Adverse Effects of Mercury Exposure in DDW Strain Mice during Organogenesis

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Abstract

Mercury (Hg) was known as a teratogenic which is distributed in tissue. This study aims to determine the retention and embryotoxicity of Hg-exposed pregnant mice. Thirty female mice was treated with $HgCl_2$ (mercuric chloride). $HgCl_2$ (5 and 6 kg/mg BW) was inducted in pregnant mice at 9 and 11 gestational days. Hg levels were measured in hair, uterus, liver, kidney, brain, blood, placenta, visceral fetus, and fetus brain on the 18th day of gestation using the atomic absorption spectrophotometry (AAS) method. Embryotoxicity test on the fetus was carried out after the surgery took place. Scales and calipers are used to calculate fetal weight and crown rump length. Statistical tests were analyzed using the SPSS 21 program. The results showed that the liver, kidney, brain, visceral fetal, and fetal brain were significantly increased ($P < 0.05$) in the treatment group at pregnant mice. Hg also produced a significant difference ($P < 0.05$) on the decrease in live fetuses, fetuses, body weight, and crown rump length and an increase in resorbed fetuses. Hg accumulation in the body apparently can cause adverse effects in pregnant mice.

Key words: *Fetus, Mercury, Mice, Organogenesis, Retention, Toxicity.*

Introduction

Hg is a very toxic heavy metal whose very high quantity in the environment. Humans can be exposed to Hg from dental amalgams, drug treatments, and food sources[1,2]. The effects of Hg toxicity in humans depend on its composition, transportation pathway, and the length of Hg accumulated in the body. In the blood, Hg will undergo an oxidation process by catalase enzymes so that it turns into Hg^{2+} ions, then through the blood circulation will be accumulated in the liver and kidneys[3]. Furthermore, microorganisms also support the conversion of Hg to Hg^{2+} and MeHg[4]. Hg has a limited ability to penetrate biological membranes, but it can pass the placenta after dissolving in the blood and will be distributed to the body. Heavy metal Hg enters the placenta along with food that is carried through blood circulation and then enters the fetus so that it can cause fetal disability[2]. Hg has a long retention time in the body so it requires a long process to eliminate it. That is because this heavy metal has a half-life of about 70 days in the body[5].

One of the developmental processes is organogenesis which in this process occurs the formation of organs in the fetus. The developmental process in mice begins at 9 days of gestation with the appearance of the forelimb bud and its differentiation continues until 18 days of gestation[6]. Based on this description, Hg can be distributed into tissues that are very vulnerable and sensitive so that it is teratogenic. Since the environment has been exposed to toxic metals such as Hg, knowledge of its adverse effects on organs is the most important global health problem. This study aims to determine the retention and embryotoxicity effect in fetuses after Hg exposure.

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Materials and Methods

The study was conducted at the Animal Laboratory of the Department of Biology, Faculty of Science and Technology, Airlangga University. The experimental animal used in this study was female mice (*Mus musculus*) DDW strain with 6-8 weeks of age and bodyweight around 25-30 g. Thirty female mice were divided randomly into six groups, with a total of 5 mice for each group. Group I (control group) was pregnant mice with aquades at 9 days; Group II (control group) was pregnant mice with aquades at 11 days; Group III (treatment group) was pregnant mice with $HgCl_2$ (5 mg/kg BW; i.p) at 9 days of gestation; Group IV (treatment group) was pregnant mice with $HgCl_2$ (6 mg/kg BW; i.p) at 9 days gestation; Group V (treatment group) was pregnant mice with $HgCl_2$ (5 mg/kg BW; i.p) at 11 days of pregnancy; Group VI (treatment group) was pregnant mice with $HgCl_2$ (6 mg/kg BW; i.p) at gestational age 11 days. On the 18^{th} day of pregnancy, all of the groups were anesthetized with chloroform and then dissected to get the organs and fetuses. The sample that had been obtained was then inserted into a tube that contains a neutral buffer of 10% formalin solution for analysis. Furthermore, embryotoxicity testing is done by observing the condition of the fetus after surgery such as the percentage of the number of implants, number of live fetuses, number of dead fetuses, and the number of fetuses absorbed based on differences in fetal movement, shape, and color. Meanwhile, the growth retardation test uses a digital balance to measure fetal body weight and calipers to measure the length of crown rump fetuses.

