NeuroToxicology 37 (2013) 15–18

Contents lists available at SciVerse ScienceDirect

NeuroToxicology

Brief communication

Visual evoked potentials in children prenatally exposed to methylmercury

Takashi Yorifuji [a], Katsuyuki Murata [b], Kristian S. Bjerve [c,d], Anna L. Choi [e], Pal Weihe [f], Philippe Grandjean [e,g,*]

[a] Department of Human Ecology, Okayama University Graduate School of Environmental and Life Science, Okayama 700-8530, Japan
[b] Department of Environmental Health Sciences, Akita University School of Medicine, Akita, Japan
[c] Department of Medical Biochemistry, St. Olavs Hospital, Trondheim University Hospital, N-7021 Trondheim, Norway
[d] Department of Laboratory Medicine, Children's and Women's Health, Norwegian University of Science and Technology, N-7491 Trondheim, Norway
[e] Department of Environmental Health, Harvard School of Public Health, Boston, MA 02215, USA
[f] Department of Occupational Medicine and Public Health, The Faroese Hospital System, FR-100 Tórshavn, Faroe Islands
[g] Department of Environmental Medicine, University of Southern Denmark, DK-5000 Odense, Denmark

ARTICLE INFO

Article history:
Received 1 January 2013
Accepted 21 March 2013
Available online 30 March 2013

Keywords:
Evoked potentials
Food contamination
Methylmercury compounds
Neurophysiological measures
Omega-3 fatty acids
Prenatal exposure delayed effects

ABSTRACT

Prenatal exposure to methylmercury can cause both neurobehavioral deficits and neurophysiological changes. However, evidence of neurotoxic effects within the visual nervous system is inconsistent, possibly due to incomplete statistical adjustment for beneficial nutritional factors. We evaluated the effect of prenatal methylmercury exposure on visual evoked potential (VEP) latencies in Faroese children with elevated prenatal methylmercury exposure. A cohort of 182 singleton term births was assembled in the Faroe Islands during 1994–1995. At age 7 years, VEP tracings were obtained from 139 cohort subjects after exclusion of subjects with abnormal vision conditions. We used multiple regression analysis to evaluate the association of mercury concentrations in cord blood and maternal hair at parturition with VEP latencies after adjustment for potential confounders that included the cord-serum phospholipid concentration of *n*-3 polyunsaturated fatty acids (PUFAs) and the duration of breastfeeding. Unadjusted correlations between mercury exposure and VEP latencies were equivocal. Multiple regression models showed that increased mercury concentrations, especially in maternal hair, were associated with delayed latencies for VEP peak N145. After covariate adjustment, a delay of 2.22 ms ($p = 0.02$) was seen for each doubling of the mercury concentration in maternal hair. In agreement with neuropsychological findings, the present study suggests that prenatal methylmercury exposure may have an adverse effect on VEP findings despite the absence of clinical toxicity to the visual system. However, this association was apparent only after adjustment for *n*-3 PUFA status.

1. Introduction

In a large-scale poisoning incident caused by methylmercury in Minamata, Japan, patients manifested a variety of neurological signs that included sensory disturbances, such as constriction of the visual fields (Harada, 1995). Recent attention to lower prenatal exposures to methylmercury has focused on subtle cognitive effects (Grandjean and Landrigan, 2006) as well as neurophysiological changes (e.g., delayed brainstem auditory evoked potential (BAEP) latencies (Murata et al., 2007)). However, although abnormalities in visual evoked potentials (VEPs) were observed in patients with methylmercury poisoning in Minamata (Imai et al., 1991), the evidence on VEP latencies is equivocal (Murata et al., 2007).

The reason for this inconsistency may partly have been the lack of adjustment for nutritional factors (e.g., polyunsaturated fatty acids and breastfeeding) (Murata et al., 2007). Thus, certain nutritional factors are reported to be beneficial for visual function development (Chong et al., 2005; Decsi and Koletzko, 2005). In particular, *n*-3 polyunsaturated fatty acids (PUFAs), which primarily originate from fish and seafood consumption, are essential for normal brain development, especially in regard to the visual system (Innis, 1991). Such beneficial nutritional factors could be associated with methylmercury exposure due to seafood consumption as a joint source of intake (Choi et al., 2008b). Therefore, the nutrients may cause negative confounding that leads to underestimation of the true association between methylmercury exposure and visual function (Choi et al., 2008b).

* Corresponding author at: Department of Environmental Medicine, University of Southern Denmark, DK-5000 Odense, Denmark. Tel.: +45 6550 3769; fax: +45 6591 1458.
E-mail addresses: pgrandjean@health.sdu.dk, pgrand@hsph.harvard.edu (P. Grandjean).

0161-813X/$ – see front matter © 2013 Published by Elsevier Inc.
http://dx.doi.org/10.1016/j.neuro.2013.03.009

In the present study, we aim to evaluate the possible adverse effects of prenatal methylmercury exposure on VEP findings at age 7 in a Faroese birth cohort by adjusting for nutritional factors (i.e., *n*-3 PUFAs and duration of breastfeeding).

2. Methods

2.1. Study design and subjects

A cohort of 182 singleton term births at the National Hospital in Tórshavn, the Faroe Islands, was assembled during a 12-month period in 1994–1995 (Choi et al., 2008a; Steuerwald et al., 2000). Children who were born before the 36th week in gestation, or had congenital neurologic disease were excluded. At delivery, blood samples from the umbilical cord and scalp hair from the mothers (the first 3 cm from the root) were collected. Neurological examination of the children was carried out at age 90 months at a hospital clinic, and visual evoked potentials were recorded. The study protocol was approved by the Ethical Review Committee for the Faroe Islands, and written informed consent was obtained.

