In vitro study of DNA-adduct formation (8-OHdG) due to exposure of benzo[a]pyrene and Co(II) through fenton-like reaction

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Abstract. Severely increasing environmental pollution with an unhealthy pattern of life in the community can increase the source of exposure of carcinogenic substances that can cause cancer. Benzo[a]pyrene and Cobalt (II) are some examples of carcinogenic substances commonly found in the environment. Both materials can simultaneously enter the body through food or polluted air and damage the DNA. In this study, we studied the effect of B[a]P and Co(II) exposure in-vitro on 2’-deoxyguanosine. An increase in the amount of the 8-hydroxy-2’-deoxyguanosin compound is used as an indicator of the occurrence of DNA damage and a cancer risk biomarker. It was found that the synergistic effect on the combination of B[a]P and Co (II) through Fenton-like reaction is influenced by the concentration of H.O. The amount of 8-OHdG that formed with the combination of B[a]P and Co (II) is 126.23 ppb. It does not vary much with the amount that resulted from B[a]P exposure alone at 136.53 ppb. Exposure of Co(II) through Fenton-like reaction alone gives the highest yield of 8-OHdG formation at 282.67 ppb. Followed by B[a]P and Co(II) at 258.98 ppb, B[a]P and H.O. at 153.16 ppb.

Keywords: Benzo[a]pyrene, Cobalt(II), Fenton-Like reaction, 8-hydroxy-2’-deoxyguanosin.

1. Introduction
The high level of pollution in the environment and an unhealthy lifestyle can leads to an increasing risk of cancer. These two phenomena increase the source of exposure to chemicals that can lead to cancer, referred as carcinogenic substances. Carcinogenic is a trait of a material that can cause the emergence of cancer cells by damaging biomolecules such as DNA.

One example of the carcinogenic substance is benzo[a]pyrene (B[a]P). This compound falls under the class of polycyclic aromatic hydrocarbon formed by an incomplete combustion of organic matter. B[a]P can be easily found in environment due to pollution from burning organic waste, forest fires, and vehicle fumes. B[a]P can also be found in foods, especially from grilled red meat [1]. B[a]P as much as 18.2 +/- 0.5 ng/rod can also be found in cigarette smoke [2].

Cobalt(II) is also another carcinogenic pollutant that can be found in the environment. Since the beginning of the 20th century, mined cobalt has been used to produce metal alloys such as superalloys and magnetic alloys, as well as high-strength steel and hard-metal cemented carbides. In the late 1980s, around one-third of the cobalt employed was in the fabrication of cobalt chemicals, mainly as catalysts and pigments. As metal alloys, cobalt is also widely used in the health equipment such as braces and implants [3].

B[a]P and Cobalt (II) can produce reactive oxygen species (ROS) [4,5]. These radical molecules can induce oxidative damage to DNA and lead to cancer [6]. When DNA is damaged by ROS, it is generally found an increase in the amount of metabolite compound 8-hydroxy-2’-deoxyguanosine.
Moreover, this superoxide can react with $B[a]P$ to form 6-oxo-$B[a]P$ and superoxide [8].

$$B[a]P + H_2O \rightarrow 6-OH-B[a]P$$  \hspace{1cm} (1)

$$6-OH-B[a]P + O_2 \rightarrow [6-OH-B[a]P] \rightarrow 6-oxy-B[a]P$$  \hspace{1cm} (2)

Moreover, this superoxide can further reacts to form H$_2$O and hydroxyl radical [8].

(8-OHdG). An increase in the number of 8-OHdG compounds can be used as an indicator or biomarker against an increasing risk of cancer due to exposure to carcinogenic compounds [7].

2. Materials and methods

2.1. Chemicals and reagent

2’-deoxyguanosine monophosphate, Benzo[a]pyrene, DMSO, CH$_3$COONa, K$_2$HPO$_4$, KH$_2$PO$_4$, NaHPO$_4$, and Na$_2$HPO$_4$ were used to make buffer (Sigma-Aldrich). H$_2$O, Co(NO$_3$)$_2$ (Merck), HCl, NaOH, and aquabides.

2.2. Instrumentations

Incubator shaker (Julobo SW22), Sonicator and Degasser 8510 (Bronson) were used to remove gas bubble from High Performance Liquid Chromatography (HPLC) solvents, pH meter to measure buffer solution pH (ZORBAX Eclipse Plus C18, 4.6 x150 mm, 5-Micron) and HPLC (Primaide) for chromatography analysis to detect and measure 8-OHdG.

2.3. In vitro studies

The in-vitro studies were done in seven variations of carcinogenic substances exposure, incubated at 37 °C for 18 hours. 200 µL of 2’-deoxyguanosine (6 ppm) was dissolved in phosphate buffer 0.01 M (pH 7.4 and 8.4), in variation one, it was reacted with 200 µL of benzo[a]pyrene (60 ppm). In variation two, it was reacted with 200 µL of benzo[a]pyrene (60 ppm) and 100 µL H$_2$O (120 ppm). In variation three, reacted with 200 µL benzo[a]pyrene (60 ppm) and 100 µL Co(II) solution (120 ppm). In variation four, reacted with 100 µL of Co(II) solution (120 ppm) and 100 µL of H$_2$O (120 ppm). In variation five, reacted with 100 µL of Co(II) solution (120 ppm), 100 µL of H$_2$O (120 ppm), and 200 µL of ascorbic acid solution (60 ppm). In variation six, reacted with 200 µL of benzo[a]pyrene (60 ppm), 100 µL of Co(II) solution (120 ppm), and 100 µL of H$_2$O (120 ppm). And in variation seven, it was reacted with 200 µL of benzo[a]pyrene (60 ppm), 100 µL of Co(II) solution (120 ppm), 100 µL of H$_2$O (120 ppm), and 200 µL of ascorbic acid solution (60 ppm).

