Event-Related Potentials in Medical Workers with Long-Term Exposure to Xylene

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A B S T R A C T

The effects of chronic exposure to xylene on cognitive ability were studied in a group of 35 medical workers occupationally exposed to low-level concentrations of xylene for at least five years by using event-related potentials (ERPs), and compared with a control group of 21 subjects. The exposure to xylene was confirmed through determination of m-methylhippuric acid, a reliable biological indicator of xylene exposure, in pre- and post-shift urine. A dose-effect relationship between log m-methylhippuric acid and ERP log latency (p = 0.032), and the ERP amplitude (p = 0.047) was statistically significant. The group of medical workers showed significantly longer ERP log latency (p < 0.001) than did the control group with respect to factors of exposure to smoking, education and age as covariates. For the ERP amplitude the difference was found not to be significant (p = 0.263), probably due to high between subject variability. The cognitive impairment may occur in workers chronically exposed to xylene.

Introduction

Xylene is a widely used organic solvent. In pathological laboratories medical workers who prepare histopathologic slides are inevitably exposed to xylene through inhalation on a daily basis[1,2]. Xylene is a well-known human neurotoxicant. It is a central nervous system (CNS) depressant that produces lightheadedness, headache, and ataxia at low concentra-tions (100 to 690 ppm), as well as confusion and coma at high concentrations (greater than 3000 ppm)[3]. Low concentrations produce subtle effects on short-term memory, mild dizziness, drowsiness, headache, giddiness, and lightheaded-ness[4–6]. Chronic low-level exposure to xylene may be featured by depression, impaired memory, dizziness, weakness, and fatigue[7]. The event-related potentials (ERPs) have been proposed as objective

electrophysiological tool of cognitive processes. The ERPs consist of early »sensory« (P100, N100, P200) and late »cognitive« (N200, P300) components of the ERPs as a function of memory processes[8]. The P300 is a positive brain wave which develops from 300 ms after presentation of a target stimulus. It is considered to reflect the process of stimulus evaluation, reflecting the timing of neural event underlying perception and discrimination of the target stimuli, their matching against memory representation of stimulus categories and decisional processes[9].

The present study was conducted in a group of medical workers from a pathology department chronically exposed to low-level concentrations of xylene through inhalation for at least five years. Our aim was to determine the effects of such exposure on cognitive ability by using the ERPs.

Subjects and Methods

The study comprised 35 medical workers employed between February and April 1997 in a pathology department. The group consisted of 4 men and 31 women with the mean age of 36   7.7 years, who had been exposed to daily inhalation of xylene for at least five years (7.5   4.8). The study included all exposed employees, pathologists, technicians, and cleaning personnel. The exposed subjects were compared to a non-exposed control group (N = 21), mean age of 40   9.4 years (Tables 1 and 2). Each subject gave informed consent as to the proceedings and the study protocol was reviewed and approved by a local ethical committee. The daily routine in the pathology department included fixation of specimens in a 10% buffered formalin, sampling, processing in histochinet, paraffin embedding, cutting, staining, and mounting in Canada balsam. The workers were exposed to formalin vapor for only few minutes during fixation and sampling and to xylene vapor during the section mounting.

Biological monitoring

To estimate exposure to xylene, biological monitoring should take in account all routes of absorption and should include large interindividual toxicokinetic variation. The samples of urine were taken from all exposed subjects on Wednesday or Thursday before and after the 8-hour shift (Table 3). The control group was not included in the biological monitoring due

TABLE 1

AGE, DURATION OF EXPOSURE AND EDUCATION IN SAMPLE OF MEDICAL WORKERS (N = 35) CHRONICALLY EXPOSED TO LOW-LEVEL CONCENTRATIONS OF XYLENE THROUGH INHALATION, AND IN CONTROL GROUP (N = 21)

	Medical workers Mean SD		Control group Mean SD	
Age (years)	36	7.7	40	9.4
Duration of exposure (years)	7.5	4.8	None	
Education	14.74	3.5	14.95	3.6

TABLE 2

SEX AND SMOKERS/NONSMOKERS RATIOS IN SAMPLE OF MEDICAL WORKERS (N = 35) CHRONICALLY EXPOSED TO LOW-LEVEL CONCENTRATIONS OF XYLENE THROUGH INHALATION, AND IN CONTROL GROUP (N = 21)

	Medical workers	Control group
Sex ratio (women/men)	31 / 4	12 / 9
Smokers / nonsmokers	7 / 35	7 / 21

to the fact that m-methylhippuric acid (MHA) do not normally occur in urine[10]. According to the detailed questionnaire none of the control subjects had any contact with xylene. The same day the samples were chilled and transported to an analytical laboratory, where they were stored at –20 °C until analyzed. In the absence of standards for o- and p-isomers, the urine analysis was limited to m-methylhippuric acid. However, this did not affect the study objective as m-xylene is the major constituent of a typical xylene mixture. Analysis of m-methylhippuric acid included 2-propanol esterification, hexane extraction, and gas chromatographic determination with a Pye Unicam 304 (Pye Unicam Inc., Cambridge, UK). The exposed workers did not wear masks and seldom wore protective gloves for prevention of possible skin penetration by xylene[11,12].

