In-Vitro Study of DNA Adduct 8-Hydroxy-2’-Deoxyguanosine Formation from 2’-Deoxyguanosine Exposure Against Benzo[a]pyrene and Ni(II) Compounds Through Fenton-Like Reactions

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Abstract. This research was performed to study the process of 8-hydroxy-2’-deoxyguanosine (8-OHdG) adduct formation as biomarker that used for early cancer detection in humans. This in-vitro study was conducted by reacting 2’-deoxyguanosine as one of the bases of DNA against benzo[a]pyrene (B[a]P) as xenobiotic, Ni(II), and H$_2$O$_2$, to study the effects of 2’-deoxyguanosine (2’-dG) exposure against xenobiotic through Fenton-like reaction. The reaction was incubated at 37°C for 18 hours in a shaker incubator. The reactions were varied at pH 7.4 and 8.4. The reaction products were then analyzed by using reverse phase HPLC with UV-Vis detector. The mobile phase used was a mixture of phosphate buffer and methanol with composition of 90:10. It was observed that exposure of 2’-dG against B[a]P and H$_2$O$_2$ and also B[a]P, Ni(II), and H$_2$O$_2$ both produced 8-OHdG. The higher 8-OHdG yield was obtained from the exposure of 2’-dG against B[a]P, Ni(II), and H$_2$O$_2$ compared to exposure against B[a]P and H$_2$O$_2$, which can be attributed to stronger effects from Fenton-like reaction. It was also observed that at similar exposure conditions, the 8-OHdG formation was higher at pH 7.4 compared to pH 8.4.

1. Introduction

Nowadays, it is known that there are many organic compounds that can cause DNA damage from oxidative stress. One of which is Benzo[a]pyrene (B[a]P), a polycyclic aromatic hydrocarbon (PAH) compound known for causing cancer formed by incomplete combustion of organic materials from roasted food, vehicle exhausts, and cigarettes [1]. It is also known from a study done by Thyssen et al in 1981, B[a]P exposure against rats are known to increase the occurrences of tumor and decrease the lifespan of the exposed group [2].

The interaction of reactive oxygen species such as hydroxyl radical with nucleobases of DNA, such as guanine, leads to the formation of DNA Adduct 8-hydroxy-2’-deoxyguanosine (8-OHdG). DNA Adduct is a damage DNA that will be repaired enzymatically and commonly excreted in blood and urine [3]. The increase of 8-OHdG levels in urine can be used as an indicator of increasing risk of DNA damage that leads to the risk of carcinogenesis.
Meanwhile, nickel is a common metal used in industry such as manufacture of coin, magnets, and electroplating industry. Nickel as a compound is commonly found at oxidation state +2 and is commonly used as pigments [4], such as Yellow 53 which is an inorganic complex consisting of titanium oxide, nickel (II), and antimony (V) [5].

Exposure against nickel in human can occur as occupational risk through inhalation of airborne nickel particles and contact with nickel working instruments with the skin. Exposure against nickel can also occur from other ways, such as nickel alloys in bone surgery. Research has shown that skin contact with nickel increased the occurrence of skin allergy [4]. Meanwhile, in a study done by ATSDR, nickel is known to increase the occurrences of lung and nose cancer and therefore nickel is classified as a Type A carcinogen [6]. Nickel is also known to be able to generate a reactive oxygen species that can cause oxidative stress to DNA [7]. The aim for this research is to understand the mechanism of DNA adduct formation in human from exposure against xenobiotics such as metals and polycyclic aromatic hydrocarbons.

2. Experiment

2.1. Materials

2’-deoxyguanosine, Benzo[a]pyrene, and 8-hydroxy-2’-deoxyguanosine (8-OHdG) standard solutions were obtained from Sigma. Methanol liquid chromatography grade, hydrochloric acid, sulfuric acid, DMSO, NiCl$_2$, and NaOH were obtained from Merck.

2.2. In-Vitro study

2’-dG was in phosphate buffer solutions of pH 7.4 and 8.4. There was xenobiotic mixture variations of 2’dG, B[a]P, Ni(II), and H$_2$O$_2$ (shown in Table 2).

