In-vitro Interpretation of 8-OHdG Formation as Cancer Risk Biomarker due to Malondialdehyde (MDA) and Cr (VI) Exposure through Fenton – Like Reaction

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Abstract. Malondialdehyde (MDA) has been widely reported as a biomarker. Endogenous genotoxic products formed from the results of lipid peroxidation and oxidative stress can bind and modify proteins, phospholipids, and DNA to form stable adducts. The increase of oxidative stress triggers how the adducts are formed—formation of adducts linked to various patterns of diseases such as cancer, cardiovascular, and neurodegenerative diseases. The research objective was to interpret 8-OHdG formation, which is triggered by oxidative damage of genetic code (2’-deoxyguanosine) exposure to chemical compounds MDA and chromium (VI). This research was done by using in-vitro studies using MDA compound and Fenton-Like reactions. The variation of reaction conditions were at 7.4 and 8.4 pH condition, 3 and 16 hours of the incubated period at the temperature of 37°C and 60°C. The reaction products were analyzed by HPLC. From this analysis, 8OHdG target compounds were found. The correlation coefficient’s value is 0.9973. The value of LOD is 11.03 μg/L, and the LOQ value is 36.77 μg/L. In vitro test to MDA compounds at 60°C incubation temperature conditions for 3 hours, and pH 7.4 produced the highest concentration of 8-OHdG, 404.09 μg/L.

1. Introduction

Many studies have reported that one of the leading causes of death is cancer. Its incidence is increasing worldwide. More than half of cancers and 60% of deaths occur in less developed countries, whereas, with lifestyle changes similar to the Western countries, cancer rates in developing countries are increasing [3] as a very complex disease; formation of cancer cells is associated with a wide variety of risk factors. One group that causes cancer is lifestyle factors (routine smoking, diet (junk food, red meat), alcohol consumption, radiation, environmental pollutants, infections, and stress [9]. These factors can produce reactive species that will interact with macromolecules, such as DNA, protein, and lipids. In the current study, oxidative stress was induced by exposure to xenobiotics (Malondialdehyde (MDA) and Hexavalent chromium (Cr VI)) from K₂Cr₂O₇ towards nucleotide bases (2’-deoxyguanosine). Considering the adverse effect of xenobiotics (MDA and Cr VI), the objective of this research was to detect and to identify 8-OHdG generation for cancer risk biomarker by using 2’-deoxyguanosine exposed by MDA and Cr(VI) through a Fenton-like reaction.

Malondialdehyde (MDA) is known widely as one of the ultimate lipid peroxidations. Excessive production of free radicals causes an increase in Malondialdehyde levels. Generally, Malondialdehyde levels in cancerous patients have been widely reported as a sign of oxidative stress and antioxidant availability [5]. Unsaturated fatty acids that react with free radicals conduct lipid peroxidation products, i.e., malondialdehyde (MDA) and 4-hydroxynonenal [2]. MDA can trigger the formation of
protein cross-link with e-amino groups of lysine under a physiological state. From this study, it was proven that both DNA and protein adducts could be formed due to reactions with MDA in the human liver without apparent symptoms of disease [8].

In the periodic table, chromium is listed as the 24th element. The presence of chromium has an oxidation number with a valence series from -2 to +6, where Cr (III) and Cr (VI) oxidation state is the most common and stable [11,12]. Cr (III) from the environment is turning into Cr (VI) through a chemical reaction. Consequently, Cr (VI) was discovered in the seawater, groundwater, soil, rocks, and primarily from anthropogenic activity. In the biological system, the toxicity of Cr compound has an individual oxidation state. Many researchers have reported that Cr (VI) gets into the body via ingestion, dermal absorption, and inhalation. Adverse health effects can occur if Cr (VI) ingests and accumulates in the liver, kidney, and brain while not all reduced in the bowel to Cr (III) [10]. In more than a century, the carcinogenicity of chromium was firstly identified over a century ago. Based on the IARC and EPA decisions in 1990, chromium was considered dangerous for the environment and classified in group A, a carcinogen for humans [1].

The indication of exposure or susceptibility to biological systems is known as a biomarker because the presence of a biomarker indicates exposure to xenobiotics from the environment. Meanwhile, the adverse effect of biomarker shows the response of the biological system to exposure xenobiotics from the environment. A biomarker that function as a target compound explains the source and chronology of a disease [7].