At the follow-up, 25 children did not participate and therefore lacked information on VEPs. We excluded 5 births with medical risks. Moreover, we excluded 4 children with strabismus and 9 children requiring eye-glasses. Accordingly, 139 children were included in the analyses.

2.2. Measurement of exposure

We used the cord-blood and maternal hair mercury concentration as indicators of prenatal exposure to methylmercury (Grandjean et al., 1992, 1997). Details of the analytic methods and quality control procedures are described elsewhere (Grandjean et al., 1992). For subjects with a missing mercury analysis, the result was calculated from the other exposure biomarker using the average ratio between mercury concentrations in hair and cord blood.

2.3. Outcome measurements

Details of the VEP recording methods are described elsewhere (Grandjean et al., 1997; Murata et al., 1999b). In short, pattern-reversal VEPs with binocular full-field stimulation were conducted in a darkened room. The subjects sat in a relaxed position 127 cm from the front of a 17-in. monitor screen and were asked to stare at the center of the screen. The checkerboard pattern on the screen consisted of white and black squares (mean luminance, 371 and 5 cd/m^2, respectively), reversing at a rate of 2/s (sampling time, 0.2 ms). Two kinds of the squares were used (30 min and 15 min). The latencies of one positive and two negative peaks (P100, N75, and N145) were recorded using standard electroencephalography (EEG) electrodes fixed to the skull above the occipital cortex, the forehead, and the left mastoid (ground).

2.4. Measurement of covariates

At delivery, the midwives collected information on the course of the pregnancy and the delivery, including nutritional habits and use of alcohol and tobacco during pregnancy. A parent also completed a self-administered questionnaire, which included questions about their demographic characteristics. Fatty acid concentrations in cord-serum total phospholipids were measured using gas chromatography with a flame ionization detector after extraction, isolation and transmethylation. The concentrations were recalculated from mg phospholipid fatty acid/L serum to relative concentrations, i.e., as weight percent of all 22 phospholipid fatty acids measured (Steuerwald et al., 2000). All results were reported as relative concentrations in percent of total phospholipids fatty acids.

2.5. Statistical analyses

Logarithmic (base 10) transformations of the cord-blood and maternal hair mercury concentrations were conducted due to highly skewed distributions. Geometric means of these exposure biomarkers were calculated. We evaluated the effects of mercury exposure (cord-blood and maternal hair concentrations) on VEP latencies using multiple linear regression models. All of the VEP latencies approximated a Gaussian distribution, thus they were used as continuous variables without transformation. We first adjusted for children's age at examination (as a continuous parameter) and their sex as mandatory covariates (Murata et al., 1999a,b). We then adjusted for nutritional factors (including cord-serum total *n*-3 PUFAs and duration of exclusive breastfeeding) thought to have beneficial effects on visual development (Chong et al., 2005; Decsi and Koletzko, 2005). Finally, to examine the robustness of the beta coefficients, we adjusted for the following variables: maternal smoking during pregnancy (yes/no); previous births (0/1/at least 2); and maternal alcohol drinking during pregnancy (yes/no).

By multiplying beta coefficients for the log transformed mercury concentrations by 0.301 (i.e., log2), we report the absolute change of the outcome variables for each doubling of the exposure. PASW Statistics software (SPSS Japan Inc., version 18.0J) was used for descriptive analyses and regression models. We report two-sided *p*-values.

3. Results

Geometric averages of cord-blood and maternal hair mercury were 22.8 μg/L and 4.6 μg/g, respectively (Table 1) in accordance with the increased levels of methylmercury exposure. Cord-serum fatty acids and mercury concentrations (both in cord blood and maternal hair) were positively correlated, with EPA showing

Table 1
Characteristics of 139 Faroese birth cohort members participating in the clinical examination.

	N	Result
Age at examination (years)[a]	139	7.5 ± 0.1
Sex of child (boy/girl) (%)	139	48.2/51.8
Total *n*-3 polyunsaturated fatty acids (weight% of total in cord serum phospholipids)[a]	117	10.9 ± 1.9
Total *n*-3 polyunsaturated fatty acids (weight% of total in maternal serum phospholipids)[a]	134	11.8 ± 2.0
Duration of exclusive breast-feeding (months)[a]	139	3.5 ± 2.1
Previous births (0/1/at least 2) (%)	139	28.8/28.8/42.4
Smoking during pregnancy (no/yes) (%)	139	70.5/29.5
Alcohol consumption during pregnancy (no/yes) (%)	139	85.6/14.4
Cord blood mercury (μg/L)[b]	125	22.8 (13.7–41.2)
Maternal hair mercury (μg/L)[b]	133	4.6 (2.7–8.2)
VEP latency (30 min) (ms)[a]		
N75	138	72.8 ± 3.1
P100	138	105.9 ± 4.7
N145	138	138.3 ± 10.8
VEP latency (15 min) (ms)[a]		
N75	139	77 ± 3.7
P100	139	109.6 ± 5.6
N145	139	146.1 ± 10.3

VEP: visual evoked potential.
[a] Arithmetic mean ± SD.
[b] Geometric mean (interquartile range).