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ORIGINAL ARTICLES

Neurotoxic Effect of Mercury

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ABSTRACT

Mercury exposure remain a problem since it is ubiquitous and human exposure is inevitable. Its potent neurotoxic effect is associated with permanent disability and death. Microtubulin and microglia are vulnerable to neurotoxict substance .The alteration of their number in respond to toxic agent will be destructif and deadly to central nervous system.Therefore the present study investigates the dose relationship between mercury exposure and their count in brain. Male rats *(Rattus Novergicus)* were used to determine the neurotoxic effect of methylmercurychloride on their brain using microglia and microtubulin as parameter. Varying dose of MeHgCl3 from 0,2 mg/BW to 0,8 mg/BW had been administered via nasogastric tube for 21 days, then the brains were removed and microglia & microtubulin count were scored. Microtubulin count were 54.3; 48.1; 39.25; 25.85; 15.65 (CI 95%) respectively. Microglia count were 9,75; 23.95; 32.5;44.4;58.35 (CI 95%) respectively. It could be conclude that methylmercury chloride exposure will decrease microtubulin count in an inverse dose relationship whereas microglia count will increase in dose dependent manner.

Key word: methylmercurychloride; microglia, microtubulin

Introduction

Pollution remains the big problem in developing and under developing countries and one of the very hazardous heavy metal polutant is mercury. Mercury is ubiquitous, it exists naturally in the earth's crust with concentration ranges from 0,1 to 1 ppm. It also can be detected in air, soil, water, industry waste product, medical equipment and medicine. The availibility of this substance contributes to the rising number of mercury exposure and mercury intoxicity cases (Yu, 2005) 25% of the world mercury emission is the result of oil fueled waste burning (US Department of Health and Human Services, 2011).

Humans are exposed to mercury from eating fish, amalgam, mercury containning vaccine, industrial and mining activities. Fish and shell are the main source of methylmercury exposure to human. Plankton eating fish contains higher concentrantion than that of predator fish, the higher fish position in chain food the higher concentration of mercury will be detected in their body (Risher *et al*., 2002). A series of mercury intoxications have been well documented, ie. methylmercury in Minamata Bay in Japan in 1950s, when seed grain coated with a methylmercury is consumed as flour in Iraq in 1970s. Human exposure to mercury in any occasion will continue to happen (Brandao *et al*., 2006, Clarkson *et al*., 2003).

The main target of mercury poisoning is central nervous system although other organs are still at risk including kidney, eyes, cardiovaskuler. Neurotoxic effect of mercury can lead to permanent disability and death (Patrick, 2002). Neurotoxic substance have direct effect on many type of cells on brain but indirect effect on glial cells is caused by inflammatory respond. Inflammatory process begins with the activation of astrocyte and glial cells as a respond to many stimulation including brain damage and neurotoxic substance(Monnet-Tschudi *et al*., 2007). The effect become more severe as duration and concentration of exposure increase(Poulin and Gibb, 2008).

Microglia can be a target or mediator of neurotoxic substance such as mercury (Blaylock and Strunecka, 2009), once activated microglia will secrete proinflammatory cytokine, chemokine, metaloprotein which lead to more severe inflammatory respond in central nervous system (Pardo *et al*., 2005). Microtubule, a cytoskeleton of neuromembran plays an important role in neuron life and is involved in processes of cell movement, cell division and chromosomal segregation. The damage of this microtubule system results in disruption of gen and genocity (Stoiber *et al*., 2004).

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This study is a true experimental research, aimed at determining direct effect of mercury exposure on microtubule and indirect effect on inflammatory respond using microglia as parameter.

Material and Methods

Animal model:

Male rats (*Rattus Novergicus)* aged 4 months, weighed 100 to 200 g were used as animal models and were exposed to methylmercurychloride via nasogastric tube. Subjects were devided into 5 groups. The 1^{st}, 2^{nd}, 3^{rd}, 4^{th} and 5^{th} group treated with aquabidest (control group), methylmercurychlorid 0,2; 0,4 ; 0,6 ;0,8 mg/BW respectively. Treatment period lasted 21 days. At the end of the treatment the brains (hemisphere cerebri) were removed, sliced and stainned with nitric silver and microtubulin was scored using immunohistochemistry.

