Neurophysiological Changes in Rats Subchronically Treated with Styrene or Its Metabolites

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Abstract: Neurophysiological Changes in Rats Subchronically Treated with Styrene or Its Metabolites: Junichi Misumi, *et al.* Department of Public Health and Hygiene, Oita Medical University—The purpose of this study was to clarify the causative agent(s) in the peripheral neuropathy induced by styrene. Styrene 600 or 300, and its metabolites; hippuric acid 600 or 300; mandelic acid 300; styrene oxide 100; mg/kg were subcutaneously injected into rats for 10 to 12 wk. The changes in maximum sensory conduction velocity (SCV), maximum motor conduction velocity (MCV), and motor distal latency (DL) in the rat's tail nerve were tested. Compared with the control group, decreases in MCV, SCV, and an increase in DL were observed in the rats injected with styrene 600, styrene oxide 100 and mandelic acid 300 mg/kg. No significant changes were found in the rats treated with hippuric acid 300 or 600 mg/kg. The MCV and SCV values in the styrene oxide 100 and mandelic acid 300 mg/kg groups were significantly lower, and DL values were significantly longer than those in the styrene 600 mg/kg group. It is presumed that the neuropathy caused by styrene is related to the neurotoxicity of its intermediate metabolites, namely mandelic acid and styrene oxide. It appears that the neurotoxicity of mandelic acid needs to be further evaluated in styrene-produced neuropathy.
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Styrene is an aromatic hydrocarbon produced in large quantities throughout the world. According to the United States's Toxic Release Inventory, styrene is 23rd on the list in terms of total releases and among the top in the list of carcinogenic VOCs[1, 2]. Wide range effects of occupational styrene exposure have been reported from barely detectable to severe acute neurotoxic effects. Such symptoms include neuropsychological changes, color vision loss, decreased nerve conduction velocity and electroencephalography, and functional and psychiatric impairment[2–20]. Welp *et al.*[21] concluded that exposure to styrene may contribute to chronic disease of the central nervous system from their international historical cohort study which involved 35,433 workers employed in the reinforced plastics industry, where high exposure to styrene occurs. Murata *et al.*[7] observed a significant difference in the median sensory nerve conduction velocity between 11 styrene workers in whom the mean exposure level was 30 ppm and 11 age-matched referents. Yuasa *et al.*[22] reported that the ulnar and peroneal motor distal latency of the workers with higher urinary mandelic acid (>250 mg/l) were significantly longer than in workers with lower urinary mandelic acid (<250 mg/l) and higher than in no exposure references. A few case reports have also shown that styrene is able to induce peripheral neuropathy[14, 20].

Animal experiments should play an important role in certifying the neurotoxic changes and mechanism induced by styrene. A few reports on peripheral neurotoxicity have been found[23–26], and there is conclusive evidence that styrene causes peripheral neurotoxicity, but the underlying mechanism of the peripheral neurotoxicity induced by styrene still remains unclear in both epidemiological and animal studies[27–30]. Ladefoged *et al.* reported that phenylglyoxylic acid, one of the styrene metabolites, reduced the peripheral nerve myelin sheath thickness[18], but it is not clear whether the reduction is connected with the reduction of conduction velocity in the peripheral nerve because Ladefoged *et al.* did measure the conduction velocity simultaneously.

On the other hand, there are some reports indicating that exposure to a relatively low concentration of styrene in the atmosphere caused no significant reduction in motor and sensory conduction velocity or no acute adverse effects on the nervous system[31–34]. The reason for these

inconsistencies in styrene-induced neurotoxicity is unclear.

In the present study, rats were treated with styrene or its metabolites, styrene oxide, mandelic acid and hippuric acid, in order to further confirm that styrene is able to induce peripheral neuropathy, and to clarify the causative agent(s) in the peripheral neuropathy induced by styrene.

Materials and Methods

Animal and grouping

Experiment I: Thirty 12-wk-old Donryu male rats, weighing 345 ± 10 g (mean $\pm$ SD) were divided into five groups named Styrene I-300, Styrene I-600, Hippuric acid-300, Hippuric acid-600 and Control I group, with six rats in each group. Styrene 300, 600 mg/kg, hippuric acid 300, 600 mg/kg and saline were subcutaneously injected into the above mentioned groups, once a day for six days a week, for a total of twelve weeks. The dosages of styrene 300, 600 and styrene oxide 100 mg/kg were about 1/10 of the LD_{50} for styrene and styrene oxide, respectively. We were also interested in seeing whether styrene 300 mg/kg, that has no carcinogenic or teratogenic effects, could induce peripheral neurotoxic changes. The rats were given access to pellet chew (Nihon Nosan MR-3-A) and tap water *ad libitum*.