Currently, DNA adduct measurement has been developed in a variety of different methods, such as 32P-post labeling, immunoassay methods based on adduct DNA antibodies, Fluorescence, and mass spectrometry-based on physicochemical properties of the adduct, high-pressure chromatography. Quantitatively, these methods have high similarity even though the endpoints of detection are different [6,4].

2. Materials and Method

2.1. Materials

2'deoxyguanosine, Malondialdehyde (MDA), and standard solutions of 8-OHdG were obtained from Sigma-Aldrich. Methanol liquid, HCl, sulfuric acid, Dimethyl Sulfoxide, Sodium Acetate, K2Cr2O7, and sodium hydroxide were purchased from Merck. All solvents and chemicals used were in analytical grade standards.

2.2. In vitro Studies

The work scheme in vitro was carried out by reacting 2'deoxyguanosine (2dG) with the xenobiotics (MDA, Cr VI, and H2O2). The 2'deoxyguanosine (100 ppm) in a solution of buffer pH 7.4 and 8.4, K2Cr2O7 (100 ppm), H2O2 (100 ppm) and demineralized water, incubation period at 3 and 16 hours, at 37 °C and 60 °C of temperatures, 100 µL of the samples to be reacted was taken so that total of mixing volume was 400 µL (shown in the following table).

| Group | 2dG (100 ppm) | MDA (100 ppm) | Cr (VI) (100 ppm) | H2O2 (100 ppm) | Demineralized water (200 microliters) | Total Volume (400 microliters) |
|-------|---------------|---------------|-------------------|----------------|--------------------------------------|-----------------------------|
| I     | 100 microliters | 100 microliters | -                 | -              |                                      | 400 microliters            |
| II    | 100 microliters | 100 microliters | 100 microliters   | -              | 100 microliters                      | 400 microliters           |
| III   | 100 microliters | 100 microliters | 100 microliters   | 100 microliters|                                      | 400 microliters           |

Table 1. Sample Mixing Variation
2.3. Apparatus
8-OHdG contents as the target compound were interpreted by using Ultra High Performance Liquid Chromatography (U-HPLC) Ultimate 3000 with UV-VIS detector by Laboratorium of Biology, University of Indonesia. The reverse-phase C-18 column was injected with the mobile phase in the form of a mixture of phosphate buffer solution and methanol (85:15) followed by 50 microliters of sample, flow rate one µL/minute.

2.4. Validation of Instrument
The validation parameters that have been done are linearity, Limit of Detection (LOD), and Limit of Quantitation (LOQ).

3. Results and Discussion
3.1. Validation of Instrument

![Chromatogram of 8-OHdG Standard Interpretation](image)

In this study, Ultra-High-Performance Liquid Chromatography (UHPLC) was used to interpret the in vitro incubated sample. The 8-OHdG standard was prepared with various concentrations of 10, 25, 50, 100, and 150 µg/L. The 8-OHdG concentration series was used to see the 8-OHdG retention time and determine the 8-OHdG levels based on the regression equation.

From the validation of the instrument, the coefficient of correlation of linearity data was 0.997 with regression equation was $y = 0.0098x - 0.0455$. Following the equation of linear regression, The LOD and LOQ have been obtained of 10.34 µg/L and 34.47 µg/L, successively.

3.2. 8-OHdG Formation by Reacting 2'Deoxyguanosine with Xenobiotics (MDA, Cr VI, H₂O₂)
The reaction of DNA bases 2'deoxyguanosine with radical triggering compounds such as MDA and Cr VI can produce DNA adduct 8-OHdG, which is placed as the primary adduct biomarker of DNA damage. This in-vitro study was carried out by several variations of experiments, including incubation period, temperature, and pH. Variation of pH 7.4 and 8.4 parameters with temperatures of 37°C and 60°C were selected to compare 8-OHdG formed between human physiological conditions and the possible effects that occur at higher conditions.
The effect of xenobiotics exposure on the formation of DNA adduct 8-OHdG which was carried out at 60°C with incubation period at 3 and 16 hours, pH 7.4, and 8.4 showed that in each of these treatment conditions and variation of reactions 2 dG+MDA, 2dG +MDA+Cr VI, and 2dG + MDA + Cr VI + H2O2 can generate 8-OHdG as target compound.
3.2.1. Interpretation of 8-OHdG Formation by Malondialdehyde Exposure

