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Myeloperoxidase as an Indicator of Liver Cells Inflammation Induced by Mercury

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ABSTRACT

Mercury (Hg) is one of the dangerous heavy metal. Exposure to Hg can cause several health problems, including liver cells damaged. However, the mechanism of liver cells damages by Hg still not clear. In this present study, we proposed that Hg could induce liver cells damaged via inflammation pathway, and in this present study we have a different approach to measure the inflammation, i.e with myeloperoxidase (MPO) activity measurement. To determine that, we correlated the MPO activity with AOPPs level which has been known as a marker of inflammation. Also, we correlated Hg and hydrogen peroxide (H₂O₂) level, Hg level and MPO activity, H₂O₂ level and MPO activity, and thiocyanate (SCN) level and MPO activity for investigated the mechanism of inflammation by Hg. The results revealed that MPO is positively correlated with AOPPs level, Hg concentrations is positively correlate with H₂O₂ level and MPO activity, H₂O₂ and SCN level is positively correlated with MPO activity. From the results, it can be concluded that Hg-induced liver cell inflammation through influencing the MPO activity. It seems Hg increase H₂O₂ and SCN level which can be utilized by MPO to form HOCl and promote an inflammation.

Keywords: Inflammation, Liver, Mercury, Myeloperoxidase.

INTRODUCTION

Mercury (Hg) is a toxic heavy metal that is considered as one of the most important toxic metals¹. Its release can occur through natural sources such as volcanic activities or anthropogenic activities such as industrial processes, agriculture, and mining, consequently making human exposure to Hg almost impossible to avoid². The most famous known cases is Minamata bay tragedy in 1950. It has been since recognized that the multiple pathways of mercury contamination through air, water, food, pharmaceuticals, cosmetic products, etc., pose serious concern because it persists in the environment and accumulates in the food web^{1,3}. The natural global biogeochemical cycling of Hg includes a conversion of Hg from inorganic into an organic form (methylmercury, MeHg) primarily in microorganisms in the aquatic environment; they are consumed by fish, and MeHg is biomagnified in the aquatic food chains so that the highest concentrations occur in large and long-lived predatory fish and marine mammals at the top trophic levels⁴. It is well known that Hg poisoning can result from inhalation, ingestion, or absorption through the skin and may be highly toxic and corrosive once absorbed into blood

stream⁵. The poisoning depends on exposure route and chemical form. Critical target organs of mercury chloride trophic exposure are kidney, liver, blood, intestinal epithelium and lungs, whereas liver, nervous system, intestinal epithelium, kidney, and muscle are common targets of organic mercury⁶. The liver is one of the central of metabolism for heavy metal and xenobiotics, including Hg. Hg mainly accumulate in several organs including liver, resulting in severe hepatic damages⁷. Previous research revealed that Hg-chloride caused histopathological and ultrastructural lesions in the liver evidenced by periportal fatty degeneration and cell necrosis⁵. One of the main and most common mechanisms behind Hg toxicity is ascribed to oxidative stress. Hg induces oxidative stress and the production of reactive oxygen species (ROS), such as hydrogen peroxide (H₂O₂) by binding to intracellular thiols (GSH and sulfhydryl proteins) and acting as a catalyst in Fenton-type reactions, with concomitantly begetting oxidative damage⁸. Also, another mechanism behind Hg toxicity is inflammation. Previous reports show that Hg potentiated LPS-induced TNF- α expression by regulating p38 MAPK. Another previous study demonstrated that oral exposure to mercury