Chemical:

Methylmercury(II) chloride, produce of Arema.Sigma-Aldrich, Co, St. Louis, USA.

Microglia assay:

Brain tissues (cortex cerebri) was fixed with formalin buffer 10%, stained with nitric silver. Microglial cells were scored for each mercury concentration group.The counting was done by 2 different examiner using olympus BX51 microscope, 400 x magnification on different 10 fields. The mean values and standard deviation were finally calculated for the total number of cells assessed per group.

Microtubulin assay:

Glass slide containing brain tissue was washed with PBS (pH 7.4) then added with 3% H_2O_2 drops for 20 min. The glass slides was rinsed 3 times with of PBS (pH 7,4) for 5 min, then treated with 5% FBS (Fetal Bovine Serum) which contained 0,25% triton x-100 for 1 hour. The slide then washed 3 times with PBS (pH 7.4) for 5 min. The slide was incubated with primary antibody anti rat microtubulin overnight at 4^oC and then washed 3 times with PBS (pH7.4) for 5 min. After that, the slides was incubated using secondary antibody anti rabbit biotin (Santa Cruz) for 1 hour at room temperature then washed 3 times with PBS (pH 7,4) for 5 min. After that the slide was treated with SA-HRP drops (*Strep Avidin-horse radin peroxidase*), then incubate it for 40 minutes, then washed 3 times with PBS (pH 7,4) for 5 min. DAB (*Diamano Benzidine*) was added and then incubated for 10 min and washed again 3 times PBS for 5 min. Counterstainning was conducted using Mayer Hemotoxylen for 10 min. The last step was washing the slides with running water and rinsed thoroguhly in aquadest. Lastly, dried the slide, and covered it with coverslip. Microtubulin could be identified by the presence of brown stain.

Result:

Table 1: The mean values of microtubulin in each group.

The mean values of microtubulin number in each group					
Group	P-0	P-1	P-2	P-3	P-4
Mean	54.3	48.10	39.25	25.85	15.65

Table 2: The mean values of microglial cells number in each group.

The mean values of microglial cells number in each group					
Group	P-0	P-1	P-2	P-3	P-4
Mean	9.75	23.95	32.5	44.4	58.35

Microtubulin analyzis:

Neuron microtubulin were counted in control group and treatment group with the dose of 0.2; 0.4; 0.6; 0.8 mg/kg BW were 54.3; 48.10; 39.25; 25.85; 15.65 respectively. This result clearly demonstrated an inverse dose relationship between the amount of microtubulin and the dose of exposure. Normality test with one-Sample Kolmogorov-Smirnov Test showed the normality of microtubulin distribution in every group ($p>0.05$). Anova test is conducted to obtain mean microtubulin count in every group and finds it significantly different with p-value 0.000 (<0.05) (Data not shown)

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Microglial cells analyzis:

Microglial cells were counted in control group and treatment group with the dose of 0.2; 0.4;0.6; 0.8 mg/kg BW were 9.75; 23.95; 32.5; 44.5; 58.35 respectively. This findings showed a dose relationship between the amount of microglial cells and the dose of exposure. Using the same statistical analyzer this study found that the miccroglial cells were counted in every group were normaly distributed with p–value >0,05 (0.26) and significantly different with p-value 0.0009<0.05 (Data not shown)