Experiment II: Thirty six 17–18-wk old Donryu male rats, weighing 449 ± 9 g (mean $\pm$ SD) were divided into four groups named Styrene II-600, Styrene oxide-100, Mandelic acid-300 and Control II group, with 9 rats of each. Styrene 600, styrene oxide 100, mandelic acid 300 mg/kg and saline were subcutaneously injected into the above-mentioned groups, once a day for five days a week, for ten weeks. In the Styrene oxide-100 group, styrene oxide dissolved in bean oil was injected. Pellet Chew (Nihon Nosan MR-3-A) was given by a paired-feeding method to diminish the influence of different food intake on the development of neuropathy. The paired-feeding chow volume was chosen as the least volume that could be eaten by any of the animal groups.

All of the rats were maintained under a 12-h light/dark cycle in an air-conditioned laboratory (temperature $25 \pm 1°C$, humidity 50%). The treatment of rats in this study was performed according to the Guidelines of the Ethnical Committee for Animal Experiments at Oita Medical University.

Chemicals

All chemicals used were purchased from Wako Co, Tokyo, Japan without any purification. The purity for styrene oxide was more than 90%, and more than 98% for the others chemicals.

Electrophysiological examinations

An electrophysiological method was employed by using the tail nerve of the rats to quantitatively estimate the induced neurotoxic effects of the above chemicals. The techniques used have been described in detail by Misumi, and by Misumi and Nagano[36, 37]. An electric stimulator (MNS-1101. Nihon Kohden), an Addscope (ATAC-250, Nihon Kohden) and an X-Y recorder (Yodogawa Denki, 3086-22) were used in the determination. The maximum sensory conduction velocity (SCV), maximum motor conduction velocity (MCV), and motor distal latency (DL, the motor conduction time in distal part (6 cm) in the tail) were determined every four weeks in experiment I, and at the 4th, 6th, 8th and 10th weeks in experiment II. Three points named A, C and B represented a point 1 cm from the anus, 2.5 cm from the tail tip, and the midpoint between A and C in the tail, respectively. MCV in AB (MCV_{AB}), and SCV in AB (SCV_{AB}) and BC (SCV_{BC}) were determined. During the measurement, room temperature was maintained around 29°C. The rat's tail temperature was maintained from 33 to 36°C with a thermic ray lamp and detected by means of a needle probe thermistor (Nihon Kohden Co.) The animals were anesthetized with amobarbital sodium (60 mg/kg) before the examination and all the measurements were carried out within ten minutes.

Statistical analysis

ANOVA in the SPSS statistical software for repeated measuring design was used to analyze differences according to treatment, for different measuring times and according to the interaction between the treatment and the measuring time. Because the interactions between the treatment and the measuring time were significant, ONE-WAY analysis of variance, followed by Duncan multiple range tests in the same software were used for each measuring time.

Results

General conditions and neurological signs

Experiment I: There was no significant difference in body weight between the control and treated, or between the treated groups (data not shown). Fur loss was noticed in the Styrene I-600 group after three weeks of treatment. Three of the six rats in this group died suddenly just after the injection of styrene in the 8th wk, perhaps as a result of shock. In the Hippuric acid-600 group, the rats showed less movement, and there was more fur loss than in the control group. The rats in the Styrene I-300 and Hippuric acid- 300 groups were normal as in the Control I group.

Experiment II: Body weight in the Styrene oxide-100 group decreased significantly in comparison to that in the Control II group (437 ± 35 vs. 468 ± 20) from the 5th wk of treatment. This was the same as for the Mandelic acid-300 group from the 7th wk (437 ± 35 vs. 468 ± 20). These differences were not induced by difference in food intake as the rats in each group consumed the same

amount of chow as the rats in the Mandelic acid-300 group, which was the smallest of all the groups. Before the significant decrease in body weight, the rats in the Mandelic acid-300 group began to move less, walk erratically, and show signs of incontinence in passing urine from the 4th wk. The rats in the Styrene oxide-100 group showed similar but milder changes in erratic walking and incontinence. The rats in the Styrene oxide-100 group were excitable, and had showed shivering of the whole body and hopping. There was no definite appearance of neurotoxic change in the Styrene II-600 group.

Neurophysiological changes

In the present experiments, ANOVA analysis showed that the group treatment, the measuring time, and the interaction between these two factors were all able to influence SCV_{AB}, SCV_{BC}, MCV_{AB} and DL significantly. These indicate styrene and its metabolites can influence the SCV_{AB}, SCV_{BC}, MCV_{AB} and DL after sub-chronic administration, and can also influence the changes in SCV_{AB}, SCV_{BC}, MCV_{AB} and DL in rats with age. The increase in SCV_{AB}, SCV_{BC} and MCV_{AB} and the decrease in DL with age were consistent with results reported elsewhere[36–38]. Because the interactions between the treatments and measuring times were significant, ONE-WAY analysis of variance followed by Duncan's multiple range tests were carried out at each measuring time. In experiment I the values for SCV_{AB} in the treated groups were not decreased except for the decrease in SCV_{AB} in Styrene I-300 at the 4th wk (data not shown). Significant decreases in SCV_{BC} were observed in Styrene I-300 and Styrene I-600 at the 4th and 8th wk (Fig. 1-a), and of MCV_{AB} in Styrene I-300 (8th and 12th wk), and in Styrene I-600 (4th wk) (Fig. 1-b). The values for SCV_{AB}, SCV_{BC} and MCV_{AB} were not decreased in the Hippuric acid-300 and Hippuric acid-600 groups compared with those in the Styrene I-300 or Styrene I-600 group.