### Table 1. Variation of xenobiotic mixtures

| No | 2’-dG (4 ppm) | B[a]P (60 ppm) | Nickel (II) (120 ppm) | H$_2$O$_2$ (120 ppm) | Aquabidest | Total Volume |
|----|--------------|---------------|----------------------|---------------------|------------|--------------|
| 1  | 100 uL       | 100 uL        | -                    | -                   | 200 uL     | 400 uL       |
| 2  | 100 uL       | 100 uL        | 50 uL                | -                   | 100 uL     | 400 uL       |
| 3  | 100 uL       | 100 uL        | -                    | 50 uL               | 150 uL     | 400 uL       |
| 4  | 100 uL       | 100 uL        | 50 uL                | 50 uL               | 100 uL     | 400 uL       |

All of mixture samples (400 uL) were shaken in an incubator shaker at 37°C for 18 hours. The 8-OHdG was analyzed from samples by using HPLC-UV detector.

2.3. Instruments

Analysis of 8-OHdG level in samples was done by using Hitachi Primaide HPLC with UV-Vis detector provided by HIPERKES K3 Cempaka Putih Laboratory. Samples (20 uL) were injected into column C-18 octadecyl silane and mobile phase 10 mM sodium phosphate buffer pH 6.7 and methanol (90:10). Under this condition, 2’-dG and 8-OHdG were eluted at 2.433 min and 5.547 min respectively. The 8-OHdG level was expressed in ppb.

3. Results and Discussions

3.1. Results
Figure 1 shows the level of 8-OHdG from the mixture of 2’-dG exposed by B[a]P was higher at pH 7.4 as much as 213.92 ppb. And increase four fold in the presence of Ni(II) metals, approximately was 858.07 ppb at pH 7.4. The peak 8-OHdG from 2’-dG+B[a]P+Ni(II) mixture at pH 7.4 was eluted in 5.400 min, the chromatogram shows in Figure 2a.

This 8-OHdG level was higher than the exposure of H₂O₂ both at pH 7.4 and pH 8.4, as much as 832.60 ppb and 196.15 ppb respectively. Meanwhile the 8-OHdG level from the mixture of 2’-dG exposed by B[a]P, Ni(II), and H₂O₂ was the highest at pH 7.4 and pH 8.4, as much as 963.35 ppb and 706.13 ppb respectively. Chromatogram of 8-OHdG from 2’-dG, B[a]P, Ni(II), and H₂O₂ mixture at pH 7.4 was eluted in 5.407 min, as it is shown in Figure 2b.

3.2. Discussion

Based on previous studies, the reaction between 2’-deoxyguanosine and xenobiotic are known to be able to cause the oxidation of DNA at guanine base, which in turn causes oxidative stress resulting in
8-OHdG DNA adduct as seen in Figure 2 [8]. This is done by the radical oxygen formed by the xenobiotic that attack the guanine base, forming guanine radicals, one of which is C8 radical. These guanine radicals then have their electron abstracted, forming 8-OHdG adduct [3].

![Image of DNA adduct formation](image)

**Figure 3. Transformation of 2’-deoxyguanosine into 8-OHdG by oxidative stress**
(Source: Valavanidis et al. 2009, with modification)

3.2.1. Effect of Benzo[a]Pyrene in the formation of 8-OHdG. We conducted in vitro study using 2’dG and B[a]P compound then found that B[a]P compound can trigger the formation of DNA Adduct such as 8-OHdG. According to the result, it can be assumed that the formation of 8-OHdG has occurred from oxidative reaction of B[a]P which will form reactive oxygen species such as H₂O₂, O₂⁻, and HO⁻ [11]. Oxidation process of B[a]P occurs in two ways, the radical cation formed from the enzymatic reaction reacts with oxygen in water, or B[a]P compound itself reacts with molecular oxygen enzymatically, they are show in equation 1 and 2 [9].

\[
\begin{align*}
\text{BaP}^+ + H_2O & \xrightarrow{\text{kat}} \text{6-OH-BaP} + 2H^+ \quad (1) \\
\text{NADPH} + H^+ + \text{BaP} + O_2 & \xrightarrow{\text{epr,40}} \text{6-OH-BaP} + \text{NADP}^+ + H_2O \quad (2)
\end{align*}
\]