Measurement of Hg levels in samples using the AAS method based on Keil et al. (2011) method[7]. Firstly, the use of AAS is to prepare samples at a temperature of 300-400°C for 1 hour, then the sample was mashed using a mortar. Standard solutions were prepared and then put into a 50 mL volumetric flask to be diluted. The Hg standard solution was analyzed by AAS. A total sample was added with HNO_3 and H_2SO_4 then heated at 300°C for 3 hours using reflux. H_2O_2 was added until the clear solution. Then cooled and filtered using Whatman paper. The sample that had been destroyed was put in a bottle. Samples were analyzed by AAS type flame with a wavelength of 254 nm, so the levels of the samples were obtained. Existing data were analyzed using SPSS 21. It will proceed using the Two Way Anova test. A Duncan test was performed to determine significant differences between each group ($P < 0.05$).

Results

Hg concentration in pregnant mice could be seen in Table 1. Based on statistical tests, there were significant differences ($P < 0.05$) between the control group and treatment group in the liver, kidney, brain, fetal brain, and visceral fetal. However, the placenta, uterine, and hair showed insignificant differences ($P > 0.05$) in the treatment group. The embryotoxicity test showed a significant decrease ($P < 0.05$) in the treatment group compared to the control group in the percentage of live fetuses. Conversely, a significant increase ($P < 0.05$) occurred in the percentage of resorb fetuses in the treatment group compared to the control group. Meanwhile the percentage of implantance and dead fetus did not show any significance ($P > 0.05$) between the treatment group and the control group. The results of the overall embryotoxicity test in pregnant mice can be seen in Table 2. The results of the embryotoxicity test also showed a visual difference in the fetal shape of the mice. Fetal resorb is evident with the loss of limbs from the fetus of mice, even there are also fetal mice that do not have limbs after being dissected from their mothers. Results of visual embryotoxicity test can be seen in Figure 1. Growth retardation test showed a significant difference ($P < 0.05$) in the treatment group where Hg caused a significant decrease in fetuses body weight. In addition, Hg also caused a significant reduction in crown rump length ($P < 0.05$) in the control group. The results of the growth retardation test can be seen in Table 3.

Table-1: Toxicities of Hg concentration from various organs in pregnant mice

Organs	Gestational days	Hg Concentration (ppm)		
		Control	5 mg/kg BW	6 mg/kg BW
Placenta	9	0.0008±0.0001a	0.0063±0.0039a	0.0028±0.0010a
	11	0.0007±0.0001a	0.0022±0.0010a	0.0017±0.0010a
Uterus	9	0.0003±0.0001a	0.0021±0.0009a	0.0023±0.0014 a
	11	0.0002±0.0002a	0.0032±0.0024a	0.0019±0.0009a
Hair	9	0.0011± 0.0001a	0.0024±0.0015a	0.0048±0.0036a
	11	0.0006±0.0002a	0.0044±0.0023a	0.0089±0.0066a
Liver	9	0.0007±0.0002a	0.0020±0.001b	0.0022±0.0002b
	11	0.0002±0.0001a	0.0019±0.0007b	0.0020±0.0001b
Kidney	9	0.0004± 0.0001a	0.0020±0.001b	0.0024±0.0006b
	11	0.0003± 0.0001a	0.0026±0.0003b	0.0029±0.0004b
Brain	9	0.0003±0.0001a	0.0016±0.0005b	0.0022± 0.0008b
	11	0.0003±0.0001a	0.0023±0.0004 b	0.0026±0.0006b
Fetal Visceral	9	0.0003± 0.0001a	0.0021±0.0009b	0.0015±0.0004b
	11	0.0001± 0.0001a	0.0024±0.0008 b	0.0022±0.0005b
Fetal Brain	9	0.0002±0.0001a	0.0010±0.0001b	0.0015±0.0006b
	11	0.0001±0.0001a	0.0020±0.0005b	0.0019±0.0002b

Means bearing different superscripts (a,b,c) in a column within the period indicate significant difference in values ($P < 0.05$).