ANOVA analysis showed that the effects of treatment and measuring time and the interaction between them were significant in Experiment II (Table 1). In the experiment II, in comparison to Control II group, 600 mg/kg styrene induced a decrease in SCV_{AB} (8th wk), of SCV_{BC} (6th and 8th wk), of MCV_{AB} (10th wk), and an increases in DL (6th wk). Significant decreases were induced by 100 mg/kg styrene oxide of SCV_{AB} (6th and 10th wk), SCV_{BC} (6th, 8th and 10th wk), and MCV_{AB} (4th, 6th and 8th wk), and a significant increases in DL (6th wk) in comparison to those in Control II group. Additionally, significant decreases were induced by 100 mg/kg styrene oxide of SCV_{AB} (4th wk), SCV_{BC} (8th and 10th wk), and MCV_{AB} (4th and 6th wk) in comparison with those in the Styrene II-600 group. The dose of 300 mg/kg mandelic acid had similar effects on SCV_{AB}, SCV_{BC}, MCV_{AB} and DL as did 100 mg/kg styrene oxide in comparison to that in the control and Styrene II-600 groups. Detailed results are shown in Figs. 2-a, 2-b, 2-c and 2-d.

In conclusion, with the present doses, styrene was able to reduce the SCV and MCV, and to increase the DL. In comparison to that in the styrene group, the changes induced by styrene oxide and mandelic acid, but not hippuric acid, are stronger.

Discussion

It has been debated since the early 1990s that styrene, especially low dosage (20–50 ppm) styrene, results in neurotoxicity[35] but with the accumulation of the neurotoxic evidence induced by styrene, it is generally accepted that exposure to styrene can induce peripheral nerve disturbance, including a decrease in peripheral nerve conduction velocity in workers[6, 7, 12, 14, 17, 20, 38, 39] or in laboratory animals[5, 26, 40]. Little is known about the mechanism by which styrene influences neurophysiology.

It can be assumed that styrene either induces neurotoxic changes by itself or by its metabolites. It has been proven that styrene is transformed by oxidation to styrene oxide, by the cytochrome P450 enzyme complex[41]. Styrene oxide is converted to styrene glycol as a major component by styrene oxide hydrolase and styrene monooxygenase. Although styrene glycol can be partially transformed into a glucuronide, the major components are mandelic, benzoic, hippuric and phenylgloxilic acid[42].

In the present experiments, styrene and its two intermediate products, styrene oxide and mandelic acid, together with hippuric acid, one of the final metabolites of styrene, were subcutaneously injected into rats repeatedly for 10 to 12 wk and induced a significant decrease in SCV and MCV, and significant increases in DL. At the same time, the treatment of styrene and its metabolites can influence the increases in SCV and MCV, and the decrease in DL in rats with age (interaction between the treatment and measuring time). The results in detail showed that 600 mg/kg of styrene induced decreases in SCV and MCV and an increase in DL compared with those in the control group (Figs. 1-a–1-c and Figs. 2-a–2-d). From the results of these experiments it can be concluded that styrene is able to induce neurophysiological changes in the rat. Moreover, 100 mg/kg of styrene oxide, 1/6 dose of styrene 600 mg/kg, and 300 mg/kg of mandelic acid, 1/2 dose of styrene, induced greater decreases in SCV and MCV, and greater increases in DL than in the Styrene II-600 group (Fig. 2-a–2-d). It can be assumed that same dosages of styrene oxide or mandelic acid used for styrene will induce greater decreases in SCV and MCV, and greater increases in DL than the effects caused by styrene. It can also be supposed that styrene affects the peripheral nerve system by its intermediate metabolites, such as styrene oxide and mandelic acid.
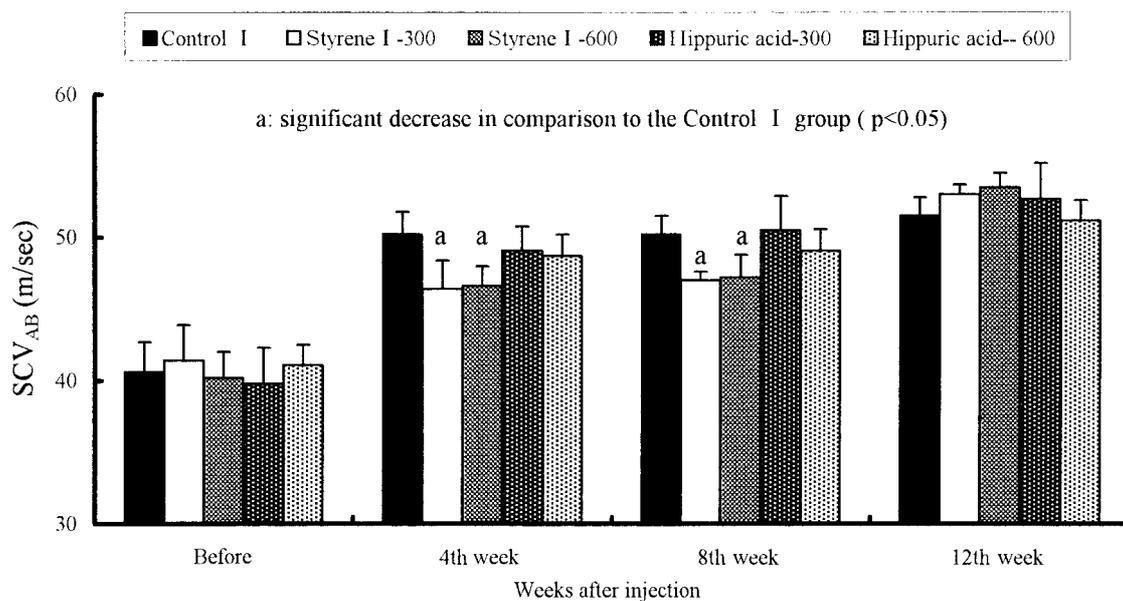
On the other hand, hippuric acid, one of the final

