Neurotoxicity Research
https://doi.org/10.1007/s12640-020-00174-4

ORIGINAL ARTICLE

Activation of MIP-2 and MCP-5 Expression in Methylmercury-Exposed Mice and Their Suppression by N-Acetyl-L-Cysteine

Muflihatul Muniroh[1] · Ainun Rahmasari Gumay[1] · Darmawati Ayu Indraswari[1] · Yuriz Bahtiar[1] · Hardian Hardian[1] · Saekhol Bakri[2] · Nani Maharani[3] · Vega Karlowee[4] · Chihaya Koriyama[5] · Megumi Yamamoto[6]

Received: 1 July 2019 / Revised: 15 January 2020 / Accepted: 2 February 2020

Abstract

Methylmercury (MeHg) is a well-known neurotoxin of the central nervous system (CNS). Neuroinflammation is one of the main pathways of MeHg-induced CNS impairment. This study aims to investigate the expressions of IL-6, MIP-2, and MCP-5, as biomarkers in relation with MeHg-induced CNS impairment and *N*-acetyl-L-cysteine (NAC) treatment in mice, as well as histopathological changes of brain tissue and clinical symptom such as ataxia. Twenty male Balb/c mice, aged 8–9 weeks, were divided into 4 groups and treated with saline (control), NAC [150 mg/kg body weight (BW) day], MeHg (4 mg Hg/kg BW), or a combination of MeHg and NAC for 17 days. MeHg induced the expression of IL-6, MIP-2, and MCP-5 in the serum, with median values (those in controls) of 55.06 (9.44), 15.94 (9.30), and 458.91 (239.91) mg/dl, respectively, and a statistical significance was observed only in IL-6 expression ($p < 0.05$). MIP-2 and MCP-5 expressions tended to increase in the cerebrum of MeHg-treated group compared with controls; however, the difference was not statistically significant. MeHg treatment also increased IL-6 expression in the cerebellum (7.73 and 4.81 mg/dl in MeHg-treated group and controls, respectively), with a marginal significance. NAC significantly suppressed MeHg-induced IL-6 and MIP-2 expressions in the serum ($p < 0.05$ for both), and slightly reduced MCP-5 expression in the cerebrum. Ataxia was observed in all MeHg-treated mice after 9-day exposure as well as the decrease of intact Purkinje cells in brain tissue ($p < 0.05$). These findings suggest that MeHg induced neurotoxicity by elevating the expression of IL-6, MIP-2, and MCP-5 and causing ataxia symptoms, and NAC reduced MeHg-mediated effects on the CNS.

Keywords Methylmercury · IL-6 · MIP-2 · MCP-5 · N-acetyl-L-cysteine · Ataxia

Introduction

Mercury (Hg) is considered one of the top 10 chemicals of major public concern (ATSDR 2017; WHO 2017). Methylmercury (MeHg) is a known neurotoxin, but its mechanism of action is not clearly understood (Farina et al. 2011). Acute or chronic inflammation in the brain is associated with brain injury and neurodegenerative disorders (Whitney et al. 2009). Inflammation involves an intricate cellular and molecular response to stress or injury. This response defends the body against insults, cleanses debris and impaired cells, and restores normalcy in the affected area. Understanding inflammation may provide insights into the pathophysiology of acute and chronic neurotoxicity following exposure to mercury compounds. For instance, DNA microarray analysis showed that certain inflammatory chemokines (e.g., CCL2, CCL4, and CCL7) were upregulated in the cerebellum of MeHg-exposed mice (Hwang et al. 2011; Kim et al. 2012). Several

✉ Muflihatul Muniroh
muflihatul.muniroh@fk.undip.ac.id

[1] Department of Physiology, Faculty of Medicine Diponegoro University, Tembalang Semarang 50275, Indonesia

[2] Department of Public Health, Faculty of Medicine Diponegoro University, Semarang 50275, Indonesia

[3] Department of Pharmacology and Therapy, Faculty of Medicine Diponegoro University, Semarang 50275, Indonesia

[4] Department of Anatomical Pathology, Faculty of Medicine Diponegoro University, Semarang 50275, Indonesia

[5] Department of Epidemiology and Preventive Medicine, Kagoshima University Graduate School of Medical and Health Sciences, Kagoshima 890-8520, Japan

[6] Department of Environment and Public Health, National Institute for Minamata Disease, Kumamoto 867-0008, Japan

Published online: 10 February 2020

studies indicated that MeHg exposure activates the expression of interleukin-6 (IL-6) in different cells such as rat C6 glioma cells, human U251HF glioma cells, and human retina pigment epithelium (ARPE-19) (Chang 2007; Chang and Tsai 2009). Similarly, our previous studies have shown that exposing U-87MG astrocytoma/glioblastoma cells and U937 macrophages to non-cytotoxic concentrations of MeHg (at 4 μM and 10 μM, respectively) caused IL-6 upregulation (Muniroh et al. 2015; Yamamoto et al. 2017). On the other hand, Macedo-Junior et al. reported that oral administration at 40 μg/ml of MeHg for 14 days reduced IL-6 expressions in the cerebellum of adult male Swiss mice (Macedo-Junior et al. 2017). Other in vitro study reported that exposure to non-cytotoxic doses of MeHg (at 0.3 μM) also induces microglial reactions and may exert neuroprotective effects by interaction with astrocytes (Eskes et al. 2002). Therefore, it is of our interest whether activation of IL-6 expression by MeHg in vivo will occur.