3. Results and discussion

This study aims to provide a scientific information on the effect of combined exposure of carcinogenic substance of both $B[a]P$ and Co(II), to 2’-deoxyguanosine on the formation of 8-OHdG. The reaction of $B[a]P$ and 2’-deoxyguanosine form 8-OHdG can be detected by using HPLC, as seen in figure 1. $B[a]P$ can react with H$_2$O to form 6-hydroxy-$B[a]P$. This substance then reacts with O$_2$ to form 6-oxo-$B[a]P$ and superoxide [8].
Figure 2. The effect Co(II) addition in 8-OHdG formation

![Figure 2. The effect Co(II) addition in 8-OHdG formation](image)

Figure 3. The effect of H₂O₂ amounts in 8-OHdG formation

![Figure 3. The effect of H₂O₂ amounts in 8-OHdG formation](image)

\[ 2O₂ + 2H \rightleftharpoons 2HO₂ \quad (3) \]
\[ 2HO₂ \rightleftharpoons H₂O₂ + O₂ \quad (4) \]
\[ O₂ + H₂O₂ \rightleftharpoons HO₂⁻ + OH⁺ + O₂ \quad (5) \]

The hydroxyl radical that formed then can oxidize 2′-deoxyguanosine and produce 8-OHdG [7]. While Co(II) reacts with H₂O₂ to produce hydroxyl radical through Fenton-like mechanism as follow:

\[ + HO₂⁻ \rightarrow 8\text{-OHdG} + e^- + H⁺ \quad (6) \]

When B[a]P and Co(II) react with 2′-deoxyguanosine, there is an increase in the amount of 8-OHdG, as seen in figure 2. This might be caused by the hydroxyl radical from the reaction of Co(II) and H₂O₂ is a byproduct from superoxide reaction as mentioned before.

The amount of H₂O₂ gives different results depending on the combination of the carcinogenic substances, as seen in figure 3. The increase of H₂O₂ as the result of 2′-deoxyguanosine and B[a]P reaction can increase the amount of 8-OHdG. While the increase of H₂O₂ in the combination of B[a]P and Co(II) is not followed by the increase of 8-OHdG. It is assumed that there is a synergistic effect of the combination between B[a]P and Co(II). H₂O₂ and Co(II) will react through Fenton-like mechanism effectively. When the amount of H₂O₂ is too high, it is possible that the Fenton-like mechanism also oxidizes the B[a]P[9]. This can decrease the amount of B[a]P in the solution[10]. This might explain why the amount of 8-OHdG that formed were not much different than the one formed in the reaction between 2′-deoxyguanosin and B[a]P alone.

Ascorbic acid is well known due to its antioxidant properties. In fact, many antioxidant substances also contain prooxidant properties. In this study, both antioxidant and prooxidant properties of ascorbic acid can be observed each in different conditions. In figure 4, it shows that when ascorbic acid is added to the mixed solution between 2′-deoxyguanosin and Co(II) + H₂O₂, ascorbic acid...
Figure 4. The effect of ascorbic acid in the formation of 8-OHdG

Figure 5. The effect of pH (a) without and (b) with Co(II) addition in 8-OHdG formation

exhibits its antioxidant properties. While in the mixed solution that contain 2’-deoxyguanosin, Co(II) + H₂O₂, and B[a]P, ascorbic acid exhibits its prooxidant properties. Ascorbic acid can reduce some metal ion thus make them to react easier with H₂O₂ through Fenton or Fenton-like mechanism [11]. In the mixed solution between 2’-deoxyguanosin and Co(II) + H₂O₂, there is high amount of hydroxyl radical, thus there is not enough unoxidized ascorbic acid that can reduce the cobalt ions and make them to react easier with H₂O₂ through Fenton-like mechanism. It indicates that ascorbic acid exhibits its antioxidant properties. While in the mixed solution that contain 2’-deoxyguanosin, Co(II) + H₂O₂, and B[a]P, the amount of hydroxyl radical are decreased by the oxidation of both 2’-deoxyguanosine and B[a]P, thus there are enough unoxidized ascorbic acid that can reduce the cobalt ions and make them to react easier with H₂O₂ through Fenton-like mechanism. It indicates that ascorbic acid shows its prooxidant properties.

pH of the solution also gives different result at different condition. If there is no Co(II) in the solution, a high pH value can increase the amount of 8-OHdG, as seen in figure 5a. On the other hand, if there is Co(II) in the solution, a high pH can decrease the amount of 8-OHdG as seen in figure 5b. The high pH value indicates a low amount of H⁺ ions. This will decrease the potential reduction of Co(II) and can turn some of Co(II) into Co(III). The decrease in the amount of Co(II) will produce a small amount of additional hydroxyl radicals through Fenton-like mechanism and it can decrease the amount of 8-OHdG.

4. Conclusions
The exposure of either B[a]P or Co (II) and H₂O₂ at 2’-deoxyguanosine leads to the formation of 8-OHdG compounds. The synergistic effect on the combination of B[a]P and Co (II) through Fenton-like reaction is influenced by the concentration of H₂O₂. At high enough concentration, the synergistic effect is worn off. If there is no Co(II) in the solution, a high pH value can increase the amount of 8-OHdG. On the other hand, if there is Co(II) in the solution, a high pH can decrease the amount of 8-OHdG. Ascorbic acid may act as both antioxidant and prooxidant. In the mixed solution between 2’-deoxyguanosin and Co(II) + H₂O₂, ascorbic acid exhibits its antioxidant properties. On the other hand,
in the mixed solution that contains 2’-deoxyguanosin, Co(II) + H₂O₂, and B[a]P, ascorbic acid shows its prooxidant properties.

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