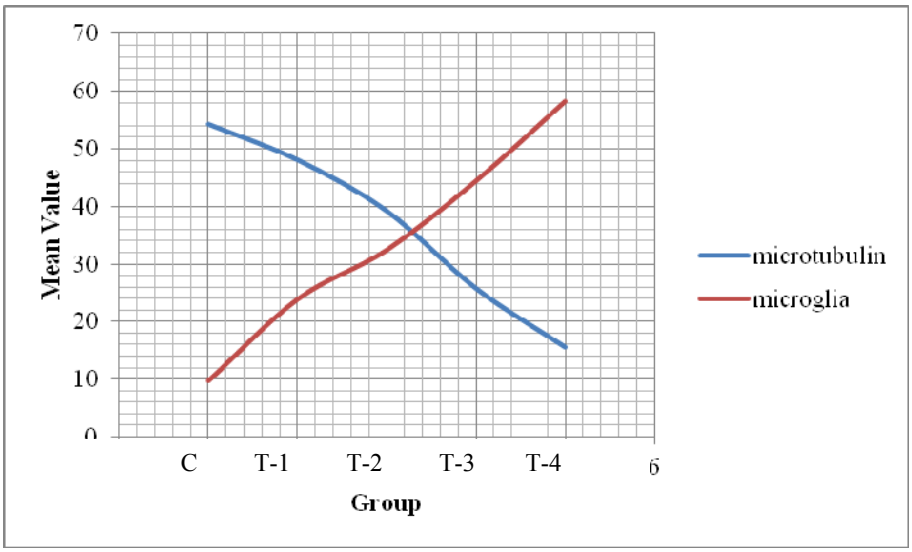

Fig. 1: Dose Relationship Between Microtubulin, Microglia Cells Count and mercury exposure

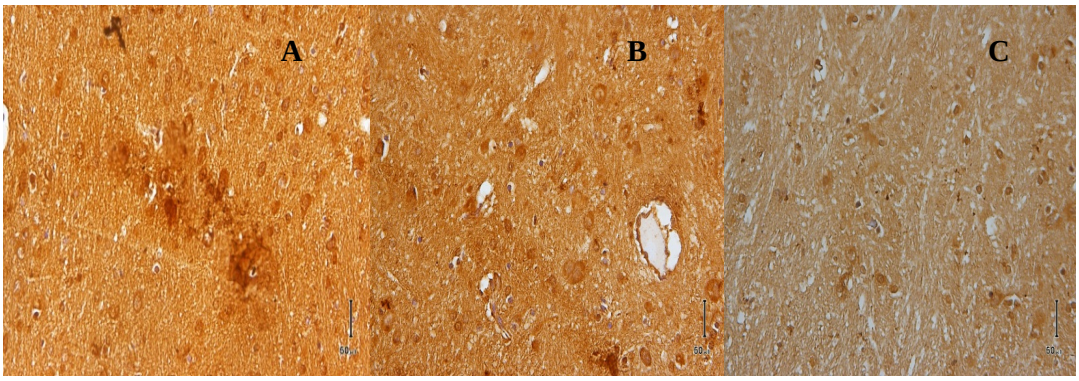

Fig. 2: Microtubule expression in control (A), group 1 (0.2mg/BW dose of exposure) (B), and group 2 (0.4mg/BW dose of exposure) (C)

Discussion:

This study was aimed to determine the neurotoxicity effect of mercury exposure on microglial cells and microtubule on brain. Male rats (*Rattus Novergicus*) were used as an animal model due to its metabolism similarity to that of human and due to its easy ways to see the direct effect of exposure on brain. The results revealed a dose-related association between the dose of methylmercury chloride exposure and the amount of microglial cells. Neurotoxic effect of mercury can directly be seen on microglial cell since the cell is part of immune system in the brain and the main source of proinflammatory factors such as (TNF-α, PGE_2, IFNγ, *oxidative stress*). Although microglial cells role is important in physiologic condition, the overtly reactivity and uncontrolled activity will be toxic for neuron and cause cell death (Block and Hong, 2007).

The presence of mercury will be considered as an antigen and will stimulate microglia reactivity. Futhermore this reactivity is stimulated by neuron death and result in the mounting of neuron death count. In otherwords the neuron damage or neuron death will stimulate glial cells reactivity and this cycle will happen over and over again a condition that we call reactive microgliosis. Glial reactivity can also be provoked by

negative gram bacteria infection and endogen peptide as well as toxic pollutant because lipopolisacharida from negative gram bacteria and toxic substance are able to activate glial cells(Block and Hong, 2007). Thus astrocyt and glial cells can be either target or mediator of neuron damage cased by neurotoxic(Blaylock, 2009). The most sensitif neuron to glial reactivity is dopaminergik neuron (neuron DA). Neuron DA is the first neuron affected by microglial whereas greater reactivity will lead to the death of other neuron (Block and Hong, 2007). Glial reactivity in physiological condition is protective and reparatif toward neuron but prolong and high dose exposure of neurotoxic substance can be destructive result in degenerative disease which is not associated with genetic disease (Monnet-Tschudi *et al*., 2007).