Fig. 1-a. Changes in sensory nerve conduction velocity (SCV) in the proximal part of the tail (SCV_{AB}) from 0 to 12 wk after the subcutaneous injection of styrene and hippuric acid 300 or 600 mg/kg and saline into the Styrene I-300, Styrene I-600, Hippuric acid-300, Hippuric acid-600 and Control-I groups, respectively. The data were obtained from 6 rats in every group for each measurement, except only from 4, 3 and 2 in the Styrene I-600 group in the 4th, 8th and 12th wk, and from 5 in the Styrene I-300 group and Hippuric acid-600 groups in the 8th and 12th wk.
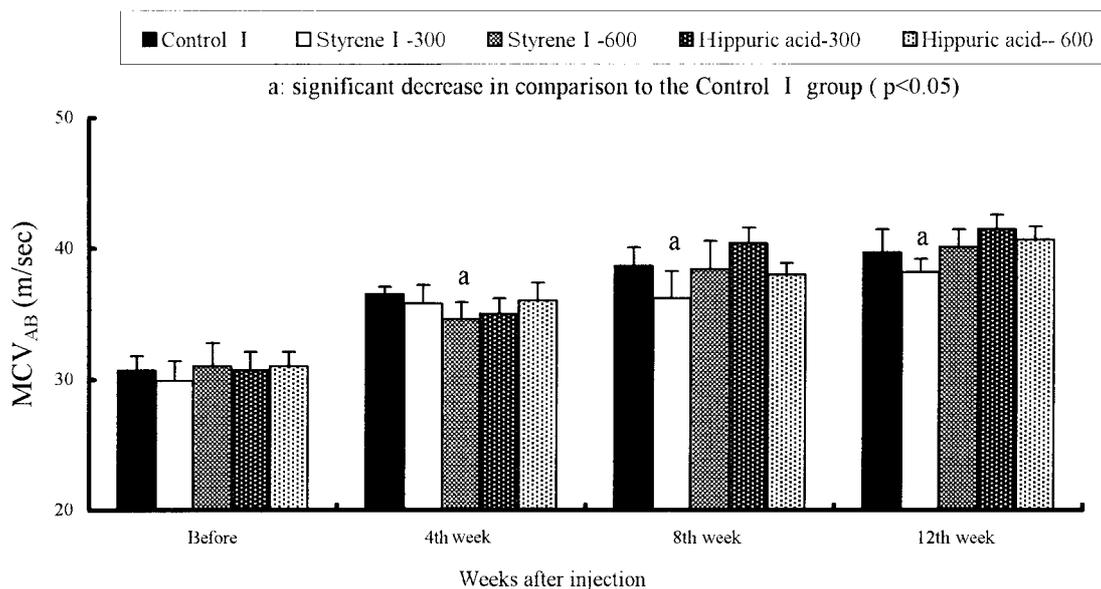

Fig. 1-b. Changes in motor nerve conduction velocity (MCV) in the proximal part of the tail (MCV_{AB}) from 0 to 12 wk after the subcutaneous injection of styrene and hippuric acid 300 or 600 mg/kg and saline into the Styrene I-300, Styrene I-600, Hippuric acid-300, Hippuric acid-600, and Control-I group, respectively. The data were obtained from 6 rats in every group for each measurement, except only from 4, 3 and 2 in the Styrene I-600 group in the 4th, 8th and 12th wk, and from 5 in the Styrene I-300 group and Hippuric acid-600 groups in the 8th and 12th wk.