The effect of exposure to malondialdehyde (MDA) on 2’-deoxyguanosine at 60°C, pH 7.4, and 8.4 with an incubation period of 3 and 16 hours was proven to trigger the formation of 8-OHdG. The highest 8-OHdG level obtained under incubation conditions for 3 hours at pH 7.4 with concentration 404.09 µg/L. 8-OHdG was obtained higher than the limit of detection and limit of quantitation. This result was significantly different from the experimental conditions carried out at 37°C, where the 8-OHdG level was beyond the detection capacity.

3.2.2. Interpretation of 8-OHdG Formation by Cr(VI) Exposure

Exposure of combination Malondialdehyde (MDA) and Cr VI toward 2’-dG, at 60°C for 3 hours at pH 7.4 and 8.4 yielded 8-OHdG levels 357.183 µg/L and 302.09 µg/L, respectively. Meanwhile, the product concentration obtained was lower at a longer incubation time. During the incubation period of 16 hours, the resulting 8-OHdG concentrations were detected at 176.31 µg/L and 235.53 µg/L, respectively.
The chart above reveals that the variation of 2'dG exposure treatment with a mixture of xenobiotics (MDA and Cr VI) incubation period of 3 and 16 hours on average increased in 8-OHdG. The highest 8-OHdG concentration was produced under the conditions of incubation for 3 hours with pH 7.4, 60°C, at 357.183 µg/L. The increase in the concentration of 8-OHdG can occur due to the presence of Cr VI because Cr VI can oxidize 2'deoxyguanosine by acting as an ion mediator in the redox cycle. Potential reduction of Cr VI was affected by pH. At lower pH, Cr VI has a more remarkable ability to generate reactive oxygen species [12].

3.2.3. Interpretation of 8-OHdG Formation due to the Presence of Fenton – Like Agent
In this study, an interpretation of the presence of H2O2 effect as a Fenton-like Agent together with radical compounds of Cr VI and MDA has been carried out in exposure to 2'deoxyguanosine. The presence of H2O2 as a catalyst helps accelerate radical reactions under oxidative stress. The addition of H2O2 has also been shown to increase the 8-OHdG. This can be seen in the following chart.
From this study, information was obtained that Fenton-like reaction increased the levels of 8-OHdG formed by 45.20%. This can be seen in figure 5a showing that it is at pH 7.4, 60°C, and incubation period of 3 hours. The presence of H₂O₂ influences the presence increased production of 8-OHdG is influenced by the presence of H₂O₂, acts as a potent oxidant which oxidizes the chromium to a higher valence state and produces byproduct in the form of hydroxyl radical, as shown in the following reaction:

\[
\begin{align*}
    \text{O}_2^{\cdot -} + \text{H}_2\text{O}_2 & \rightarrow \cdot\text{OH} + \text{OH}^- + \text{O}_2 \\
    \text{Cr}^{3+} + \text{H}_2\text{O}_2 & \rightarrow \text{Cr}^{6+} + \text{OH}^- + \cdot\text{OH}
\end{align*}
\]

Unfortunately, from graph five, it can also be seen that there is no increase in the 8-OHdG concentration. Further research is needed to study the effect of Fenton-like Agent in increasing the levels of DNA adduct produced, especially 8-OHdG.

4. Conclusion
Xenobiotic compounds in the form of Malondialdehyde, Cr (VI), exposed singly or in combination to 2'deoxyguanosine in vitro were proven to trigger 8-OHdG formation initial signal to detect potential growth of cancer cells. The formation of 8-OHdG in vitro is also influenced by the presence of
Fenton-like agents $\text{H}_2\text{O}_2$; however, it can only increase the 8-OHdG formed under certain conditions. This study does not identify the presence of 8-OHdG formation at $37^\circ\text{C}$; thus, further studies are needed to confirm the presence of 8-OHdG formation in vitro from exposure to malondialdehyde, Cr VI, and $\text{H}_2\text{O}_2$ to 2'deoxyguanosine under physiological conditions.

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