Interleukin-8 (IL-8) plays a crucial role in immune responses of the human CNS (Semple et al. 2010), particularly in the induction of chemotaxis against its target cells (e.g., monocytes and neutrophils). In our previous study, IL-8 was demonstrated to be upregulated in response to MeHg exposure in U937 macrophages and U-87MG astrocytoma/glioblastoma cells (Yamamoto et al. 2017). We also showed that murine macrophage inflammatory protein-2 (MIP-2), a functional homolog of human IL-8 (Hol et al. 2010), was activated in response to MeHg exposure in murine RAW294.7 macrophages. MIP-2 expression was also activated in vivo in the liver of MeHg-exposed mice (Wu et al. 2016). However, there are no studies reporting MIP-2 expression in the brain following MeHg exposure.

Based on experimental findings in murine MCP-1 homolog knockout mice, monocyte chemoattractant protein-1 (MCP-1) may work as an alert system in response to MeHg exposure in the brain (Godefroy et al. 2012). In addition, at a non-cytotoxic concentration of MeHg, we reported significant induction of MCP-1 expression in U-87MG astrocytoma/glioblastoma cells (Muniroh et al. 2015). Murine MCP-5 is the homologous chemokine of human MCP-1 (Mojsilovic-Petrovic et al. 2007; Sarafi et al. 1997). We have previously reported the activation of MCP-5 expression in the presence of MeHg in murine RAW 264.7 macrophages (David et al. 2017). mRNA expression of inflammatory chemokines (CCL2, CCL4, CCL7, CCL9, CCL12) was reported to be upregulated in MeHg-exposed mouse cerebellum (Hwang et al. 2011; Kim et al. 2012). However, there is no study reporting the MCP-5 (CCL12) expression at protein level in response to MeHg exposure in vivo. Therefore, the primary purpose of this study was to examine whether the expression of IL-6, MIP-2, and MCP-5 is activated in the serum and brain tissues of MeHg-exposed mice.

N-acetyl-L-cysteine (NAC), the acetylated precursor of L-cysteine, has been reported to suppress MeHg-induced toxicity in vitro and in vivo (Aremu et al. 2008; Chen et al. 2006). NAC is also an anti-inflammatory agent (Samuni et al. 2013). We showed that NAC suppressed MeHg-activated MCP-1 and IL-6 expression in the U-87MG astrocytoma/glioblastoma cells (Muniroh et al. 2015), and IL-6 and IL-8 expression in U937 macrophages (Yamamoto et al. 2017). In this study, we also examined the suppressive effect of NAC on MeHg-induced IL-6, MIP-2, and MCP-5 expression in mice.

Materials and Methods

Samples and Treatments

Twenty male Balb/c mice, aged 8–9 weeks, were used in this study.

Mice were randomly divided into 4 groups with the following treatments for 17 days:

- Group 1 was a negative control that was administered saline solution (vehicle).
- Group 2 was treated with NAC at a dose of 150 mg/kg body weight (BW)/day.
- Group 3 was treated with MeHg at 4 mg Hg/kg BW/day.
- Group 4 was treated with both MeHg 4 mg Hg/kg BW/day and NAC 150 mg/kg BW/day.

MeHg (Sigma–Aldrich, Switzerland) stock solution (10 mM) was dissolved in saline with L-cysteine (Nacalai Tesque, Kyoto, Japan) (Hg:Cys = 1:1.2) and stored at − 80 °C until use (Yamamoto et al., 2014, 2019). The stock solution was diluted with saline just before administration and orally administered to mice at a dose of 4 mg/kg BW for 17 days. Regarding the choice of MeHg dose in this study, we referred to previous studies in mice. Yasutake et al. reported that oral administration of 5 mg MeHg (4 mg Hg)/kg BW per day for 15 days induced BW decrease in Balb/c mice (Yasutake and Hirayama 1988), and MeHg treatment at 5 mg Hg/kg BW three times per week for 5 weeks caused brain injury in KK-Ay mice (Yamamoto et al. 2019; Yamamoto et al. 2014). Therefore, we expected that treatment condition of MeHg in this study (4 mg Hg/kg BW per day) would induce neurotoxicity in 2–3 weeks.

NAC (Wako Pure Chemical Industries, Osaka, Japan) solution was freshly prepared everyday by mixing with normal saline solution before orally administering to mice at a concentration of 150 mg/kg BW. This dose was determined based on a previous study (Chen et al. 2006), which reported that daily NAC oral administration at 150 mg/kg BW for 2–4 week effectively suppressed MeHg-induced pancreatic β cell

dysfunction in ICR mice. Regarding the improvement of neurological dysfunction by NAC in mice, Roseguini et al. reported that intraperitoneal administration of NAC at 150 mg/kg BW for 15 days improved fatigue resistance in Balb/c mice (Roseguini et al. 2015), and oral administration of NAC at 100 mg/kg BW for 11 days reduced restraint stress-activated hippocampal microglial cells and astrocytes in Balb/c mice (Lee et al. 2017). Therefore, we expected that treatment of NAC in this study (150 mg/kg BW per day) would suppress MeHg toxicity in this experimental condition.

All mice were given oral administration twice to ensure all mice were similarly handled. For mice that only received MeHg or NAC solution, normal saline solution was administered during the second oral gavage.

In this study, we aimed to observe changes during the early stages of MeHg intoxication without drastic loss in BW. All mice were sacrificed on day 18 at 24 h after the last treatment. One mouse was sacrificed on day 14 because of severe symptoms of ataxia, fatigue, and BW loss. Blood was collected from the periorbital veins before euthanizing animals with diethyl ether anesthesia. Brain tissues of cerebrum (cerebral hemisphere without olfactory bulb, pons or medulla) and cerebellum (Spijker 2011) were collected, weighed, and then immersed in 10% formalin buffer solution. The experimental procedures were approved by the Ethical Committee of the Faculty of Medicine Diponegoro University and Dr. Kariadi Hospital, Semarang, Central Java, Indonesia (No. 80/EC/H/FK-RSDK/XI/2017).