Neurophysiological assessment

The investigation was performed with a Neuroscience Brain Imager (Neuroscience Inc., San Jose, CA, USA). The auditory ERPs are elicited by a tone discrimination »oddball« paradigm when a subject hearing two kinds of acoustic stimuli is asked to keep count of the »target« stimulus in a regular train of standard stimuli so called »non-oddball« stimuli[13.] Auditory sensory cortical activity in humans during an auditory short-term memory task reflects the N100 and P200 changes during both memorization and memory scanning[14]. The P300 component is elic-ited by task-relevant stimuli under condition of active attention. If a subject forgets to count from time to time, the P300 is not expected to be considerably affected. Counting accuracy depends on memory and attention. We used 25% target stimuli[15]. The ERP activity was recorded at the Cz, Fz, Pz, F3, F4, C3, C4, P3, and P4 electrode sites of the 10–20 electroencephalography system[16], using Ag-AgCl electrodes affixed with electrode paste and tape, referred to linked earlobes (A1+A2), with a forehead ground and impedance at 5 kOhm or less. The filter bandpass was between 800 and 1000 Hz. Two kinds of stimulus tone, high (1000 Hz) and low (800 Hz), were presented binaurally through earphones in random series. For the target stimulus tone 1000 Hz was used. The stimulus tone lasted for 50 ms, the intensity was 100 dB, and the interstimulus interval was between 3.5 and 5.5 ms. The experimental condition lasted about 20 minutes, ending when 32 trials were counted.

Repeated measurements analyses of covariance (ANCOVA) were performed for latency and amplitude data on all ERPs components (P100, N100, P200, N200, P300) on each subject for the factors of exposure and smoking, including data on education and age as covariates (Table 4). Smoking and age as covariates were studied due to the fact that they reflect risk factors to cerebrovascular disorders and related possible cognitive impairment[17,18]. Variance of latencies was proportional to the mean. Logarithmic transformation

TABLE 3

CONCENTRATIONS OF M-METHYLHIPPURIC ACID (MHA) IN PRE- AND POST-SHIFT URINE OF MEDICAL WORKERS (N = 35) CHRONICALLY EXPOSED TO LOW-LEVEL CONCENTRATIONS OF XYLENE

MHA (mg/g creatinine) in urine	N	Median	Range
Pre-shift	31[1]	10.81	< 2.0–48.00
Post-shift	35	30.42	3.94–278.85

[1] Urine samples with creatinine concentrations < 0.5 g/L (N = 2) and > 3.0 g/L (N = 2) were not taken into consideration as recommended by Alessio et al.[22]

was used to attain homoscedasticity. The Greenhouse-Geisser correction to the degrees of freedom[19] was taken into account in calculations of significance levels. The Tukey method was used for post hoc unplanned comparisons. Probabilities below 0.05 were considered statistically significant. The data analyses were performed using SAS 6.12 PROC GLM and SAS//INSIGHT[20,21].

Results

The m-methylhippuric acid concentrations in the exposed group ranged from 3.94 to 278.75 mg/g of creatinine after the 8-hour shift. The m-methylhippuric acid concentrations in the pre-shift urine samples of four workers were below the detection limit i.e. < 2.0 mg/g of creatinine. Urine samples with creatinine concentrations < 0.5 g/L (N = 2) and > 3.0 g/L (N = 2) were not taken into consideration[22] (Table 3). A dose-effect relationship between log m-methylhippuric acid (MHA), a reliable biological indicator of xylene exposure, and ERP log latency for the oddball paradigm was statistically significant (p = 0.032). A dose-effect relationship between log m-methylhippuric acid and ERP amplitude for the oddball paradigm was also found to be statistically significant (p = 0.047) (Figure 1). The group of exposed medical workers (N = 35) showed significantly longer ERP log latencies (p < 0.001) than did the control group (N = 21) with respect to factors of exposure to smoking, education and age as covariates. For the ERP amplitude the difference was found not to be significant (p = 0.263) (Table 4). For the ERP log latency there is a significant difference (p < 0.001) between the group of workers exposed to xylene and the control group. For the ERP amplitude the difference is not significant (p = 0.263). This is probably due to high between subject variability and rather small number of subjects.

Discussion

Methylhippuric acids do not normally occur in urine of subjects not exposed to xylene[10]. The increase in m-methylhippuric acid in all analyzed urine samples after work confirmed that the examined workers were occupationally exposed to xylene. None of the three isomers (o-, m-,

TABLE 4

RESULTS OF THE REPEATED MEASUREMET ANALYSES OF COVARIANCE FOR ERPs AMPLITUDE AND LOG LATENCY. WITHIN SUBJECT EFFECTS P VALUES WERE ADJUSTED USING GREENHOUSE-GEISSER (G-G) CORRECTION

