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A Preliminary Study on Delayed Vestibulo-Cerebellar Effects of Tokyo Subway Sarin Poisoning in Relation to Gender Difference: Frequency Analysis of Postural Sway

Kazuhito Yokoyama, MD
Shunichi Araki, MD
Katsuyuki Murata, MD
Mariko Nishikitani, MHE
Tetsu Okumura, MD
Shinichi Ishimatsu, MD
Nobukatsu Takasu, MD

To evaluate delayed (long-term) effects of acute sarin poisoning on postural balance, nine male and nine female victims of the Tokyo Subway Sarin Poisoning in Japan (sarin cases) were examined by computerized posturography 6–8 months after the poisoning. Their plasma cholinesterase activities (ChE) on the day of the poisoning (March 20, 1995) were 13–95 (mean 68.2) IU/l for females and 19–131 (mean 75.9) IU/l for males, which were not significantly different between the two sexes. In females, the postural sway of low frequency (0–1 Hz) in the anterior-posterior direction and area of sway with eyes open was significantly larger in the cases than in the controls. Romberg quotients for the low-frequency sway in the anterior-posterior direction for females and low-frequency sway and length of sway in the medio-lateral direction for males were significantly related to log ChE. It is suggested that a delayed effect on the vestibulo-cerebellar system was induced by acute sarin poisoning; females might be more sensitive than males.

From the Department of Public Health, Faculty of Medicine, University of Tokyo, Tokyo, Japan (Dr Yokoyama, Dr Araki, Dr Murata, Ms Nishikitani); and the Emergency Department, St. Luke's International Hospital, Tokyo, Japan (Dr Okumura, Dr Ishimatsu, Dr Takasu).

Address correspondence to: Kazuhito Yokoyama, MD, Department of Public Health, Faculty of Medicine, University of Tokyo, Bunkyo-ku, Tokyo 113, Japan.

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On June 27, 1994, about 600 residents in Matsumoto city, Japan, were poisoned with sarin (methyl phosphonofluoridic acid 1-methylethyl ester), which is a organophosphate compound known as a chemical warfare nerve agent and potent acetylcholinesterase inhibitor, presumably by the Aum Shinrikyo Cult.[1] All affected people recovered and showed no abnormal findings on routine neurological and laboratory examinations, except for one patient, who had severe anoxic encephalopathy due to respiratory arrest.[2] On March 20, 1995, approximately 5500 people were poisoned with sarin in subways in Tokyo, Japan (Tokyo Subway Sarin Poisoning), which has also criminated the Cult.[1] According to a follow-up study on 640 cases,[3] no clinical abnormalities were detected 3 months after the poisoning, except for two cases who died just after being admitted to the hospital. Thus delayed (long-term) neurological effects of acute sarin poisoning have not been reported in these two studies.

On the other hand, we have recently found significant decrease in performance on neurobehavioral testing in cases of the Tokyo Subway Sarin Poisoning with no clinical signs 6–8 months after the poisoning.[4] Also, electroencephalogram abnormalities have been observed in chemical plant workers one year after accidental exposure to sarin.[5] Therefore, delayed sequelae with subtle neurological changes may be caused by acute sarin poisoning; extensive investigations using sensitive methods are necessary.

A case report on organophosphate poisoning performed in an attempt to commit suicide showed that cerebellar ataxia started about 5 weeks after the poisoning and persisted for more than 2 months.[6] Significant increase in postural sway has been demonstrated in organophosphate pesticide applicators by computerized static posturography, a technique for measuring displacements of the body's center of pressure in a horizontal plane by the use of a force platform connected to a microcomputer.[7] Because evidence has been given that acetylcholine mediates vestibular and cerebellar functions,[8,9] inhibition of acetylcholinesterase may lead to postural dysequilibrium.