Evaluation of Ataxia

The BW of each mouse was daily measured and the severity of ataxia symptoms was checked by the hind-limb clasping test (Guyenet et al. 2010). Briefly, a mouse was held near the base of its tail and the clasping of its hind-limbs was observed for 10 s. Hind-limb retraction toward the abdomen was considered a positive symptom. For the ledge test, a mouse was placed on the cage's ledge and its balance was observed while walking along the ledge. Losing balance, not using its hind legs, landing on its head, shaking, motionlessness, and falling down were considered as positive symptoms. The tests were repeated thrice for each mouse.

Determination of IL-6, MIP-2, and MCP-5 Expression

The collected blood was placed in BD Vacutainer® Plus Plastic Serum Tubes (Ref. 367,812; Becton, Dickinson and Company, NJ, USA) and centrifuged (1008 rcf, 4 °C, 10 min) to separate the serum. Tissues of the cerebrum and cerebellum were separately homogenized using phosphate buffer saline solution (1 mg:9 μl) and centrifuged (1008 rcf, 4 °C, 10 min) to obtain the supernatant. The levels of IL-6, MIP-2, and MCP-5 expressions in the serum and brain (cerebrum and cerebellum) supernatant were measured by an enzyme-linked immunosorbent assay kit (R&D system, MN, USA). Only IL-6 expression was measured in the cerebellum owing to the small amount of supernatant available. The detection limit of IL-6, MIP-2, and MCP-5expressions was typically < 1.6, 1.5, and 2.1 pg/ml, respectively. Cytokine levels were compared between MeHg and/or NAC-treated groups and the vehicle group.

Histopathological Examination

Brain tissues were divided into cerebrum (right and left) and cerebellum and were immersed in 10% formalin buffer solution. Each tissue specimen was subjected to hematoxylin and eosin staining. The number of intact Purkinje cells was determined by a certified pathologist in 10 fields with × 100 and × 400 magnification for all blinded samples.

Data Analysis

Difference of BW change between groups was examined using Kruskal–Wallis test, then followed by Wilcoxon signed rank test to see the change of BW before and after the treatment. Comparison of cytokine expressions and intact Purkinje cell number between different treatment and presence of ataxia groups was examined using Mann–Whitney *U* test. Comparison of IL-6 expression in cerebellum and intact Purkinje cells number was analyzed using Spearman's rho test. The frequency of ataxia symptoms was compared between groups by Fisher's exact test, and the correlation between ataxia symptoms and intact Purkinje cells number in each group was analyzed using Mann–Whitney *U* test. The level of significance was considered by $p < 0.05$.

Results

Effect of MeHg on Mice Body Weight Change

Figure 1 shows the BW change of mice over the entire duration of the study. Although the mean BW values of MeHg and MeHg+NAC groups were higher than those of control and NAC groups, this difference was not statistically significant ($p = 0.846$, Kruskal–Wallis test). In the MeHg-treated group, BW decreased from day 7 to 17. However, the difference in BW before and after treatment was not significant (35.32 ± 4.16 vs. 30.02 ± 6.18 g; $p = 0.068$; Wilcoxon signed rank test). One mouse in MeHg-treated group was sacrificed on day 14 because of severe symptoms. Cotreatment with NAC suppressed MeHg-mediated decrease in BW.

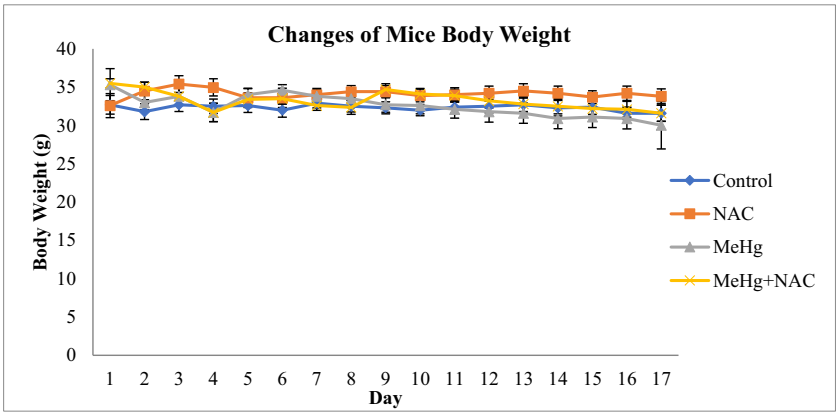

Fig. 1 Change in mean BW of Balb/c mice from day 1 to day 17 of treatment. Error bars indicate standard error

Effect of MeHg on IL-6, MIP-2, and MCP-5 Expressions

Table 1 shows the concentration of IL-6, MIP-2, and MCP-5 in the serum and brain of each treatment group. The expression level in the serum was higher than that in the brain for all cytokines, except IL-6 which had higher expressions in the cerebrum. NAC only treatment did not significantly affect the expression of cytokines. Following MeHg treatment, IL-6 expression was significantly increased in the serum ($p = 0.016$), and similar trends were observed for serum levels of MIP-2 expression ($p = 0.075$). NAC significantly suppressed MeHg-mediated IL-6 and MIP-2 expressions increase in serum ($p = 0.028$ and $p = 0.047$, respectively). In contrast, a MeHg-mediated increase in cytokine levels was less significant in brain tissues.

