in diameter were traced for 2 minutes using an image shearing monitor (Model 908, IPM, San Diego, CA, USA), and AD converted voltages were recorded into the hard disk at a sampling frequency of twice per second. Time series data recorded in the disk were analyzed by the autoregressive spectral analysis[11] to determine the vasomotor frequency. The autoregressive model fitting was performed on the 240 time series data, and autospectrum was computed. Following this, the spectra of individual spectral component were calculated with their center frequencies, associated powers and component coefficients of variance by the component analysis. The frequency of the spectral component showing the greatest component coefficient of variance was employed as the vasomotor frequency. As amplitude showing a range of vascular calibers varied, a standard deviation of the time series data divided by the mean value of vascular calibers and expressed in percentage was used. Number of observed animals were five in each of the experimental groups.

Observation of Lymph Circulation : On a day before observation the tail of mice was treated with a depilatory cream. At the time of observation the tail was fixed on the stage of microscope with care not to press. Without the toluene inhalation, and immediately after the x4 and x8 inhalations, 1μ*l* of dextran (2,000,000 mol wt) labelled with fluorescein isothiocyanate (FITC) and dissolved to 10% (w/v) in physiological saline was injected intracutaneously at the tip of the tail using a 30G needle. Fixing the gain of video camera to the fluorescence microscope employing a x3 objective (NA 0.03, Planl, Olympus, Tokyo, x2.5 camera lens), images of the FITC spot were printed immediately and 10 minutes after injection by the video copy processor (SCT-P67, Mitsubishi Denki, Ltd., Tokyo). As high molecular dextran was transferred from the injected spot to the lymphatic capillaries and moved through the collecting lymphatics to the transport channels, distance of the FITC spot moved on the printed picture was measured to evaluate the rate of the matter travelled in the lymph circulation. Three animals were examined in each of the experimental groups.

Blood Pressure : To know the effect of toluene inhalation on the blood pressure, a polyethylene catheter (0.28 mm in inner diameter) was canulated into the left common carotid. The catheter was induced subcutaneously to the back and detained safely not to be droped off. After 24 hours the blood pressure at the common carotid was determined using the blood pressure measuring device (AP-611G, Nihonkodenkogyo, Ltd., Tokyo) at 0, 10, 20 and 30 minutes after inhalation of toluene under awakening conditions.

Statistical Analysis : Values at 0 and 30 minutes after inhalation in comparison with one before inhalation were shown in percentage. Significance at the level of 5 % examined by Student's t-test and population mean test.