Table 1. Significant levels of treatment, measuring time and interaction between them in Experiment II

	MCV_{AB}	SCV_{AB}	SCV_{BC}	DL
Treatment [a]	0.041	<0.0001	0.05	0.042
Measurement time [b]	0.005	<.0001	0.023	0.001
Interaction between the treatment & determination time	0.033	0.015	0.041	0.051

[a]: Subcutaneous injection of styrene (600 mg/kg), styrene oxide (100 mg/kg), or mandelic acid (300 mg/kg) and saline into four groups of rats. [b]: Five measurements of MCV_{AB}, SCV_{AB}, SCV_{BC} and DL at the 0, 4th, 6th, 8th and 10th wk.
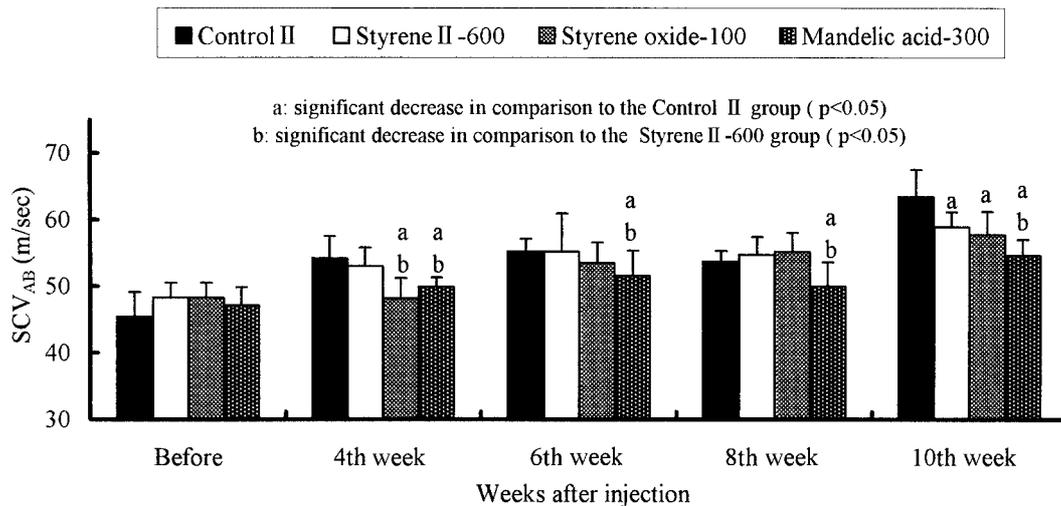

Fig. 2-a. Changes in sensory nerve conduction velocity (SCV) in the proximal part of the tail (SCV_{AB}) from 0 to 10 wk after the subcutaneous injection of styrene (600 mg/kg), styrene oxide (100 mg/kg), or mandelic acid (300 mg/kg) and saline into the Styrene II-600, Styrene oxide-100, Mandelic acid-300 and Control II groups, respectively. The data were obtained from 9 rats in every group for each measurement.
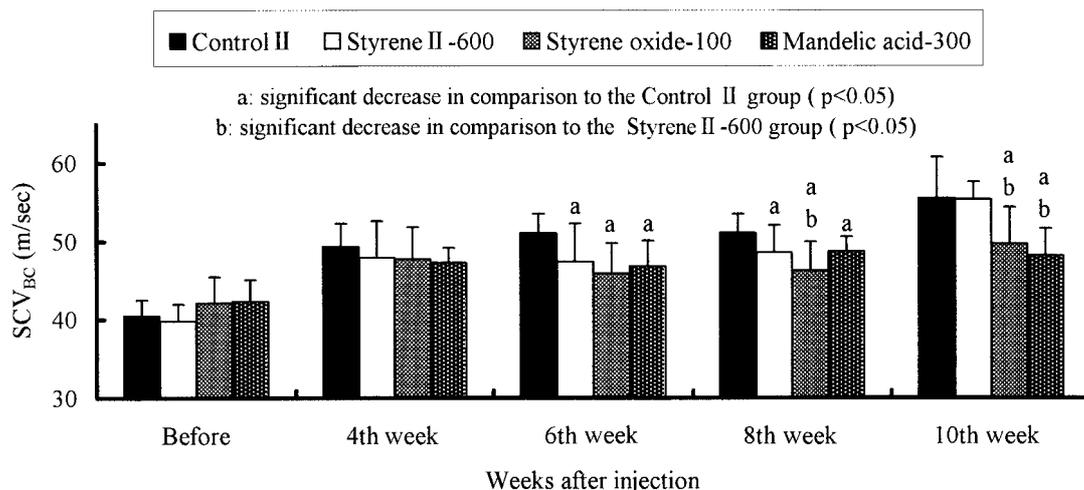