Effect of MeHg on Ataxia Symptoms and Histopathological Changes in the Brain

Ataxia symptoms were observed on day 9 in one mouse from the MeHg-treated group. This increased to 2 symptomatic mice on day 10, 3 on day 11, and finally all mice ($n = 5$) showed delayed in the MeHg+NAC combination group, with only 2 mice developing symptoms on days 13 and 14.

As described in Table 2, the occurrence of ataxia symptoms was significantly different between control and MeHg-treated groups ($p = 0.003$; Fisher's exact test). No mice developed ataxia symptoms in the control and NAC-treated groups. However, all MeHg-treated mice displayed ataxia symptoms, which first became apparent on day 9. NAC treatment reduced the effect of MeHg-induced ataxia ($p = 0.038$, Fisher's exact test) and rescued intact Purkinje cells ($p = 0.039$; Mann–Whitney U test).

Association Between Cytokine Expression, Ataxia Symptoms, and Brain Histopathological Changes

As shown in Table 3, the levels of IL-6 and MCP-5 expressions in serum and cerebrum were significantly increased by the presence of ataxia. The number of Purkinje cells was negatively correlated with IL-6 expression in the cerebellum

Table 1 IL-6, MIP-2, and MCP-5 expression levels by treatment groups

		Cytokine expression level: median (range), pg/ml						
Treatment group		Control	NAC	p^a	MeHg	p^b	MeHg+NAC	p^c
IL-6	Serum	9.44 (6.14–35.06)	18.50 (5.7–45.01)	0.602	55.06 (29.35–77.99)	0.016**	28.62 (5.48–41.41)	0.028**
	Cerebrum	12.05 (6.59–28.08)	10.36 (7.69–13.13)	0.465	16.89 (8.13–29.14)	0.754	9.88 (5.48–31.68)	0.347
	Cerebellum	4.81 (3.45–8.35)	6.69 (4.8–8.57)	0.142	7.73 (4.58–10.75)	0.175	4.81 (3.9–7.91)	0.172
MIP-2	Serum	9.30 (6.58–10.69)	8.01 (1.99–17.83)	0.075	15.94 (8.06–23.17)	0.075*	8.65 (6.58–12.99)	0.047**
	Cerebrum	2.31 (1.99–2.93)	1.73 (0.33–3.25)	0.169	3.98 (1.35–6.87)	0.116	2.31 (0.68–3.86)	0.116
MCP-5	Serum	239.91(132.14–353.04)	229.92(173.22–285.27)	0.917	458.91(217.37–559.43)	0.047**	378.19(232.64–574.08)	0.602
	Cerebrum	106.57 (39.2–188.58)	84.08 (26.31–180.87)	0.347	121.18 (63.01–700.35)	0.251	44.34 (10.98–207.94)	0.076*

[a] Comparison between control and NAC-treated groups. p values were obtained by Mann–Whitney U test

[b] Comparison between control and MeHg-treated groups. p values were obtained by Mann–Whitney U test

[c] Comparison between MeHg-treated and MeHg+NAC-treated groups. p values were obtained by Mann–Whitney U test

*Marginal significance, p value < 0.1

**Significant different, p value < 0.05

Table 2 Ataxia symptoms and Purkinje cell number by treatment group

Group	Number of mice		p^a	Number of intact Purkinje cells/10 fields[#] Median (range)	p^b
	No ataxia	Ataxia			
Control	5	0		7.65 (7–8.6)	
NAC	5	0	-	8.0 (6–11)	0.675*
MeHg	0	5	0.003*	6.3 (4.8–6.8)	0.009*
MeHg+NAC	3	2	0.038**	7.2 (6.3–8.9)	0.039**

[#] × 400 magnification

[a] p values were obtained by Fisher's exact test

[b] p values were obtained by Mann–Whitney U test

*Comparison with control group

**Comparison with MeHg-treated group

(rho = − 0.482, $p = 0.032$; Spearman's rho test, data not shown). The cytokine expressions were different between ataxia ($n = 2$) and non-ataxia ($n = 3$) mice in the MeHg+NAC group. For instance, IL-6 expression in cerebrum (22.68 vs. 7.68 pg/ml) and MCP-5 expression in serum and cerebrum (476.14 vs. 312.89 and 141.75 vs. 31.41 pg/ml) were higher than those in non-ataxia mice (Fig. 2).

Correlation of Ataxia Symptoms and Purkinje Cell Number Following MeHg Exposure

MeHg treatment significantly reduced the number of intact Purkinje cells in MeHg-treated group compared with control group ($p = 0.009$; Mann–Whitney U test). The intact Purkinje cell number was different between ataxia and non-ataxia mice, where non-ataxia mice have higher number of intact Purkinje cells than that of mice with ataxia (7.65, range 6–11 vs 6.4; range 4.8–8.9; $p = 0.039$; Mann–Whitney U test), as described in Fig. 3.