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altered inflammatory cytokine expression during endotoxin treatment⁹. It is widely accepted that inflammation can be seen from several markers, such as pro-inflammatory cytokines level. Recently, inflammation can be seen from Myeloperoxidase (MPO) activity and or Advanced Oxidation Protein Products (AOPPs). MPO is an enzyme linked to both inflammation and oxidative stress¹⁰. MPO is a heterodimeric, cationic and glycosylated haem enzyme. MPO use H_2O_2 to catalyzes the oxidation several substrates including pseudohalide (thiocyanate ion, SCN) to form HOSCN¹¹⁻¹³. MPO can use as a marker of lung inflammation, inflammation in infection, cardiovascular inflammation, and other inflammation^{14,15}. AOPPs are diytrosine-containing and cross-linking protein products formed mainly by exposure of the protein to HOCl. AOPPs has known related to markers of monocyte activation, and may serve as mediators of inflammation¹². Previous study shows that AOPPs related to the chronic inflammation in early-stage alcoholic-cirrhosis¹⁶. Also, in another study AOPPs may represent a novel class of proinflammatory mediators acting as a mediator of oxidative stress and monocyte respiratory burst¹⁷. Since Hg can induced liver cells damage via inflammation pathway, and inflammation can be seen from MPO activity. Thus, our present study aimed to investigate the liver cells inflammation by Hg with the measurement of correlation between MPO activity and AOPPs level with the presence of Hg in different concentrations. Also, in this present study we investigated the correlation between Hg exposure and H_2O_2 level and MPO activity to determine the impact of Hg exposure to those two parameters. Since MPO use H_2O_2 as a substrate and SCN as a co-substrate, we also measure the correlation between H_2O_2 and SCN level with MPO activity with the presence of Hg in different concentrations.

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MATERIAL AND METHODS

Animals and Homogenate Preparation

Male rats (*Rattus norvegicus*) weighing 200–250 gram with 2-3 months old were obtained from the Abadi Jaya farm at Yogyakarta, Indonesia, in healthy condition. The experiment was approved by the Ethical Committee of the Lambung Mangkurat University, South Kalimantan, Indonesia. Animals were fed under standard conditions and acclimatized with a 12 hours light/dark cycle. The animals were sacrificed by surgical procedure and the livers were removed. Then, the organs homogenized in phosphate buffer saline (pH 7.0) and were ready to use for in vitro experimental models.

Experimental Models

Liver cells will be exposed to different concentration of HgCl. The HgCl concentrations were 0 mg/l, 0.1 mg/l, 1 mg/l, 10 mg/l, and 100 mg/l. Each solution then incubated at 37°C for 3 hours. After incubation, The H_2O_2 , HOSCN, and AOPPs level, and MPO activity were estimated.

H_2O_2 level analysis

The H_2O_2 level was calculated by the FOX2 method with slight modification. Solutions measured spectrophotometrically at $\lambda = 505$ nm. Standard and test solutions consisted of 1 M H_2O_2 200 μ L and 200 μ L serum,

respectively, with the addition of 160 μ L PBS pH 7.4, 160 μ L $FeCl_3$ (251.5 mg $FeCl_3$ dissolved in 250 ml distilled water) and 160 μ L o-phenanthroline (120 mg o-phenanthroline dissolved in 100 ml distilled water) for both solutions. The composition of the blank solution was identical to that of the test solution, except for an absence of $FeCl_3$ in the blank. Subsequent to preparation, all solutions were incubated for 30 minutes at room temperature, then centrifuged at 12,000 rpm for 10 minutes, and the absorbance of the standard (As), test (Au) and blank (Ab) solutions measured at $\lambda=505$ nm, using the supernatant of each solution^{18,19}.

MPO activity analysis

MPO activity was measured spectrophotometrically using o-dianisidine (Sigma-Aldrich) and H_2O_2 . In the presence of H_2O_2 as an oxidizing agent, MPO catalyzes the oxidation of o-dianisidine yielding a brown colored product, oxidized o-dianisidine, with a maximum absorbance at 470 nm. One unit (U) of MPO activity was defined as that degrading 1 μ mol of H_2O_2 per minute at 25°C²⁰.