Fig. 2-b. Changes in sensory nerve conduction velocity (SCV) in the distal part of the tail (SCV_{BC}) from 0 to 10 wk after the subcutaneous injection of styrene (600 mg/kg), styrene oxide (100 mg/kg), or mandelic acid (300 mg/kg) and saline into the Styrene II-600, Styrene oxide-100, Mandelic acid-300 and Control II groups, respectively. The data were obtained from 9 rats in every group for each measurement.
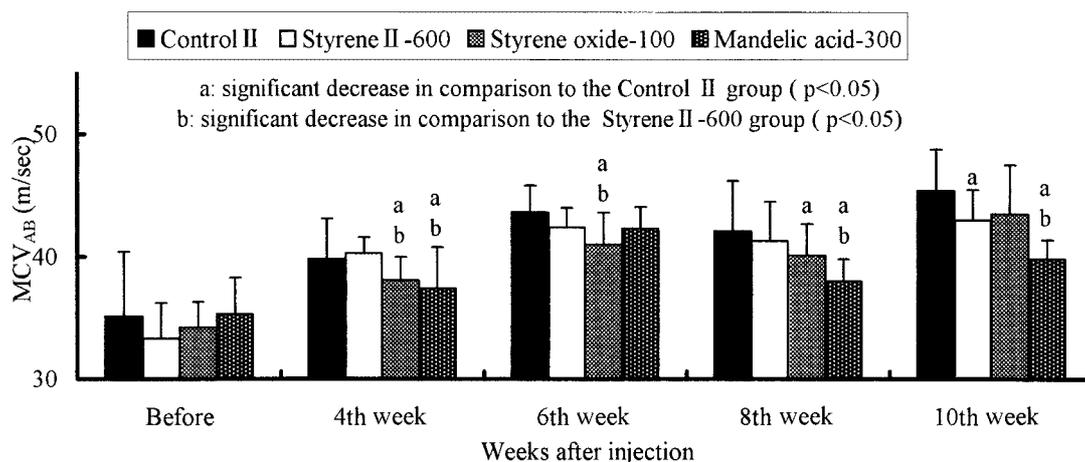
Fig. 2-c. Changes in motor nerve conduction velocity (MCV) in the proximal part of the tail (MCV_{AB}) from 0 to 10 wk after the subcutaneous injection of styrene (600 mg/kg), styrene oxide (100 mg/kg), or mandelic acid (300 mg/kg) and saline into the Styrene II-600, Styrene oxide-100, Mandelic acid-300 and Control II groups, respectively. The data were obtained from 9 rats in every group for each measurement.

Fig. 2-d. Changes in distal latency (DL) in the proximal part of the tail from 0 to 10 wk after the subcutaneous injection of styrene (600 mg/kg), styrene oxide (100 mg/kg), or mandelic acid (300 mg/kg) and saline into the Styrene II-600, Styrene oxide-100, Mandelic acid-300 and Control II groups, respectively. The data were obtained from 9 rats in every group for each measurement.
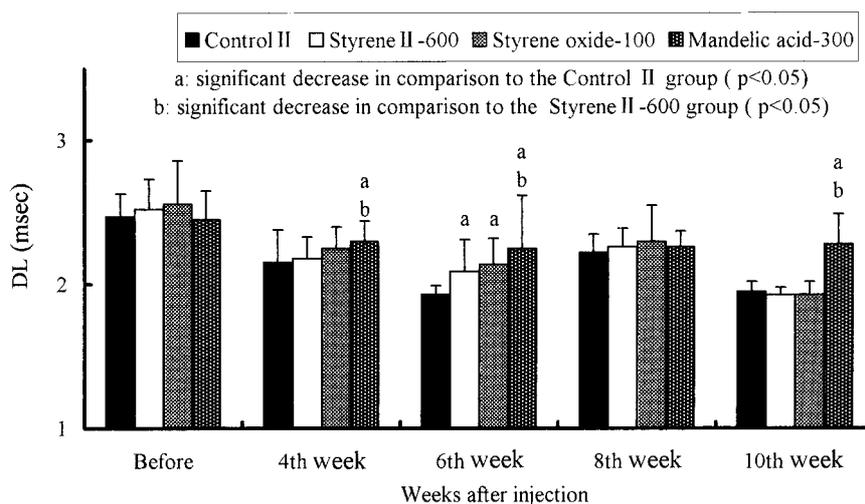
metabolites of styrene, had no significant effect on the SCV, MCV and DL in doses of 300 or 600 mg/kg compared with those in Control I. These results indicate that hippuric acid has no significant effect on peripheral nerves, and plays no role in the peripheral neuropathy induced by styrene.

There are two possible ways to explain why small doses of styrene oxide or mandelic acid produced greater changes in SCV, MCV and DL than those caused by relatively higher doses of styrene (6:1 and 2:1 in mg/kg).

One possibility is that styrene metabolizes to other nonneurotoxic metabolites other than styrene oxide and mandelic acid. Metabolites other than styrene oxide and mandelic acid have been demonstrated in previous reports[2, 41], but their neurotoxic effect is unknown. Another possibility is that either styrene oxide or mandelic acid metabolized from styrene can be eliminated rapidly, or that the rate of metabolism from styrene to styrene oxide is slow.