Discussion

MeHg exposure has been found to be a potent stimulator of neurotoxicity by upregulating several pro-inflammatory cytokines in the CNS cells of MeHg-exposed in vivo (Hwang et al. 2011; Kim et al. 2012) and in vitro studies (Muniroh et al., 2015; Yamamoto et al., 2017). However, the activation of pro-inflammatory cytokines such as IL-6, MIP-2, and MCP-5 expression at protein level in blood and brain tissues in relation with brain histopathological change and its clinical manifestation are not well understood. In this study, we examined the expression of IL-6, MIP-2, and MCP-5 as well as intact Purkinje cell number as biomarkers and signs of early stage MeHg intoxication related with brain inflammation and injury without a drastic loss in BW. Although there was a decrease in BW in MeHg-treated mice, there was no significant difference in BW between the control and MeHg-treated groups. It was concurrent with previous report that MeHg exposure at similar dose for around 2 weeks could reduce the BW in

Table 3 IL-6, MIP-2, and MCP-5 expression in blood and brain tissue with respect to ataxia symptoms following MeHg exposure

		Cytokine expression level: median (range), pg/ml		p
		No ataxia ($n = 13$)	Ataxia ($n = 7$)	
IL-6	Serum	14.25 (5.48–45.01)	51.99 (28.62–77.99)	0.003**
	Cerebrum	9.88 (5.48–28.08)	16.89 (8.13–31.68)	0.043**
	Cerebellum	5.13 (3.45–8.57)	7.69 (3.9–10.75)	0.475
MIP-2	Serum	8.64 (1.99–17.83)	13.85 (6.58–23.17)	0.132
	Cerebrum	1.99 (0.33–3.53)	3.25 (1.35–6.87)	0.074*
MCP-5	Serum	239.91 (132.14–447.76)	458.91 (217.37–574.08)	0.006**
	Cerebrum	49.27 (10.98–188.58)	121.18 (63.01–700.35)	0.029**

The difference in IL-6, MIP-2, and MCP-5 expression between non-ataxia and ataxia mice was analyzed using Mann–Whitney U test

*Marginal significance, p value < 0.1

**Significance difference, p value < 0.05

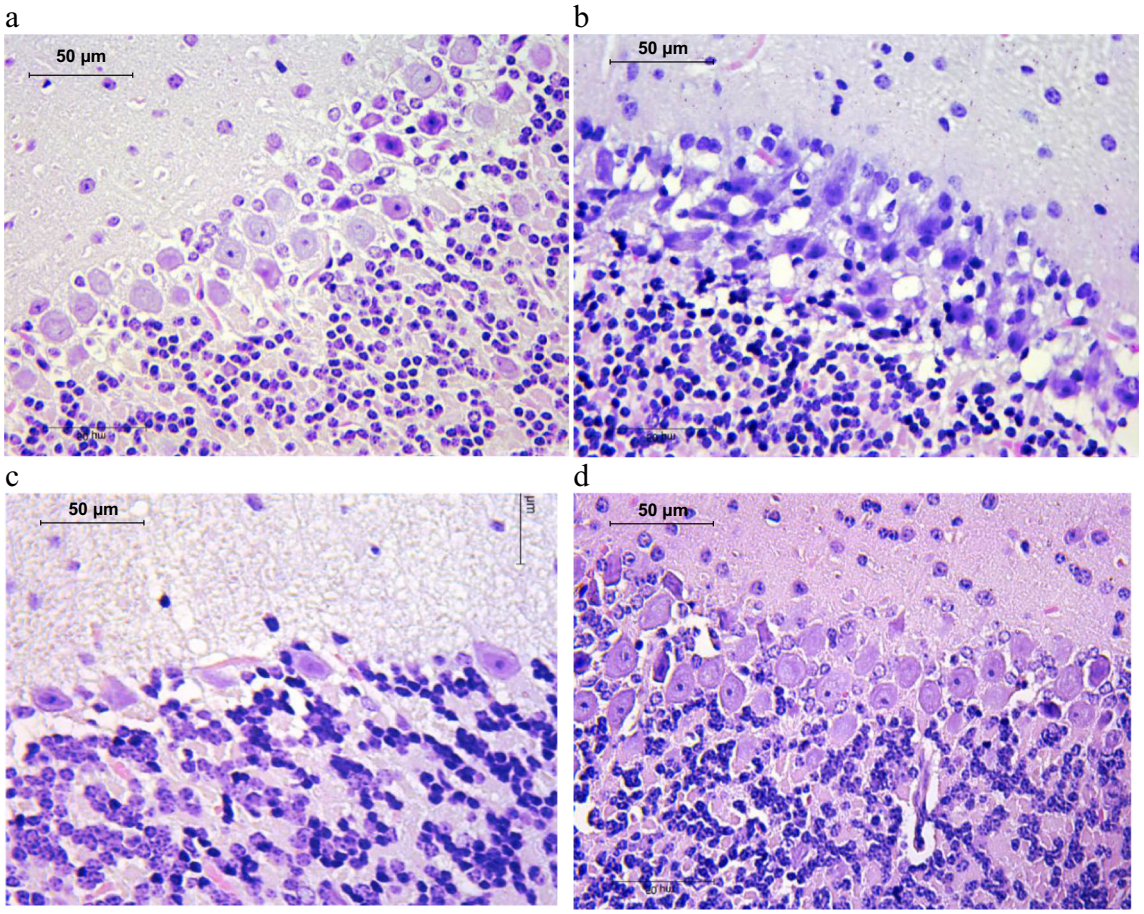

Fig. 2 Representative images (× 400 magnification) of histopathological changes in the cerebellum of control (**a**), NAC 150 mg/kg BW/day (**b**), MeHg 4 mg Hg/kg BW/day (**c**), and combination of MeHg 4 mg Hg/kg BW/day+NAC 150 mg/kg BW/day (**d**) group. Images show differences in the number of Purkinje cells in the various groups

Balb/c mice (Yasutake and Hirayama 1988). The suppressive effect of NAC in MeHg-exposed mice was shown by protecting the decrease of BW as well as other biomarkers of neurological disturbance, indicating NAC could protect from MeHg neurotoxicity in vivo.