AOPPs concentration analysis

AOPPs concentration analysis was calculated by spectrophotometric methods which were first performed by Witko-Sarsat *et al*¹⁵, with slight modification. 200 μ l of supernatant from the liver homogenate were diluted with phosphate buffer solution. Then, placed on 96-test wells. Add 20 ml of acetic acid in each test well. For the standard, add 10 ml of 1.16 mol potassium iodide, 200 ml of chloramine-T solution (0–100 mmol/l), and 20 ml of acetic acid. Placed the standard mixture into standard wells. Then, read the absorbance of the mixture at 340 nm. The absorbance was read against a blank solution. A blank solution is a mixture of 200 ml of phosphate buffer solution, 10 ml of potassium iodide, and 20 ml of acetic acid. AOPPs concentrations were expressed as μ mol/l of chloramine-T equivalents¹¹.

SCN level analysis

SCN concentration was measured spectrophotometrically as described by Aune and Thomas. In brief, 50 μ l of the sample was added to a mixture of 400 μ l of 0.1 M HCl and 100 μ l of 0.1 M ferric chloride. After centrifugation at 1000 g for 1 min, the absorbance of the supernatant due to $FeSCN^{2+}$ was measured at 450 nm¹¹.

Statistical analysis

The results were expressed as mean \pm SE for six replicates. The data was analysed between each parameter level and cyanide concentration. For analyzing the data, microsoft excell 2010 was used and was examined by simple correlation regression.

RESULTS AND DISCUSSION

The present investigation has been carried out to demonstrated that inflammation involves in liver toxicity induced by Hg. In this study, we used MPO as a marker of liver inflammation. Since AOPPs is also a marker of inflammation, we determine the correlation between MPO activity and AOPPs lever in liver cells homogenate with the presence of Hg in several concentrations. The result shows in figure 1. From figure 1, we can see that MPO

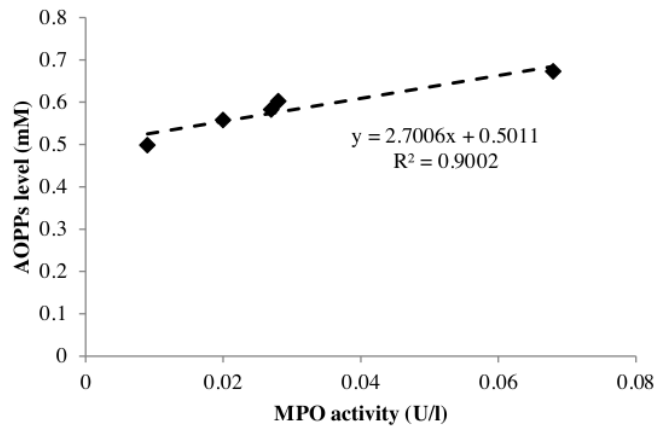


Figure 1: The correlation between MPO activity and AOPPs level with the presence of Hg in different concentrations in liver cells homogenate. MPO: myeloperoxidase; AOPPs: Advanced Oxidation Protein Products; Hg: mercury.

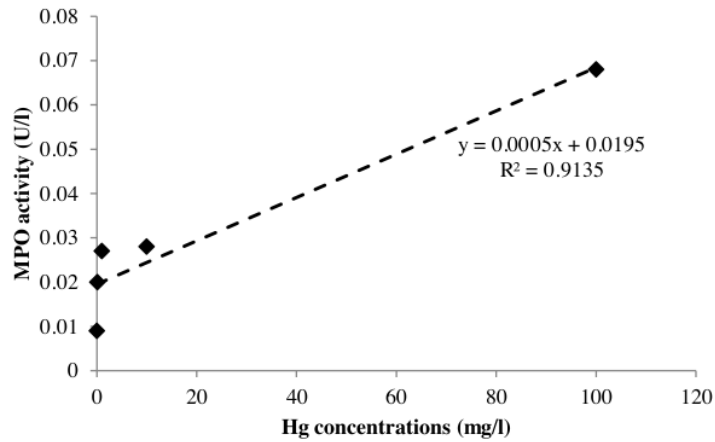


Figure 2: The correlation between Hg concentrations and MPO activity in liver cells homogenate. Hg: mercury; MPO: myeloperoxidase.