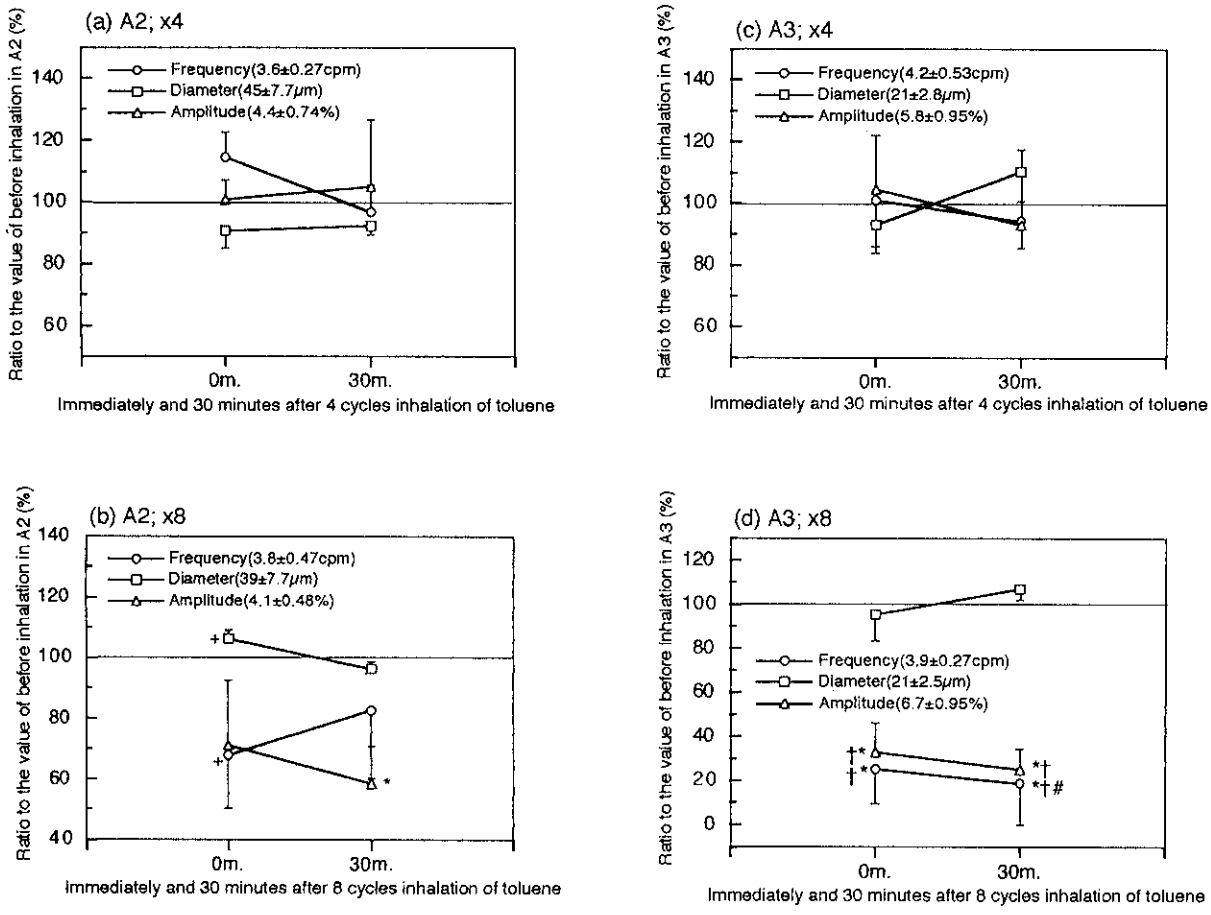

Fig. 2 Changes of vasomotion-related parameters of dorsal subcutaneous microcirculation in mice after inhalation of 1000 ppm toluene. Numbers in each parenthesis show values before inhalation as the mean±standard errors. Symbols for significances of $p<0.05$ in population test and in comparison with the corresponding values of (a), (c) and (b) present as * and +, †, #, respectively.

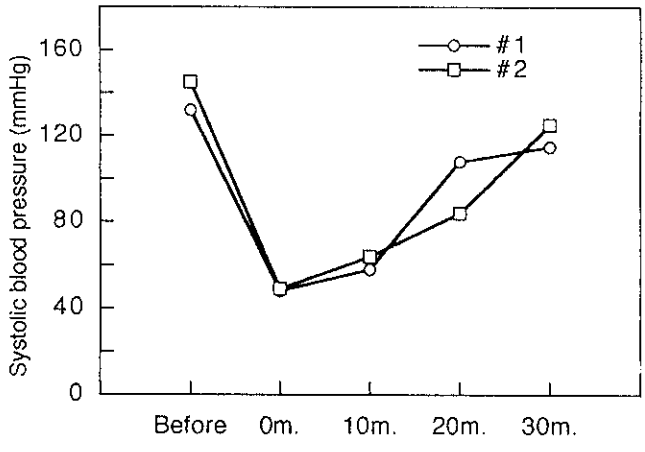

Fig. 3 Blood pressure change immediately and 10, 20 and 30 minutes after 4 cycles inhalation of 1000 ppm toluene.

Table 1 Lymphatic transport in tail of mice. Control and x4 or x8 indicate each group without and with 4 or 8 cycles of toluene inhalation.

No.	Control	×4	×8
#1	580	580	670
#2	670	580	830
#3	630	580	330

(μm/10min.)