Response	Predictor	Between subjects effects		Within subject effects	
		F (d.f. = 1.44)	p	F (d.f. = 4.176)	G-G adjusted p
Amplitude G-G = 0.8728	Site[1]	–	–	1.44	0.2285
	Age	0.00	0.9799	0.87	0.4696
	Smoking	1.51	0.2254	0.90	0.4534
	Education	1.52	0.2240	0.91	0.4507
	Group	1.28	0.2633	1.19	0.3167
Log Latency G-G = 0.7971	Site[1]	–	–	85.47	0.0001
	Age	0.17	0.6779	2.47	0.0607
	Smoking	0.26	0.6115	0.72	0.5486
	Education	0.57	0.4539	1.14	0.3377
	Group	16.81	0.0002	0.06	0.9839

[1] Event-related potentials (ERPs) consist of several components (P100, N100, P200, N200, and P300).
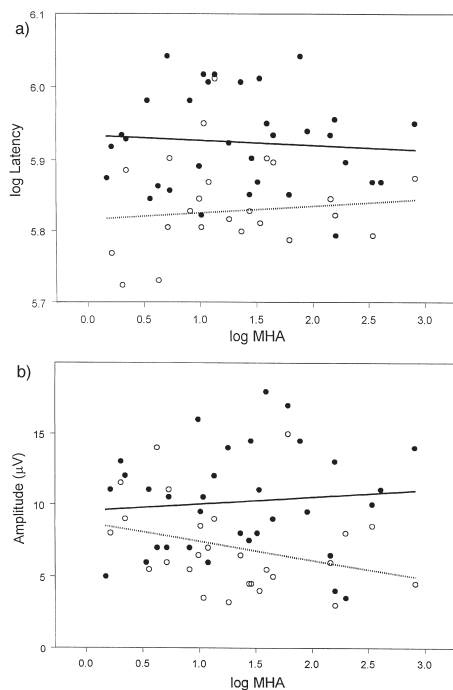
Fig. 1. Dose-effect relationship between log me-thylhippuric acid (MHA), a reliable biological indicator of exposure to xylene, and ERP log latency (a) and ERP amplitude (b) for oddbal (——) and non-oddball (······) stimuli.

p-) of methylhippuric acid did not reach the biological tolerance value of 1,500 mg/g of creatinine set by the American Conference of Governmental Industrial Hygienists[23]. We assume that this is due to the fact that workers do not spend the entire 8-hour shift in laboratories. Although the concentrations of the m-methylhippuric acid were still within the biological range values the dose-effect relationship between log m-methylhippuric acid and ERP log latency as well as ERP amplitude was found to be significant. These results confirm that exposure to xylene may influence the cognitive ability.

Multivariate analyses showed a significantly longer ERP latency in the group of medical workers exposed to xylene

than in the control group. For the ERP amplitude a difference was found not to be significant (p = 0.263). This is probably due to high between subject variability and rather small number of subjects. The ERP amplitude varies with the improbability of the targets. Its latency varies with the difficulty of discriminating the target stimulus from the standard ones. Aging and increased task-difficulty prolong P300 latency. The P300 amplitude is smaller and P300 latency is longer in patients with decreased cognitive ability than in age-matched normal subjects[24]. The neural origin of P300 is still controversial[25].

For most neurotoxic agents, there is a substantial margin of safety between the current permissible exposure levels and levels that would be expected to produce overt signs of neurotoxicity in humans[26]. However, this is not the case with xylene, as the neurologic effects were observed at or below the current Threshold Limit Value[23]. In the histopathologic laboratory during the daily preparation of samples it is not possible to separate xylene from formaldehyde exposure. Although the exposure to formaldehyde may impair the memory[27] the medical workers in our study were exposed to formaldehyde for a maximum of few minutes. We, therefore assume that significantly longer ERP log latencies we found in the group of exposed workers are the result of xylene exposure rather than formaldehyde exposure We recommend, therefore, that protective devices such as digestors, forced ventilation, and/or air conditioning system will be used in order to reduce exposure to xylene in the working environment.

Acknowledgement

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KONGNITIVNI EVOCIRANI POTENCIJALI KOD MEDICINSKIH RADNIKA DUGOTRAJNO IZLOŽENIH KSILENU

SAŽETAK

Značaj kronične izloženosti ksilenu na kognitivne funkcije ispitivali smo u grupi od 35 medicinskih radnika profesionalno izloženih niskim koncentracijama ksilena tijekom najmanje pet godina, pomoću *event-related potencijala* (ERP). Rezultate smo usporedili sa kontrolnom skupinom od 21 ispitanika. Izloženost ksilenu potvrdili smo određivanjem m-metilhipurne kiseline, biološkog indikatora izloženosti ksilenu, u urinu prije i nakon završetka posla. Odnos između log m-metilhipurne kiseline i ERP log latency (p = 0.032) i ERP amplitude (p = 0.047) bio je statistički značajan. ERP log latency u grupi medicinskih radnika bila je statistički značajno produžena (p < 0.001) u odnosu na kontrolnu skupinu, uzimajući u obzir pušenje, obrazovanje i starost kao kovariante. ERP amplituda nije se statistički razlikovala (p = 0.263) u obje skupine, vjerojatno radi velike varijabilnosti unutar pojedinih ispitanika. Kognitivne smetnje mogu se javiti kod radnika kronično izloženih ksilenu.