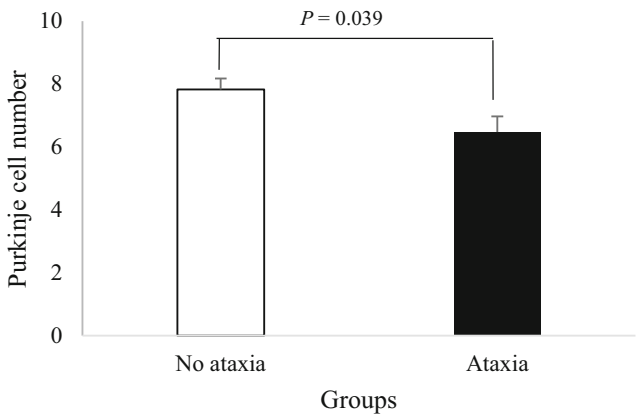

Fig. 3 The differences in the number of Purkinje cells between no ataxia and ataxia groups. *p* value was obtained by Mann–Whitney *U* test

The current study showed that MeHg treatment caused significant increase in serum levels of IL-6, MIP-2, and MCP-5 expressions compared with the control group, indicating that these cytokines could be potential biomarkers for MeHg-induced inflammation. Previous reports have demonstrated that IL-6 expression in numerous cell types was influenced by MeHg exposure (Chang 2007; Chang and Tsai 2009; Eskes et al. 2002; Muniroh et al. 2015; Yamamoto et al. 2017). MeHg-induced activation of IL-6 may be a general response that may be involved in several processes underlying MeHg toxicity, including protection against MeHg exposure. In this study, MIP-2 and MCP-5 expression in the cerebrum was enhanced following MeHg exposure but was not significantly different from that in controls. Cytokine expression was only transiently activated following MeHg exposure in vitro (Muniroh et al. 2015; Yamamoto et al. 2017). Therefore, significant differences may not be detected in vivo. MIP-2 elevation has been reported in the brains of mice and rats during the acute stages of bacterial meningitis (Diab et al. 1999; Klein et al. 2006; Koedel et al. 2004), in association with reactive astrocytes (Luo et al. 2000) and MCP-5 (Fowler et al. 2004;

Kastenbauer et al. 2005; Moller et al. 2005). This indicates an important role for MIP-2 and MCP-5 in early neuroinflammatory processes. The mRNA levels of MCP-5 tended to increase in mouse brain tissue after MeHg treatment (Hwang et al. 2011; Lee et al. 2012). Therefore, the increase in MIP-2 and MCP-5 expression in serum and brain tissue may serve as biomarkers of early stage of MeHg-induced neurotoxicity.

The expression of IL-6 clearly increased in serum and cerebrum of mice with ataxia, but not in cerebellum. In previous studies, cerebral cortex as well as hypothalamus and hippocampus have been reported as the prominent regions in the brain to release high levels of IL-6 expression compared with other brain regions (Aniszewska et al. 2015; Gadient and Otten 1994; Gonzalez-Hernandez et al. 2006). IL-6 immunoreactive cells were found mostly from astrocytes in all border zones of four ventricles, and microglial cells in the hippocampus, cerebral cortex, and brain stem regions (Aniszewska et al. 2015). Activation of IL-6 expression in the cerebral hemisphere might be correlated with ataxia symptom compared with that in the cerebellum.

In the cerebellum of patients with Minamata disease, the loss of granule cells was more apparent than that of Purkinje cells (Takeuchi and Eto 1999). Conversely, injury in Purkinje cells was clearly observed in this experiment; therefore, we used the number of Purkinje cells as an indicator for MeHg-induced brain injury. This study demonstrates that the number of Purkinje cells differed significantly between groups, with the lowest number in the MeHg-treated group. Reduction in the number of intact Purkinje cells correlated with IL-6 (both in the serum and brain tissue) and MIP-2 expression (in the serum) as well as ataxia symptoms. Several in vitro studies have reported that MeHg blocked Ca^{2+} channels in both granule cells (Sirois and Atchison 2000) and Purkinje cells (Peng et al. 2002). Therefore, the neurological symptoms in mice in this study may be due to release of the inhibitory neurotransmitter GABA.

We found that cotreatment with NAC significantly reduced MeHg-induced increase in serum levels of IL-6 and MIP-2. The data also suggest that oral administration of NAC suppressed the expression of IL-6 in the cerebellum and MCP-5 in the cerebrum, rescuing Purkinje cells, and consequently reducing ataxia symptoms. NAC is an anti-inflammatory agent with anti-oxidant and chelating effects (Aremu et al. 2008). Therefore, early administration of NAC after MeHg exposure may suppress MeHg-mediated neuroinflammation.

Conclusion

In present study, we reported for the first time that MeHg exposure activated the expression of MIP-2 and MCP-5 at protein level in the blood and brain tissues in mice. These cytokines may serve as an important biomarker during the early stages of MeHg intoxication. We also indicated that NAC was able to suppress MIP-2 and MCP-5 expressions, suggesting its potentially protective agent against MeHg neurotoxicity if these cytokines have harmful effects on brain tissues.

Funding Information This study was financially supported by Research Grant Number 38/UN.7.3.4/HK/2017 from Faculty of Medicine, Diponegoro University, Semarang, Central Java, Indonesia.

References

Aniszewska A, Chlodzinska N, Bartkowska K, Winnicka MM, Turlejski K, Djavadian RL (2015) The expression of interleukin-6 and its receptor in various brain regions and their roles in exploratory behavior and stress responses. J Neuroimmunol 284:1–9. https://doi.org/10.1016/j.jneuroim.2015.05.001

Aremu DA, Madejczyk MS, Ballatori N (2008) N-acetylcysteine as a potential antidote and biomonitoring agent of methylmercury exposure. Environ Health Perspect 116:26–31. https://doi.org/10.1289/ehp.10383

ATSDR (2017) ATSDR's substance priority list. Agency for Toxic Substances and Disease Registry. https://www.atsdr.cdc.gov/spl/.