Results

Changes of Vasomotor Frequency: No significant change was noted in A2 and A3 after the x4 toluene inhalation (Fig. 2(a), (c)), but at A2 it tended to increase immediately after inhalation, being noted on 4 cases out of 5. Significant reduction was shown in A2 and A3 after the x8 toluene inhalation (Fig. 2(b), (d)). Reduction of A3 was more remarkable than that of A2. Further, recovery noted at 30 minutes after the inhalation in A2 was not available in A3. Frequencies before the inhalation were 3.7 ± 0.81 cpm in A2 and 4.1 ± 0.90 cpm in A3.

Changes of Vasomotor Amplitude: After the x4 toluene inhalation both A2 and A3 showed no significant change (Fig. 2(a), (c)), though after the x8 inhalation it reduced significantly, which continued further without recovery even 30 minutes later (Fig. 2(b), (d)). Being the same as to the frequency, reduction in A3 was remarkable.

Changes of Mean Vascular Caliber: No significant change was noted, but patterns of change were different between A2 and A3. Namely, contraction at A2 was noted immediately after the x4 inhalation and remained on, while it dilated after the x8 inhalation and then recovered to the former state. On the other hand, at A3 it showed contraction immediately after the x4 inhalation followed by a trend of dilatation. The same trend was noted in the x8 inhalation (Fig. 2(a)-(d)).

Effects on Lymph Circulation: A definite trend of lymph circulation being inhibited by the toluene inhalation was not noted, but as shown in Table 1, travel rate of the FITC spot was fluctuated greatly in the x8 group, being not homoscedastic compared with variances of other two groups (Test for the equity of two variances, $p < 0.05$).

Changes of Blood Pressure by Toluene Inhalation: As shown in Fig. 3, it rapidly decreased by inhalation and recovered after that, but it did not return upto the level before inhalation for 30 minutes.

Discussion

In general anesthesia reduction of blood pressure by 20-30%, dilation of microvascular vessels and loss of vasomotion are noted[1]. On the other hand, after toluene inhalation no significant vascular dilation was noted, although marked hypotension by ca. 65% was noted immediately after the inhalation. Patterns of change in vascular calibers at A2 and A3 were different, which suggests that, even if it branched from the common blood vessel, changes of the blood pressure might differ by orders of branching. It means that at A3 vascular walls contracted immediately after the inhalation with marked reduction of blood pressure, and dilated at 30 minutes later when blood pressure was recovered. This indicates that vascular calibers passively changed by pressures received on vascular walls, while such change was not noted at A2.

Vasomotion was not lost completely at the level of exposure in this study. Vasomotor frequency and amplitude were decreased by the x8 toluene inhalation, which showed reduction of the vasomotor activity. Such reduction of vasomotor activity differed by A2 and A3, being noted more markedly at A3, which suggested that an incidence of vasomotor inhibition of the thinner blood vessels easily might be caused by toluene. It was not tried in this study, but further studies on vasomotion of the terminal arterioles (less than 15μm of diameter) should have to be made. Further, since vasomotor frequency of A2 was increased by the toluene inhalation of x4 and decreased by the x8 inhalation, it is considered that in small arteries the diphasic vasomotor changes might be activated at low concentrations of and inhibited at high concentrations of toluene.

Passive changes of vascular diameter of A3 are probably related to the less amount of smooth muscle in the vascular wall of A3. Difference in smooth muscle amount in vascular walls of A2 and A3 may give one of the reasons why effects of toluene on vasomotion differs between A2 and A3. Energy of vasomotion derives from contractive action of smooth muscle, therefore, it is considered that as the resistant power to vasomotor inhibitive action of toluene is weak at A3 with less muscles, vasomotion is easily inhibited.

Since vasomotion is considered to participate in transfer of fluid in the tissue spaces and formation of lymph[7,8], it is supposed that lymph circulation is inhibited by the suppressed vasomotion. After the x8 toluene inhalation vasomotion was inhibited significantly, however, as shown in Table 1, rate of travel in the lymphatic vessels of FITC-dextran was not delayed. Travel rate of the substance by lymph was fluctuated significantly by the x8 toluene inhalation, which might indicate the complexity of the effect of toluene inhalation on lymph circulation.

It is suggested by findings reported above that modulation of microcirculation would be participated in onset of encephalatrophy, hepatic and renal disturbances and others so far considered to be caused by harmful actions of toluene[3].

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