Urinary metabolites have been used as monitors in

humans after exposure to 168 or 840 mg/m^3 of styrene for 4 to 8 h[43]. Half times of 3.9 and 24.7 h (initial and final, respectively) were reported for mandelic acid. This means that the elimination of mandelic acid changes with time[43, 44]. The exposure doses can also affect the rate of metabolism of styrene. It has been supposed that metabolic saturation occurs at concentrations of 100 to 200 ppm styrene in humans, depending on the level of physical activity[45] but repeated exposure to styrene can stimulate the metabolic rate[46].

According to the above reports, it can be assumed that the styrene doses (300 and 600 mg/kg) in the present study were beyond metabolic saturation in the rats. Thereafter, styrene oxide and mandelic acid produced from styrene in the styrene groups were less than those used in the Styrene oxide and Mandelic acid groups in the present experiments (100 and 300 mg/kg, respectively), and caused fewer changes in SCV, MCV and DL.

Much attention has been paid to the toxic effects of styrene oxide. In the present experiments, mandelic acid also induced significant toxic changes in the peripheral nervous system. Further study on the toxic effects of mandelic acid is necessary.

References

1) Doa MJ. The toxics release inventory. J Hazard Waste Hazard Mat 1992; 9: 61–72.

2) Gibbs BF, Mulligan CN. Styrene toxicity: an ecotoxicological assessment. Ecotox Environ Safe 1997; 38: 181–194.

3) Mutti A, Mazzucchi A, Rustichelli P, Frigeri G, Arfini G, Franchini I. Exposure-effect and exposure-response relationships between occupational exposure to styrene and neuropsycholoical functions. Am J Ind Med 1984; 5: 275–286.

4) Flodin U, Ekberg K, Andersson L. Neuropsychiatric effects of low exposure to styrene. Brit J Ind Med 1989; 46: 805–808.

5) Rosengren LE, Haglid KG. Long term neurotoxicity of styrene. A quantitative study of glial fibrillary acidic protein (GFA) and S-100. Brit J Ind Med 1989; 46: 316–320.

6) Cherry N, Gautrin D. Neurotoxic effects of styrene: further evidence. Brit J Ind Med 1990; 47: 29–37.

7) Murata K, Araki S, Yokoyama K. Assessment of the peripheral, central and autonomic nervous system function in styrene workers. Am J Ind Med 1991; 20: 775–784.

8) Matikainen E, Forsman-Gronholm L, Pfaffli P, Juntunen J. Nervous system effects of occupational exposure to styrene: a clinical and neurophysiological study. Environ Res 1993; 61: 84–92

9) Groth-Marnat G. Neuropsycholoical effects of styrene exposure: a review of current literature. Percept Mot Skills 1993; 77: 1139–1149

10) Jegaden D, Amann D, Simon JF, Habault M, Legoux B, Galopin P. Study of the neurobehavioral toxicity of styrene at low levels of exposure. Int Arch Occup Environ Health 1993; 64: 527–531.

11) Edling C, Anundi H, Johanson G, Nilsson K. Increase in neuropsychiatric symptoms after occupational exposure to low levels of styrene. Brit J Ind Med 1993; 50: 843–850.

12) Stetkarova I, Urban P, Prochazka B, Lukas E. Somatosensory evoked potentials in workers exposed to toluene and styrene. Brit J Ind Med 1993; 50: 520–527.

13) Chia SE, Jeyaratnam J, Ong CN, Ng TP, Lee HS. Impairment of color vision among workers exposed to low concentrations of styrene. Am J Ind Med 1994; 26: 481–488.

14) Gobba F, Cavalleri F, Bontadi D, Torri P, Dainese R. Peripheral neuropathy in styrene-exposed workers. Scand J Work Environ Health 1995; 21: 517–520.

15) Tsai SY, Chen JD. Neurobehavioral effects of occupational exposure to low level styrene. Neurotoxical Terratol 1996; 18: 463–469.

16) Campagna D, Gobba F, Mergler D, Moreau T, Galassi C, Cavalleri A, Huel G. Color vision loss among styrene-exposed workers neurotoxicological threshold assessment. NeuroToxicology 1996; 17: 367–374.

17) Yuasa J, Kishi R, Eguchi T, et al. Study of urinary mendelic acid concentration and peripheral nerve conduction among styrene workers. Am J Ind Med 1996; 30: 41–47.

18) Ladefoged O, Lam Hr, Ostergaard G, et al. Toxicity of the styrene metabolite, phenylglyoxylic acid, in rats after three months' oral dosing. Neurotoxicology 1998; 19: 721–737.

19) Coccini T, Randine G, Li B, Manzo L, Costa LG. Effect of styrene on monoamine oxidase B activity in rat brain. J Toxicol Environ Health 1999; 56: 59–68.

20) Fung F, Clark RF. Styrene-induced peripheral neuropathy. J Clin Toxicol 1999; 37: 91–97.

21) Welp E, Kogevinas M, Andersen A, et al. Exposure to styrene and mortality from nervous system diseases and mental disorders. Am J Epidemiol 1996; 144: 623–633.