Chang JY (2007) Methylmercury causes glial IL-6 release Neurosci Lett 416:217-220. https://doi.org/10.1016/j.neulet.2007.01.076

Chang JY, Tsai PF (2009) IL-6 release from mouse glia caused by MeHg requires cytosolic phospholipase A2 activation. Neurosci Lett 461: 85–89. https://doi.org/10.1016/j.neulet.2009.06.004

Chen YW et al (2006) The role of phosphoinositide 3-kinase/Akt signaling in low-dose mercury-induced mouse pancreatic beta-cell dysfunction in vitro and in vivo. Diabetes 55:1614–1624. https://doi.org/10.2337/db06-0029

David J, Nandakumar A, Muniroh M, Akiba S, Yamamoto M, Koriyama C (2017) Suppression of methylmercury-induced MIP-2 expression by N-acetyl-L-cysteine in murine RAW264.7 macrophage cell line. Eur J Med Res 22:45. https://doi.org/10.1186/s40001-017-0287-4

Diab A et al (1999) Neutralization of macrophage inflammatory protein 2 (MIP-2) and MIP-1alpha attenuates neutrophil recruitment in the central nervous system during experimental bacterial meningitis. Infect Immun 67:2590–2601

Eskes C, Honegger P, Juillerat-Jeanneret L, Monnet-Tschudi F (2002) Microglial reaction induced by noncytotoxic methylmercury treatment leads to neuroprotection via interactions with astrocytes and IL-6 release. Glia 37:43–52. https://doi.org/10.1002/glia.10019

Farina M, Aschner M, Rocha JB (2011) Oxidative stress in MeHg-induced neurotoxicity Toxicol Appl Pharmacol 256:405–417. https://doi.org/10.1016/j.taap.2011.05.001

Fowler MI, Weller RO, Heckels JE, Christodoulides M (2004) Different meningitis-causing bacteria induce distinct inflammatory responses on interaction with cells of the human meninges. Cell Microbiol 6: 555–567. https://doi.org/10.1111/j.1462-5822.2004.00382.x

Gadient RA, Otten U (1994) Expression of interleukin-6 (IL-6) and interleukin-6 receptor (IL-6R) mRNAs in rat brain during postnatal development. Brain Res 637:10–14. https://doi.org/10.1016/0006-8993(94)91211-4

Godefroy D et al (2012) The chemokine CCL2 protects against methylmercury neurotoxicity. Toxicol Sci 125:209–218. https://doi.org/10.1093/toxsci/kfr252

Gonzalez-Hernandez T et al (2006) Interleukin-6 and nitric oxide synthase expression in the vasopressin and corticotrophin-releasing

factor systems of the rat hypothalamus. J Histochem Cytochem 54: 427–441. https://doi.org/10.1369/jhc.5A6845.2005

Guyenet SJ, Furrer SA, Damian VM, Baughan TD, La Spada AR, Garden GA (2010) A simple composite phenotype scoring system for evaluating mouse models of cerebellar ataxia. J Vis Exp. https://doi.org/10.3791/1787

Hol J, Wilhelmsen L, Haraldsen G (2010) The murine IL-8 homologues KC, MIP-2, and LIX are found in endothelial cytoplasmic granules but not in Weibel-Palade bodies. J Leukoc Biol 87:501–508. https://doi.org/10.1189/jlb.0809532

Hwang GW, Lee JY, Ryoke K, Matsuyama F, Kim JM, Takahashi T, Naganuma A (2011) Gene expression profiling using DNA microarray analysis of the cerebellum of mice treated with methylmercury. J Toxicol Sci 36:389–391. https://doi.org/10.2131/jts.36.389

Kastenbauer S, Angele B, Sporer B, Pfister HW, Koedel U (2005) Patterns of protein expression in infectious meningitis: a cerebrospinal fluid protein array analysis J Neuroimmunol 164:134–139. https://doi.org/10.1016/j.jneuroim.2005.03.009

Kim MS, Takahashi T, Lee JY, Hwang GW, Naganuma A (2012) Methylmercury induces CCL2 expression through activation of NF-kappaB in human 1321N1 astrocytes. J Toxicol Sci 37:1275–1278. https://doi.org/10.2131/jts.37.1275

Klein M, Paul R, Angele B, Popp B, Pfister HW (2006) Koedel U, Protein expression pattern in experimental pneumococcal meningitis. Microbes Infect 8:974–983. https://doi.org/10.1016/j.micinf.2005.10.013

Koedel U, Rupprecht T, Angele B, Heesemann J, Wagner H, Pfister HW, Kirschning CJ (2004) MyD88 is required for mounting a robust host immune response to Streptococcus pneumoniae in the CNS. Brain 127:1437–1445. https://doi.org/10.1093/brain/awh171

Lee HY, Lee JS, Kim HG, Kim WY, Lee SB, Choi YH, Son CG (2017) The ethanol extract of Aquilariae Lignum ameliorates hippocampal oxidative stress in a repeated restraint stress mouse model. 17:397–BMC Complement Altern Med. https://doi.org/10.1186/s12906-017-1902-1

Lee JY, Hwang GW, Kim MS, Takahashi T, Naganuma A (2012) Methylmercury induces a brain-specific increase in chemokine CCL4 expression in mice. J Toxicol Sci 37:1279–1282. https://doi.org/10.2131/jts.37.1279