This study demonstrated the increasing number of microglial cell along with the increasing dose of exposure. The result was in accordance with the study conducted by Fuyuta *et al* who increased the dose of methylmercury exposure on rat from 4 to 6 mg/BW. The animal model died as the dose increased (Fuyuta *et al*., 1978). Other studies conducted by Geyer *et al* and Gellen *et al* also provide evidence of the similar dose – related association between clinical outcome and mercury exposure, the higher the dose the worst clinical outcome and the more death number (Geyer *et al*., 1985, Geelen *et al*., 1990).

Similar data on exposure – respond relationship was also observed by Hua J *et al* who identified the elevated number of glial cell from proliferation of Bergman glia cells as the dose of exposure increased (Hua *et al*., 1995) whereas Charleston Js *et al* also noted the increasing number in glial population following the increasing dose of methylmercury exposure on female macaca fascicularis(Charleston *et al*., 1994). Eskes *et al* who studied exposure of MeHgCl on rat has shown the increasing glial activity as the dose of exposure increased from 10-6 M to 10-10 M for 5-10 consecutive day (Eskes *et al*., 2002).

As already demonstrated, this study provide evidence that MeHgCl affected microtubulin count in an inverse dose-response manner, the higher the dose the lower the amount of microtubulin. Microtubule is cytoskeletal of neuromembrane in central nervous system and is composed of polymerized tubulin dimer subunits and plays an important role in neuron life cycle (leong *et al*, 2001). The disruption of the microtubule structure will lead to neuron damage.

Methylmercury has capability to interact with cytoplasma cytoskeletal component including microtubule (Nascimento *et al*, 2008). Mercury is known for strong affinity towards functional group of protein such as sulfhydril tubulin (-SH) and β-subunit tubulin has a pair of a very reactive sulfhidryl compound which is easily bound to Hg2+ (Stoiber *et al*., 2004). Microtubule is formed as result of tubulin polymerisation through the binding between GTP and β-subunit tubulin dimer. Mercury binds tubulin at β-subunit tubulin dimer. Disrupting tubulus polymerisation will cause the disruption of microtubule formation (Pendergrass *et al*., 1997). In other words mercury has direct impact on depolymerisation of cerebral microtubules and preventing tubule to assemble its structure (Sager *et al*., 1983, Vogel *et al*., 1989). MeHg is also said to be the cause of microtubules damage in other type cells such as human fibroblast, glioma cells and neuroblastoma cells (Miura *et al*., 1984). Leong *et al* (2001) also found that mercury is responsible for the number of neurodegeneration ganglia cells on snail brain (*Lymnaea stagnalis*). The damage will affect microtubule structure and nerve fiber aggregation. Pendergrass (1997) who investigated elemental mercury (Hg) exposure also observed the toxic effect of this type of mercury on tubulin.

Conclusion:

Methylmercury Chloride exposure could increase the amount of microglial cells and damage neuron microtubulin. Increasing the dose of exposure will result in more deteriorating effect.

References

Blaylock, R.L., 2009. A possible central mechanism in autism spectrum disorders, part 3: the role of excitotoxin food additives and the synergistic effects of other environmental toxins. *Altern Ther Health Med.,* 15: 56-60.

Blaylock, R.L. & A. Strunecka, 2009. Immune-glutamatergic dysfunction as a central mechanism of the autism spectrum disorders. *Curr Med Chem,* 16: 157-70.

Block, M.L. & J.S. Hong, 2007. Chronic microglial activation and progressive dopaminergic neurotoxicity. *Biochem Soc Trans,* 35: 1127-32.

Brandao, R., F.W. Santos, M. Farina, G. Zeni, D. Bohrer, J.B. Rocha, & C.W. Nogueira, 2006. Antioxidants and metallothionein levels in mercury-treated mice. *Cell Biol Toxicol.,* 22: 429-38.