In the study presented here, the postural sway of 18 cases of the Tokyo Subway Sarin Poisoning was examined by computerized static posturography with frequency analysis of sway, which has been used in our previous studies.[10–14] It is believed that the frequency analysis would be useful for understanding the effects of sarin, as clinical studies have suggested that disorders of the vestibular, cerebellar, and spinocerebellar-afferent systems are related to changes in specific frequency domains of postural sway, although such relations are not fully confirmed.[15–20] The study might suggest which part of the vestibular, cerebellar and/or spinocerebellar system is affected.

Attention was paid to differences in the effects of sarin by gender in the study presented here because it had been reported that female sex amplifies the neurologic effects of organophosphates, such as malaoxon.[21]

Methods

Subjects

The subjects of the study (sarin cases) were nine male and nine female victims of the Tokyo Subway Sarin Poisoning. The 18 sarin cases were transported to St. Luke's International Hospital to receive emergency medical checks and treatment just after the poisoning.

On admission to the hospital, plasma cholinesterase (ChE) activities measured between 13 and 95 (mean 68.2) IU/l for females and between 19 and 131 (mean 75.9) IU/l for males; except for three males, all cases showed the values lower than "normal" range (100–250 IU/l). There was no significant difference in the ChE activities between female and male sarin cases ($P > 0.05$). Major symptoms that the cases complained of at the time of poisoning were as follows: dyspnea (six females, five males), headache (six females, five males), nausea (four females, five males), feeling of asthenia (four females, four males), diplopia (four females, three males), ocular pain (four females, three males), diarrhea (three males), sneezing (one female, two males), paresthesia (one female, two males), vomiting (two females, one male), darkness of visual field (two males), decreased visual acuity (two males), anxiety (two males), cough (one female, one male), loss of consciousness (one female, one male), and heart arrest (one female).

The sarin cases were examined from September to November 1995, ie, 6–8 months after the poisoning. At the time of the study, their ages were 19–58 (mean 29.4) and 23–51 (mean 31.8) years for females and males, respectively. Similarly, height was 153–163 (mean 158.2) and 167–178 (mean 171.1) for females and males, respectively; body weight was 45–50 (mean 47.7) and 60–78 (mean 64.8) kg, respectively. In an interview, female cases reported that they drank alcoholic beverages equivalent to 0–123 (mean 25.2) ml of ethanol per week, which was calculated using ethanol content in beverages, ie, 15% for sake, 43% for whiskey, and 5% for beer; similarly, male cases consumed 1–249 (mean 131.4) ml of ethanol per week. Plasma ChE activities were measured again in 13 cases on the days of the study, and all were found to be within "normal" range.

Control subjects were 53 "healthy" volunteers, consisting of 31 males and 24 females. Their ages were 23–47 (mean 35.1) for females and 19–54 (mean 31.5) years for males. Their height was 154–161 (mean 156.3) and 162–182 (mean 171.3) cm for females and males, respectively; body weight was from 45–61 (mean 50.3) and 46–100 (mean 66.4) kg, respectively; alcohol consumption was 0–400 (mean 60.5) and 0–749 (mean 117.2) ml of ethanol per week, respectively. The differences in age, height, body weight, and alcohol consumption between the cases and controls were not significant ($P > 0.05$) for both females and males. None of the cases or controls had orthopedic or other confounding disease, such as alcoholic dependency, or had been exposed in their workplace to such chemicals as pesticide or heavy metals, which might have neurotoxic effects.

The nature of the procedure in this study was fully explained to all subjects, and the study was carried out with each subject's informed consent.

Computerized Static Posturography

Postural balance was quantitatively measured by the method we reported previously.[10–14] Measurement was conducted in a quiet room with flat floor by the use of a strain gauge–type force platform (Static Sensograph 1G06; NEC Sanei, Tokyo, Japan) connected to a microcomputer (PC9801; NEC, Tokyo, Japan) via an analog-to-digital converter (ANALOG PRO; Canopus, Tokyo, Japan). Subjects were instructed to refrain from alcohol or drugs from the day before the study (12 hours or more prior to the study).