Luo Y, Fischer FR, Hancock WW, Dorf ME (2000) Macrophage inflammatory protein-2 and KC induce chemokine production by mouse astrocytes. J Immunol 165:4015–4023. https://doi.org/10.4049/jimmunol.165.7.4015

Macedo-Junior SJ, Luiz-Cerutti M, Nascimento DB, Farina M, Soares Santos AR, de Azevedo Maia AH (2017) Methylmercury exposure for 14 days (short-term) produces behavioral and biochemical changes in mouse cerebellum, liver, and serum. J Toxicol Environ Health A 80:1145–1155. https://doi.org/10.1080/15287394.2017.1357324

Mojsilovic-Petrovic J, Callaghan D, Cui H, Dean C, Stanimirovic DB, Zhang W (2007) Hypoxia-inducible factor-1 (HIF-1) is involved in the regulation of hypoxia-stimulated expression of monocyte chemoattractant protein-1 (MCP-1/CCL2) and MCP-5 (Ccl12) in astrocytes. J Neuroinflammation 4:12. https://doi.org/10.1186/1742-2094-4-12

Moller AS, Bjerre A, Brusletto B, Joo GB, Brandtzaeg P, Kierulf P (2005) Chemokine patterns in meningococcal disease. J Infect Dis 191: 768–775. https://doi.org/10.1086/427514

Muniroh M, Khan N, Koriyama C, Akiba S, Vogel CF, Yamamoto M (2015) Suppression of methylmercury-induced IL-6 and MCP-1 expressions by N-acetylcysteine in U-87MG human astrocytoma cells. Life Sci 134:16–21. https://doi.org/10.1016/j.lfs.2015.04.024

Peng S, Hajela RK, Atchison WD (2002) Characteristics of block by Pb2+ of function of human neuronal L-, N-, and R-type Ca2+ channels transiently expressed in human embryonic kidney 293 cells. Mol Pharmacol 62:1418–1430. https://doi.org/10.1124/mol.62.6.1418

Roseguini BT, Silva LM, Polotow TG, Barros MP, Souccar C, Han SW (2015) Effects of N-acetylcysteine on skeletal muscle structure and function in a mouse model of peripheral arterial insufficiency. J Vasc Surg 61:777–786. https://doi.org/10.1016/j.jvs.2013.10.098

Samuni Y, Goldstein S, Dean OM, Berk M (2013) The chemistry and biological activities of N-acetylcysteine. Biochim Biophys Acta 1830:4117–4129. https://doi.org/10.1016/j.bbagen.2013.04.016

Sarafi MN, Garcia-Zepeda EA, MacLean JA, Charo IF, Luster AD (1997) Murine monocyte chemoattractant protein (MCP)-5: a novel CC chemokine that is a structural and functional homologue of human MCP-1. J Exp Med 185:99–109. https://doi.org/10.1084/jem.185.1.99

Semple BD, Kossmann T, Morganti-Kossmann MC (2010) Role of chemokines in CNS health and pathology: a focus on the CCL2/CCR2 and CXCL8/CXCR2 networks. J Cereb Blood Flow Metab 30:459–473. https://doi.org/10.1038/jcbfm.2009.240

Sirois JE, Atchison WD (2000) Methylmercury affects multiple subtypes of calcium channels in rat cerebellar granule cells Toxicol Appl Pharmacol 167:1–11. https://doi.org/10.1006/taap.2000.8967

Spijker S (2011) Dissection of rodent brain regions. In, vol 57. pp 13–26. https://doi.org/10.1007/978-1-61779-111-6_2

Takeuchi T, Eto K (1999) The pathology of Minamata disease : a tragic story of water pollution, with editorial collaboration of H. Nakayama and A. Sumiyoshi. Kyushu University Press, Inc., Fukuoka

Whitney NP, Eidem TM, Peng H, Huang Y, Zheng JC (2009) Inflammation mediates varying effects in neurogenesis: relevance to the pathogenesis of brain injury and neurodegenerative disorders J Neurochem 108:1343–1359. https://doi.org/10.1111/j.1471-4159.2009.05886.x

WHO (2017) Mercury and health. World Health Organization. https://www.who.int/en/news-room/fact-sheets/detail/mercury-and-health. Accessed 23 January 2019

Wu Q et al. (2016) The Tibetan medicine Zuotai differs from HgCl2 and MeHg in producing liver injury in mice Regul Toxicol Pharmacol 78:1-7. https://doi.org/10.1016/j.yrtph.2016.03.017

Yamamoto M, Khan N, Muniroh M, Motomura E, Yanagisawa R, Matsuyama T, Vogel CF (2017) Activation of interleukin-6 and -8 expressions by methylmercury in human U937 macrophages involves RelA and p50. J Appl Toxicol 37:611–620. https://doi.org/10.1002/jat.3411

Yamamoto M, Motomura E, Yanagisawa R, Hoang VAT, Mogi M, Mori T, Nakamura M, Takeya M, Eto K (2019) Evaluation of neurobehavioral impairment in methylmercury-treated KK-Ay mice by dynamic weight-bearing test. J Appl Toxicol 39:221–230. https://doi.org/10.1002/jat.3710

Yamamoto M, Yanagisawa R, Motomura E, Nakamura M, Sakamoto M, Takeya M, Eto K (2014) Increased methylmercury toxicity related to obesity in diabetic KK-Ay mice J Appl Toxicol 34:914–923. https://doi.org/10.1002/jat.2954

Yasutake A, Hirayama K (1988) Sex and strain differences of susceptibility to methylmercury toxicity in mice. Toxicology 51:47–55. https://doi.org/10.1016/0300-483x(88)90079-0