Charleston, J.S., R.P. Bolender, N.K.. Mottet, R.L. Body, M.E. Vahter, & T.M. Burbacher, 1994. Increases in the number of reactive glia in the visual cortex of Macaca fascicularis following subclinical long-term methyl mercury exposure. *Toxicol Appl Pharmacol,* 129: 196-206.

Clarkson, T.W., L. Magos, & G.J. Myers, 2003. The toxicology of mercury--current exposures and clinical manifestations. *N Engl J Med.,* 349: 1731-7.

Eskes, C., P. Honegger, L. Juillerat-Jeanneret, & F. Monnet-Tschudi, 2002. Microglial reaction induced by noncytotoxic methylmercury treatment leads to neuroprotection via interactions with astrocytes and IL-6 release. *Glia,* 37: 43-52.

Fuyuta, M., T. Fujimoto, & S. Hirata, 1978. Embryotoxic effects of methylmercuric chloride administered to mice and rats during orangogenesis. *Teratology,* 18: 353-66.

Geelen, J.A., J.A. Dormans, & A. Verhoef, 1990. The early effects of methylmercury on the developing rat brain. *Acta Neuropathol,* 80: 432-8.

Geyer, M. A., R.E. Butcher, & K. Fite, 1985. A study of startle and locomotor activity in rats exposed prenatally to methylmercury. *Neurobehav Toxicol Teratol,* 7: 759-65.

Hua, J., A. Brun, & M. Berlin, 1995. Pathological changes in the Brown Norway rat cerebellum after mercury vapour exposure. *Toxicology,* 104: 83-90.

Leong, C.C., N.I. Syed, & F.L. Lorscheider, 2001. Retrograde degeneration of neurite membrane structural integrity of nerve growth cones following in vitro exposure to mercury. *Neuroreport,* 12: 733-7.

Miura, K., M. Inokawa, & N. Imura, 1984. Effects of methylmercury and some metal ions on microtubule networks in mouse glioma cells and in vitro tubulin polymerization. *Toxicol Appl Pharmacol,* 73: 218-31.

Monnet-Tschudi, F., M.G. Zurich, & P. Honegger, 2007. Neurotoxicant-induced inflammatory response in three-dimensional brain cell cultures. *Hum Exp Toxicol,* 26: 339-46.

Pardo, C.A., D.L.Vargas, & A.W. Zimmerman, 2005. Immunity, neuroglia and neuroinflammation in autism. *Int Rev Psychiatry,* 17: 485-95.

Patrick, L. 2002. Mercury toxicity and antioxidants: Part 1: role of glutathione and alpha-lipoic acid in the treatment of mercury toxicity. *Altern Med Rev.,* 7: 456-71.

Pendergrass, J.C., B.E. Haley, M.J. Vimy, S.A. Winfield, & F.L. Lorscheider, 1997. Mercury vapor inhalation inhibits binding of GTP to tubulin in rat brain: similarity to a molecular lesion in Alzheimer diseased brain. *Neurotoxicology,* 18: 315-24.

Poulin, J. & H. Gibb, 2008. Mercury: Assessing the environmental burden of disease at national and local levels. *Environmental burden of disease series, No. 16.*

Risher, J.F., H.E. Murray, & G.R. Prince, 2002. Organic mercury compounds: human exposure and its relevance to public health. *Toxicol Ind Health,* 18: 109-60.

Sager, P.R., R.A. Doherty, & J.B. Olmsted, 1983. Interaction of methylmercury with microtubules in cultured cells and in vitro. *Exp Cell Res.,* 146: 127-37.

Stoiber, T., D. Bonacker, K.J. Bohm, H.M. Bolt, R. THIER, G.H. DEGEN, & E. UNGER, 2004. Disturbed microtubule function and induction of micronuclei by chelate complexes of mercury(II). *Mutat Res.,* 563: 97-106.

US Department of Health and Human Services, P. H. S. 2011. Agency for Toxic Substances and Disease Registry (ATSDR).

Vogel, D.G., R.L. Margolis, & N.K. Mottet, 1989. Analysis of methyl mercury binding sites on tubulin subunits and microtubules. *Pharmacol Toxicol,* 64: 196-201.

Yu, M.-H., 2005. *Environmental toxicology : biological and health effects of pollutants,* Boca Raton, CRC Press.