Subjects were asked to stand quietly on the platform for 60 seconds with their eyes open and then for 60

TABLE 1
Differences in Sum of Square Root of Power (cm) for Each Frequency Range of Postural Sway (Means with Standard Deviations in Parentheses) between Sarin Cases (Nine Females and Nine Males) and Controls (18 Females and 35 Males) by Gender: Analysis of Covariance with Age, Height, Body Weight, and Alcohol Consumption as Covariates

	Females		Males		Differences (*F* Values)	
Frequency Range	Cases	Controls	Cases	Controls	Females	Males
Eyes open						
Anterior-posterior						
0–1 Hz	3.47 (1.02)	2.72 (0.50)	3.00 (0.65)	3.00 (0.68)	9.75*	0.00
1–2 Hz	0.46 (0.15)	0.43 (0.12)	0.62 (0.13)	0.53 (0.20)	2.83	0.02
2–4 Hz	0.25 (0.11)	0.24 (0.07)	0.28 (0.09)	0.31 (0.14)	4.11	0.29
Medio-lateral						
0–1 Hz	2.82 (0.82)	2.59 (0.50)	2.66 (0.55)	2.69 (0.66)	3.04	0.00
1–2 Hz	0.58 (0.25)	0.60 (0.25)	0.62 (0.13)	0.66 (0.25)	3.51	0.18
2–4 Hz	0.25 (0.11)	0.24 (0.07)	0.28 (0.09)	0.31 (0.14)	3.01	0.35
Eyes closed						
Anterior-posterior						
0–1 Hz	3.85 (1.03)	3.63 (1.07)	3.35 (0.58)	3.47 (0.79)	0.24	0.14
1–2 Hz	0.56 (0.24)	1.10 (0.31)	0.73 (0.27)	0.69 (0.23)	0.00	0.25
2–4 Hz	0.34 (0.19)	0.37 (0.08)	0.41 (0.21)	0.41 (0.17)	0.02	0.00
Medio-lateral						
0–1 Hz	3.52 (1.02)	3.52 (0.82)	3.30 (0.75)	3.24 (0.97)	0.03	0.06
1–2 Hz	0.88 (0.48)	1.10 (0.31)	0.73 (0.27)	0.95 (0.39)	0.04	0.02
2–4 Hz	0.31 (0.13)	0.37 (0.08)	0.41 (0.21)	0.41 (0.16)	0.49	0.00

* $P < 0.01$.

TABLE 2
Differences in Length of Sway Path in Anterior-Posterior and Medio-Lateral Directions (Dx and Dy, respectively) and Area of Sway (Area) (Means with Standard Deviations in Parentheses) between Sarin Cases (Nine Females and Nine Males) and Controls (18 Females and 35 Males) by Gender: Analysis of Covariance with Age, Height, Body Weight, and Alcohol Consumption as Covariates

	Females		Males		Differences (*F* Values)	
Frequency Range	Cases	Controls	Cases	Controls	Females	Males
Eyes open						
Dx (cm)	45.1 (16.7)	44.2 (11.0)	46.3 (10.6)	50.4 (14.7)	3.47	0.67
Dy (cm)	32.0 (9.4)	35.1 (8.2)	37.9 (8.9)	41.5 (11.7)	0.15	0.88
Area (cm^2)	2.7 (1.3)	2.1 (0.7)	2.3 (0.6)	2.6 (1.2)	4.84*	0.19
Eyes closed						
Dx (cm)	60.4 (24.1)	61.4 (16.9)	63.7 (18.6)	65.8 (20.8)	0.66	0.06
Dy (cm)	45.0 (15.6)	47.5 (12.2)	58.4 (20.1)	55.4 (15.4)	0.31	0.24
Area (cm^2)	4.0 (1.9)	3.4 (2.0)	3.1 (1.0)	3.5 (1.6)	1.42	0.29

* $P < 0.05$.

seconds with their eyes closed. Strain gauges at the three corners of the platform measured vertical forces and converted them to electric voltages; the medio-lateral (right-left) and anterior-posterior location of the body's center of pressure (CP) in the horizontal plane was calculated from the voltages sampled at a frequency of 20 Hz by the microcomputer and recorded on a disk.