Table-2: Embryotoxicity of mice exposed to Hg at 9 and 11 days of gestation

Parameters	Gestational days	Hg Concentration		
		Control	5 mg/kg BW	6 mg/kg BW
Fetuses implantations (%)	9	9.20±0.58a	8.80±2.08a	10.20±0.66a
	11	9.20±0.58a	10.80±1.02a	11.40±0.93a
Live fetuses (%)	9	100.00±0.00a	54.00±22.27b	20.00±20.00c
	11	100.00±0.00a	90.10±3.00b	21.76±16.60c
Dead fetuses (%)	9	0.00±0.00a	4.00±4.00a	0.00±0.00a
	11	0.00±0.00a	6.54±2.72a	26.92±11.25a
Resorbed fetuses (%)	9	0.00±0.00a	42.00±23.75a	80.00±20.00b
	11	0.00±0.00a	3.36±2.07a	51.30±14.96b

Means bearing different superscripts (a,b) in a column within the period indicate significant difference in values ($P < 0.05$).

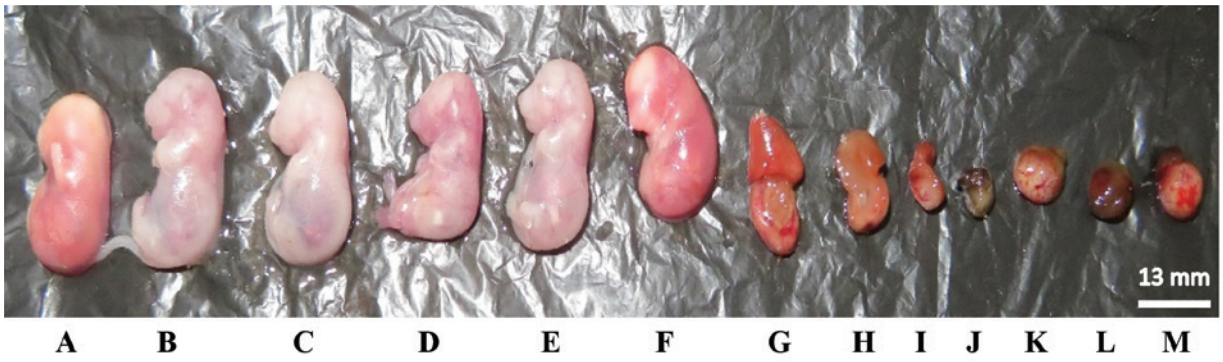

Figure-1. Embryotoxic effects on mice fetuses exposed to Hg dose of 6 mg/kg BW on gestational days 11. Dead fetuses (A, F); live fetuses (B, C, D, E); resorbed fetuses (G, H, I, J, K, L, M).

Table-3: The impact of developing fetal mice after Hg exposure.

Parameters	Gestational days	Hg Concentration		
		Control	5 mg/kg BW	6 mg/kg BW
Fetuses body weight (g)	9	1.41±0.05a	1.19±0.11b	0.93±0.01b
	11	1.41±0.05a	1.14±0.11b	0.87±0.19b
Crown rump length (cm)	9	2.42±0.04a	2.19±0.04b	1.97±0.00a,b
	11	2.42±0.04a	2.14±0.09b	1.97±0.17a,b

Means bearing different superscripts (a,b) in a column within the period indicate significant difference in values ($P < 0.05$).

Discussion

Placenta did not show a significant increase in the treatment group. This is due to the different response in Hg exposure to intrauterine which is also caused by the role of sex steroids in immune function[8]. However, uterine Hg levels can be transferred to the placenta so they can have a direct impact on the fetus[9]. The developing fetus has a high sensitivity to heavy metal toxicity. Fetal visceral organs showed significant differences in all treatment groups. This indicates the existence of a protective system in the fetus against Hg. According to Yoshida (2002), Hg that successfully crosses the placenta will be oxidized by fetal liver and bound by fetal hepatic methallotionein[10]. It shows that this protein plays an important role in protecting the fetus from Hg toxicity. Meanwhile, Oliveira et al. (2015) state that Hg accumulation can also occur in fetal brain, especially in brain motor nerve cells[11]. In addition, Hg can also accumulate in the hippocampus and fetal cerebellum[12]. Thus, the development of fetal brain is considered to have a high sensitivity to Hg[13]. However, the concentration of Hg in the parent brain is still higher than in the fetal brain. The high concentration of Hg in the brain is caused by the presence of inorganic Hg such as MeHg which can penetrate the brain barrier through diffusion and active transport[14,15,16]. The liver showed a significant difference in the treatment group. Sharma et al. (2005) revealed that exposure to Hg caused a significant increase in lipid peroxidation, ALT, and AST levels[17]. The main toxicity in Hg is related to a number of interactions in various cell metabolic processes such as in the formation of thiol complexes which can increase oxidative stress[18]. Hg^{2+} and MeHg will form covalent bonds with GSH and cysteine after they are absorbed in the cell. GSH serves as the mainline of cellular defense against Hg. However, GSH may experience a decrease in function after fighting Hg toxicity. It has been proven that Hg can reduce the antioxidant system and produce oxidative damage through the emergence of H_2O_2 and can trigger lipid peroxidation[19]. Generally, liver cells have higher GSH levels than kidney cells so that the Hg levels in the liver show lower concentrations compared to the kidneys[20,21]. In the kidney, proximal tubules are the main target tissue of Hg. Meanwhile, the half-life of Hg concentration in the kidney is around 60 days[22]. Meanwhile, the concentration of inorganic Hg has always increased since the first day until the 16th day of exposure to the Hg[23].