These reaction causes the formation of intermediate 6-hydroxy-B[a]P which quickly undergo autoxidation with molecular oxygen, and form H₂O₂ and other radical species that can react with 2’-dG and generate 8-OHdG adduct as the equation 3 to 6 show below [9].

\[
\begin{align*}
6\text{-OH-BaP} + O_2 & \rightarrow O_2^- + [6\text{-OH-BaP}]^2- -H^+ \rightarrow 6\text{-oxy-BaP} \quad (3) \\
6\text{-oxy-BaP} + H^+ + O_2 & \rightarrow \text{BaP-diols (1,6-, 3,6-, 6,12-)} + O_2 \quad (4) \\
\text{BaP-diol} + O_2 & \leftrightarrow \text{BaP-semiquinone} + O_2^- + 2H^+ \quad (5) \\
\text{BaP-semiquinone} + O_2 & \leftrightarrow \text{BaP-dione} + O_2^- \quad (6)
\end{align*}
\]

3.2.2. Effect of nickel and Fenton-Like reactions towards 8-OHdG formation. The effects of nickel towards 8-OHdG can be seen with the results that samples with nickel produced higher 8-OHdG yield compared to samples without nickel. This is because at samples with nickel, Fenton-Like reaction occurs, in which organic compounds are oxidized with metals other than iron, whereas a Fenton
reaction is a reaction in which organic compounds are oxidized with iron [10], as seen in equation 1 below.

\[
\text{Fe}^{2+} + \text{H}_2\text{O}_2 \rightarrow \text{Fe}^{3+} + \text{OH}^- + \text{OH}^-
\]

(7)

In a Fenton-Like reaction, the metal is oxidized after reacting with H$_2$O$_2$, whereas the H$_2$O$_2$ is reduced to form hydroxide ion and hydroxyl radical. These radicals, which are reactive oxygen species would then attack guanine base and form 8-OHdG adduct. Meanwhile the oxidized metal would then be reduced by the hydroperoxyl radical, whereas the hydroxyl radical would then form H$^+$ ion and O$_2$ [10], which can be seen in equations 8 and 9.

\[
\text{OH}^- + \text{H}_2\text{O}_2 \rightarrow \text{HO}_2^- + \text{H}_2\text{O}
\]

(8)

\[
\text{Fe}^{3+} + \text{HO}_2^- \rightarrow \text{Fe}^{2+} + \text{H}^+ + \text{O}_2
\]

(9)

In this reaction, other than being a reduct, nickel also serves as an ion mediator in redox cycle, which helps the transfer of electrons which results in oxidation of B[a]P to form BPDE which is the carcinogenic form of B[a]P[11].

Without nickel, the only source of reactive oxygen species would be the H$_2$O$_2$ alone which is expected to produce fewer reactive oxygen species, because H$_2$O$_2$ would only react with superoxide radicals to form hydroxyl radical that can attack guanine [11]. In this condition, B[a]P would also have lower oxidation rate compared with the condition in the presence of nickel.

3.2.3. Effect of pH towards 8-OHdG formation. Overall, the effects of pH towards 8-OHdG formation can be seen with the results that samples at pH 7.4 produce higher 8-OHdG yield compared to samples at pH 8.4. For exposure of dG against xenobiotic where nickel is present, lower 8-OHdG yield at pH 8.4 can be explained that at lower pH, nickel is easier to be reduced than at higher pH due to higher reduction potential at lower pH [12].

4. Summary

It can be concluded from this research that when 2’-deoxyguanosine is exposed to xenobiotic such as Benzo[a]pyrene, nickel, and H$_2$O$_2$, 8-OHdG as DNA adduct can form oxidative stress on guanine base. It is also found that 8-OHdG formed from 2’-deoxyguanosine exposure is higher when exposed against Benzo[a]pyrene, nickel, and H$_2$O$_2$ compared to just exposed against B[a]P and H$_2$O$_2$ due to stronger effects from Fenton-Like reactions. Finally, at similar exposure conditions, 8-OHdG formation is found to be higher at pH 7.4 compared to pH 8.4.

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