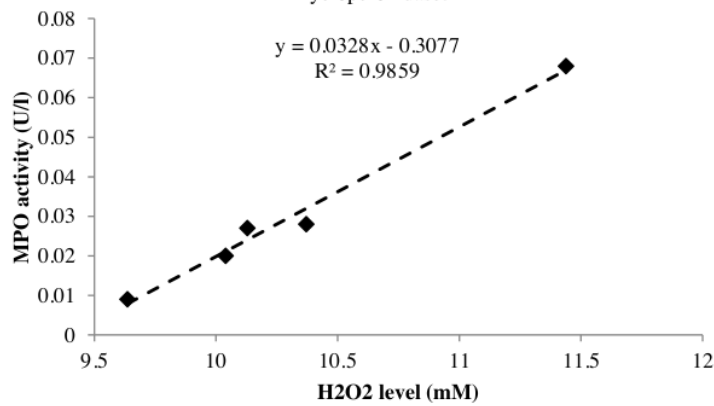


Figure 3: The correlation between H₂O₂ level and MPO activity with the presence of Hg in different concentrations in liver cells homogenate. H₂O₂: hydrogen peroxide; MPO: myeloperoxidase; Hg: mercury.

activity is strong positively correlated with AOPPs level. It means, with the presence of Hg, both MPO activity and AOPPs level are increase. It is widely accepted that MPO activity and AOPPs can use as biomarkers of inflammatory oxidative pathology²¹. This results indicated that MPO activity is strongly correlated with AOPPs level. It means Hg-induced liver inflammation which can be seen from the result in figure 1. To determine whether Hg can trigger liver cell inflammation through the activities in influencing the activity of MPO, in this present study, we correlated the different concentrations of Hg with MPO activity. The result is presented in figure 2. From figure 2 we can see that the MPO activity is increased with the increasing of Hg concentrations in liver cells homogenate. It is also consistent with another result study by Sener et al.²² who found the increasing MPO activity after Hg treatment in several rats organs. These results reinforce previous results in figure 1 that Hg exposure can cause liver inflammation through the activities in influencing the activity of MPO.

It is based on several studies that suggested that the increasing of MPO activity might be related to inflammation and infection conditions^{23,24}. The mechanism how the Hg can increase the MPO activity is still unclear. It is well known that the basic work mechanism of an enzyme is the availability of substrate. In this case, the MPO can be active when there is its substrate, ie H₂O₂. To know that, in this present study, we correlated the MPO activity with the level of H₂O₂ with the presence of Hg. The results are presented in figure 3. From figure 3 we can see that the MPO activity is increased with the increasing of H₂O₂ concentrations with the presence of Hg in different concentrations in liver cells homogenate. It means MPO is active by the increasing oh H₂O₂ level in liver cells homogenate. It is well known that MPO is a human peroxidase enzyme, an inflammatory enzyme, and a lysosomal protein stored in azurophilic granules of the neutrophil and utilize H₂O₂ as a substrate^{21,25}. In inflammatory conditions, MPO is released from the neutrophils in the extracellular environment where, in the

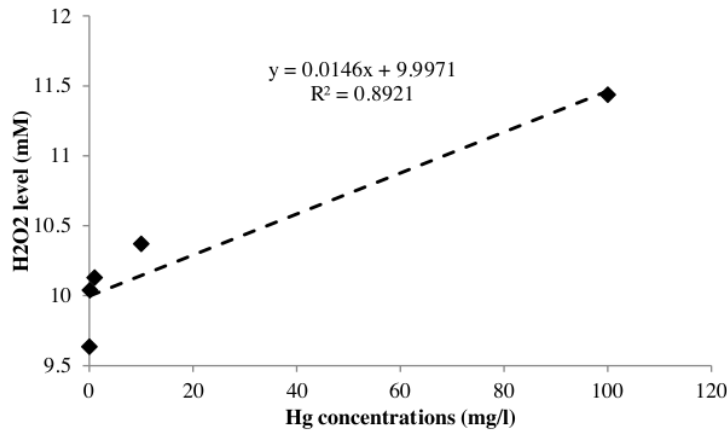


Figure 4: The correlation between Hg concentrations and H₂O₂ level in liver cells homogenate. Hg: mercury; H₂O₂: hydrogen peroxide.