Significant reduction in the percentage of live fetuses indicates a high embryotoxic exposure of Hg. Moreover, the existence of fetus live after exposure because it has a higher body resistance to outside material exposure compared to other fetuses and also can begin recovery process by forming new cells. Meanwhile, Hg can cause severe damage that makes fetuses do not have time to recover[21]. Fetuses that are exposed to Hg can significantly increase the percentage of fetal resorption. This condition occurs because of fetus inability to carry out repairing damaged cells process into normal cells. In addition, fetal death that occurs can trigger the mother's body to reabsorb the dead fetus. Fetal resorption occurs in fetuses that have the lowest immune system against teratogenic substances compared to other fetuses in one parent[22]. In this study, measurements of fetal body weight and length of crown rump could not be measured in fetuses undergoing resorption because their death occurs before the organogenesis process ends where the organs of the body have not formed completely. Hg exposure caused a significant decrease in fetal body weight compared to controls. This induction can cause obstacles to fetal development by reducing the length of the crown rump. In addition, a fetus that succeeds after being exposed to teratogenic material still compete with other fetuses to obtain nutrients for its body. The mother can not provide optimal nutrition so that it causes the fetus to be smaller in size. Weight loss and fetal crown rump length are the mildest effects of the effects of teratogenic substances[24].

Fetal development and growth begin with an increase in cell number followed by the differentiation of various organ systems. The development is also influenced by several factors such as nutrition from the parent and genetics. Furthermore, fetal nutrition comes from the mother through the placenta[21]. Hong et al. (2012) states that the presence of toxic substances that enter the body causes mice require a large amount of energy to neutralize these toxic substances[25]. Thus, resulting in less than optimal parent body in providing growth nutrition for the fetus. High toxic substances

in the body can increase cell damage resulting in cell death. The number of dead cells causes disruption of the body's metabolic processes, so that it can disrupt the normal functioning of organs, and reduce growth, body weight, and body length. Low body weight in the fetus is closely related to intrauterine growth restriction (IUGR). The factor that influence IUGR include placental dysfunction, malnutrition, developmental abnormalities, chromosomal disorders, disorders of the parent metabolism, toxic substances, and drugs[11]. According Saini et al. (2013), fetal weight loss is an appropriate and sensitive indicator of developmental obstacles[26]. Interference with blood flow to the uterus due to reduction in vascularization of the uterus can cause developmental obstacles in the fetus. Fetal weight loss accompanied by a delay in ossification in the fetal skeleton can be a factor in the development of the fetus. In addition, Hg can also inhibit the transmembrane transport of nutrients in the placenta and cause an obstacle to fetal development[2].

Conclusion

Hg can be distributed and accumulates for a long time in experimental animals in the pregnant mice. The differences of concentration in Hg due to the ability of the defense mechanism in each of these organs to be exposed with it or the ability of mercury to pass through the organ's defense mechanism. This study also reveals that the administration of teratogen material Hg causes teratogenic effects in the form of embryotoxicity and growth retardation. This teratogen when exposed to pregnant mice will cause teratogenic effects that can inhibit fetal development and cause an abnormal condition in the fetus.

Conflict of Interest : The authors declare that they have no conflict of interest.

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