Displacements of the body's CP in the both directions were then analyzed by Fast Fourier Transformation analysis.[14,16] The total duration of sampling was 51.2 seconds (1024 points). The span of frequencies analyzed ranged from 0 to 4 Hz (0.02 Hz steps). The sum of the root squares of the power calculated from the power spectrum between (1) 0 and 1 Hz, (2) 1 and 2 Hz, and (3) 2 and 4 Hz served as measures of the amount of postural sway for each frequency range with eyes open and closed.

Lengths of displacement of the CP in the medio-lateral and anterior-posterior directions within each sampling time were summed up for each direction and defined as the length of sway path of the body's CP in the medio-lateral (Dx) and anterior-posterior (Dy) directions, respectively; the area included within the sway path traveled by the CP during 60 seconds (Area) was also calculated. Romberg quotients, ie, the ratio of the value with eyes closed to that with eyes open,[14,16] for the power of the sway for each frequency band—Dx, Dy, and Area—were calculated.

Some studies[15–20] have suggested that (1) the lesion of the vestibulo-cerebellum (lower vermis), which contains the vestibulo-cerebellar pathway, shows a sway of no specific frequencies with eyes open (low Romberg quotient), (2) the lesion of the anterior cerebellar lobe is related to a sway of high frequency (2–4 Hz) predominantly in anterior-posterior direction with eyes closed (high Romberg quotient), and (3) the lesion in the spinocerebellar afferent pathway (including the spinocerebellar tract and proprioceptive nerve fibers in the legs) is associated with an omnidirectional sway of low frequency (1 Hz or less) with eyes closed (high Romberg quotient).

Statistical Analysis

Differences in all measures between the sarin cases and controls were examined by gender, using the analysis of covariance with age, height, body weight, and alcohol consumption as covariates to control for possible confounding effects of these variables. Relations of postural sway to ChE activities and log ChE on the day of the poisoning were assessed in female and male sarin cases, respectively, by Pearson's product moment correlation and by stepwise multiple regression analysis in which the ChE (or log ChE), age, height, body weight, and alcohol consumption, were entered and removed at $P < 0.05$ as predictors. All of the statistical analyses were performed by SPSS version 4.0,[22] using a microcomputer (Mac IIVx; Apple, Cupertino, CA).

Results

Differences in postural sway between sarin cases and controls by sex are shown in Tables 1 and 2. In females, the power of the sway of 0–1 Hz in sarin cases was significantly larger than that in controls in the anterior-posterior direction when

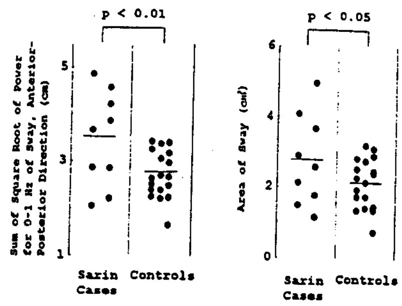

Fig. 1. Differences in the sum of the square root of power of sway and area of sway between nine female sarin cases and 18 female controls by analysis of covariance (eyes open). Transverse lines represent mean values.

eyes were open (Table 1); similarly, the area of the sway with eyes open was significantly larger in the cases than in the controls (Table 2). Figure 1 illustrates the differences in these measures of postural sway between the female cases and controls. On the other hand, none of the postural sway measures was significantly different between the male cases and controls.