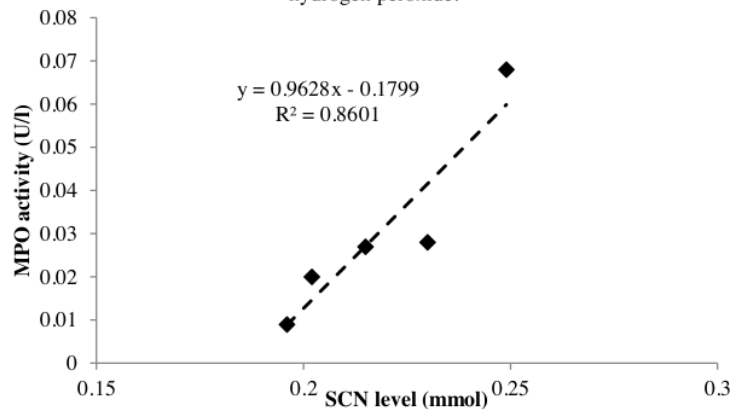


Figure 5: The correlation between SCN level and MPO activity with the presence of Hg in different concentrations in liver cells homogenate. SCN: Thiocyanate; MPO: myeloperoxidase; Hg: mercury.

presence of H₂O₂, it generates the toxic oxidant, such as, hypochlorous acid (HOCl), hypobromous acid (HOBr), and hypothiocyanite acids (HOSCN)^{19,21,26}. As mentioned above, MPO can active by the presence of H₂O₂. It is widely accepted that Hg exposure could induce the oxidative stress condition. The precise mechanism how the Hg-induced oxidative stress is still unclear. Some previous study suggested that Hg reacts with thiol groups (-SH), thus depleting intracellular thiol, especially glutathione and causing cellular oxidative stress or predisposing cells to it and forming ROS, including H₂O₂²⁷. To prove it, in this present study, we correlated the Hg concentrations with H₂O₂ level. The results are presented in figure 4. From figure 4 we can see that H₂O₂ level is a dose dependent increase with the level of Hg. This is in with previous statements above that Hg can trigger the formation of H₂O₂ which can utilize by MPO to form another toxic agent. MPO also use hypohalous acid, such as thiocyanate (SCN) as a co-substrate to form a hypothiocyanite acid (HOSCN). In this present study, we also evaluated this mechanism with the presence of Hg. The result is presented in figure 5. According to the result, it seems SCN level is positively correlated with MPO activity. It means, the increasing of SCN level will follow by the increasing of MPO activity. The SCN is a physiological co-substrate for the mammalian heme peroxidases, including MPO. A relatively abundant anion in plasma, physiological SCN concentrations is typically in the range of 20-120 μM, depending upon the vegan content of the diet. At physiological concentrations of halides (100 mM Cl⁻, 50-150 μM Br⁻, and 0.1- 0.6 μM I⁻ (23-25), oxidation of SCN accounts for approximately 40% of H₂O₂ consumed by MPO²⁸. In conclusion, the present study indicated that the Hg exposure induced liver cells inflammation through the activation of MPO since the MPO activity is strong positively correlated with AOPPs level. Also, this results indicated that Hg is induced the formation of H₂O₂ which can be used by MPO to form another toxic agent, and MPO also used SCN as a co-substrate for the reaction. Further investigation might be needed to explore the precise mechanism, how the Hg could influence the MPO activity and induced the inflammation in liver cells homogeneate.

CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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