22) Yuasa J, Kishi R, Eguchi T, et al. Study of urinary mandelic acid concentration and peripheral nerve conduction among styrene workers. Am J Ind Med 1996; 30: 41–47.

23) Savolainen H, Vainio H. Organ distribution and nervous system biding of styrene and styrene oxide. Toxicology 1977; 8: 135–141.

24) Savolainen H. Some aspects of the mechanisms by which industrial solvents produce neurotoxic effects. Chem-Biol Interact 1977; 18: 1–10.

25) Seppalainen AMK, Harkonen H. Neurophysiological findings among workers occupationally exposed to styrene. Scand J Work Environ Health 1976; 2: 40–146.

26) Teramoto K, Wakitani F, Horiguchi S, et al. Comparison of the neurotoxicity of several chemicals estimated by the peripheral nerve conduction velocity in rats. Environ Res 1993; 62: 148–154.

27) Baker EL Jr, Smith TJ, Landrigan PJ. The neurotoxicity of industrial solvents: a review of the literature. Am J

Ind Med 1985; 8: 207–217.

28) Baker EL Jr. Epidemiological issues in neurotoxicity research. Neurobehav Toxicol Teratol 1985; 7: 293–297.

29) Bleecker ML, Bolla KI, Agnew J, Schwertz BS, Ford DP. Dose-related subclinical neurobehavioral effects of chronic exposure to low levels of organic solvents. Am J Ind Med 1991; 19: 715–728.

30) Hogstedt C. Has the Scandivavian solvent syndrome controversy been solved? Scand J Work Environ Health 1994; 20: 59–64.

31) Seppalainen AM. Neurotoxicity of styrene in occupational and experimental exposure. Scand J Work Environ Health 1978; 4 (Suppl 1, 2): 181–183.

32) Edling C, Ekberg K. No acute behavioral effects of exposure to styrene: a safe level of exposure? Brit J Ind Med 1985; 42: 301–304.

33) Triebig C, Schaller KH, Valentin H. Investigations on neurotoxicity of chemical substances at the workplace. Longituding studies with determination of nerve conduction velocities in person occupationally exposed to styrene. Int Arch Occup Environ Health 1985; 56: 239–247.

34) Triebig C, Lehrl S, Weltle D, Schaller KH, Valentin H. Clinical and neurobehavioural study of the acute and chronic neurotoxicity of styrene. Brit J Ind Med 1989; 46: 799–804.

35) Rebert CS, Hall TA. The neuroepidemiology of styrene: a critical review of representative literature. Creit Rev Toxicol 1994; 24: S57–S106.

36) Misumi J. Electrophysiological studies in vivo on peripheral nerve function and their application to peripheral neuropathy produced by organic mercury in rats. II Measurement of the maximum conduction velocity of compound action potentials in the tail nerve of healthy rats. Kumamoto Med J 1979; 32: 1–14.

37) Misumi J, Nagano M. Neurophysiological studies on the relationship between structural properties and neuropathy of aliphatic hydrocarbons in rats. Brit J Ind Med 1984; 41: 526–532.

38) Misumi J, Nagano M, Futatsuka M, Zhao W, Kudo M. Different administration schedules of the same dose of 2,5-hexanedione influence the development of neuropathy and the toxicokinetics. Neurochem Res 1997; 22: 27–32.

39) Behari M, Choudhary C, Roy S, Maheshwari MC. Styrene-induced peripheral neuropathy. A case report. Eur Neurol 1986; 25: 424–427.

40) Kulig BM. The neurobehavioral effects of chronic styrene exposure in the rat. Neurotoxicol Teratol 1988; 10: 511–517.

41) Watabe T, Isobe M, Sawahata T, Yoshikawa K, Yamada S, Takabatake E. Metabolism and mutagenicity of styrene. Scand J Work Environ Health 1978; 4 (Suppl 1, 2): 142–155.

42) Ohtsuji H, Ikeda M. The metabolism of styrene in the rat and the stimulatory effect of phenobarbital. Toxicol App. Pharmacol 1971; 18: 321–328.

43) Guillemin MP, Bauer D. Human exposure to styrene. III. Elimination kinetics of urinary mandelic and phenylglyoxylic acids after single experimental exposure. Int Arch Occup Environ Health 1979; 44: 249–263.

44) Sumner SJ, Fennell TR. Review of the metabolic fate of styrene. Crit Rev Toxicol 1994; 24 (S1): S11–S33.

45) Lof A, Johanson G. Dose-dependent kinetics of inhaled styrene in man. IARC Sci Publ 1993; 1993 (127): 89–99.

46) Sumner SCJ, Cattley RC, Asgharian B, Janszen DB, Fennell TR. Evaluation of the metabolism and hepatotoxicity of styrene in F344 rats, B6C3F1 mice, and CD-1 mice following single and repeated inhalation exposures. Chem-Bio Interact 1997; 106: 47–65.