Romberg quotient was correlated significantly with the log ChE for the power of the sway of 0–1 Hz in the anterior-posterior direction in the female cases, and for the power of the sway of 0–1 Hz and the length of the sway path in the medio-lateral direction in the male cases (Figure 2). In the stepwise multiple regression analysis, only the log ChE was selected as a significant predictor for these three measures (standardized partial regression coefficient = 0.673, 0.707, and 0.742, respectively, $P < 0.05$).

Discussion

In females, the sway of low (0–1 Hz) frequency and the area of sway with eyes open were increased significantly in the sarin cases. Similarly, the Romberg quotient for the former measure was significantly related to log ChE in the cases. Therefore, it is suggested that a delayed (long-term) effect on the vestibulo-cerebellar type of sway was caused by acute sarin poisoning in females.

In males, on the other hand, none of the postural sway measures was

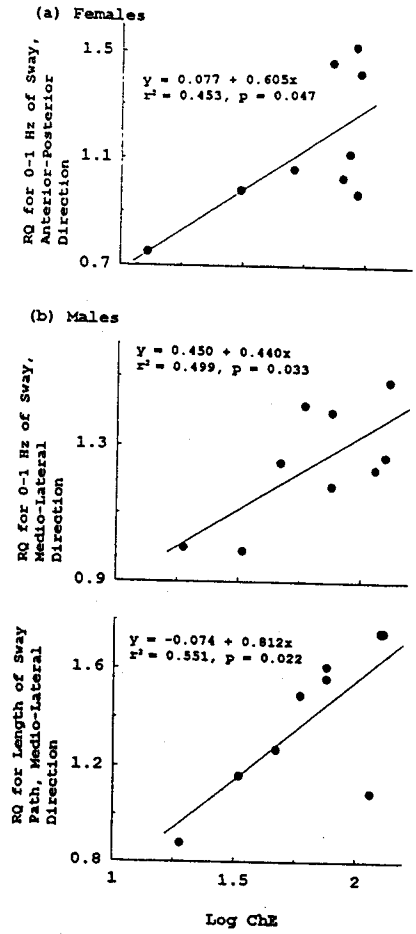

Fig. 2. Relationships of Romberg quotients (RQs) to logarithm of plasma cholinesterase activity (log ChE) in nine female and nine male sarin cases. r = Pearon's product moment correlations coefficient.

significantly increased, although Romberg quotients for the 0–1 of sway and length of sway with eyes open were significantly related to the log ChE. Thus acute sarin poisoning might have caused a delayed effect in males, but to a lesser extent than that in females. This coincides with the observation that the effects of maloxon are more severe in female than male rats[21]; a mechanism underlying the difference by gender is still unclear.

Neuronal degeneration and necrosis in brain including the cerebellum (vermis) have been observed in rats surviving convulsions after injection of soman [o-(1,2,2-trimethylpropyl)-methylfluorophosphate] (a organophosphate compound that is strongly toxic like sarin), which are similar to those observed in hypoxic encephalopathy.[23,24] In the study presented here, hypoxic damage to brain was doubtful in the sarin cases because convulsion was not observed in the cases; only one case showed cardiac arrest. Histopathological changes underlying the delayed effect of sarin on postural balance still remains unclear.

In contrast to this study, the study on pesticide applicators[7] with concurrent exposure to organophosphate showed that postural balance expressed by a total length of the sway path was affected more greatly with eyes closed than with eyes open. The discrepancy between the two studies also remains unexplained.

Thus this study suggests a subclinical delayed effect of sarin. However, because the study was based on only nine male and nine female subjects, a study on a larger number of Tokyo Subway Sarin Poisoning cases will be necessary to confirm the findings. Also, examination on the vestibulo-oculomotor system, such as the opticokinetic nystagmus test, and on the brainstem, such as measurement of brainstem auditory evoked potentials and/or neuroimaging technique such as magnetic resonance imaging, should have been performed to survey the mechanisms underlying sarin-induced impairment of postural balance.

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