Study of urinary 8-hydroxy-2’-deoxyguanosine (8-OHdG) in rats (Rattus norvegicus) due to bisphenol A (BPA) exposure based on fenton-like reaction

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Abstract. This study was conducted to analyze the formation of DNA adduct 8-OHdG due to oxidative damage of DNA caused by Bisphenol (BPA) and chromium (VI) exposure. The in vivo study was conducted in the rat (Rattus norvegicus) group with exposure of BPA (2 mg/kg BW), the mixture of BPA (2 mg/kg BW) and Cr (VI) (1.28 µg/kg BW) for 28 days, respectively. Urine of rats was collected every week as samples for tracking the 8-OHdG compounds. The formation of 8-OHdG in urine samples was analyzed using LC-MS/MS with reverse-phase chromatography. Mixture of ammonium acetate pH 4.0 20 mM and acetonitrile were used as mobile phase in LC-MS/MS for an elution gradient. The results showed that the exposure of BPA, the mixture of BPA and Cr (VI) might cause the formation of urinary 8-OHdG in rats. The longer exposure time causes the increase level of urinary 8-OHdG formations. The study also showed that urinary 8-OHdG concentration increases due to synergistic effect between BPA and Cr (VI) exposure.

1. Introduction

Bisphenol A (BPA; 2,2-bis (4-hydroxy-phenyl) propane) is a chemical compound produced in large quantities in the industrial field. This compound is used as a monomer for producing polycarbonate (PC) in plastic and epoxy resin, or as an antioxidant and stabilizer in various plastics [1]. With so many benefits, the use of BPA are currently widespread in the production of water-drain pipes, children's toys, medical devices, dental products, electronic devices, and CD/DVDs. Along with the development of industries in many countries, BPA as a chemical is commonly used in many industries in the product also continues to grow to this day.

Based on its main function as a plastic monomer and epoxy resin, BPA can enter the human body through two main lines, food and non-food intake. If it exposes to heat and in contact with acidic or alkaline solutions, the ester in BPA as polycarbonate or epoxy resins will rapidly hydrolyze [2]. This causes the migration of BPA into food or drink from containers that use BPA as polycarbonate monomer or epoxy resin. Many countries have banned or restricted the use of BPA due to the ease of BPA release and the adverse health effects. The side effects caused by BPA exposure are toxicity, teratogenic, carcinogenic, and especially estrogenic effects [3]. In the human body, BPA can also trigger the production of reactive oxygen species (ROS) [4]. BPA in the human body cannot directly undergo the reaction that causes the hydroxyl radical to form. However, BPA is notable to reduce the activity of antioxidant enzymes in mitochondria, such as superoxide dismutase enzymes, catalase, glutathione reductase, and glutathione peroxidase, accompanied by elevating levels of hydrogen peroxide and lipid peroxidation [5]. Cr (VI) is known to be easily passing through the cell membrane and get in the body cells [6]. Cr (VI) will undergo a reduction process to a lower valence state, Cr (V), Cr (IV), and Cr (III). In this process, the reduction reaction with Cr (VI) will produce metabolites in the form of a large number of reactive oxygen species [7]. This study have analyzed the DNA adduct of 8-hydroxy-2’-deoxyguanosine (8-OHdG) in rat urine after 28 days of exposure to BPA and Cr (VI) chemical compounds.
2. Materials and methods

2.1. Chemicals
The BPA, 8-OHdG and SPE cartridge DSC-18LT tube were purchased from Sigma-Aldrich (SG). HPLC grade acetonitrile and methanol, potassium dichromate, ammonium acetate, dimethyl sulfoxide and diethyl ether were from Merck (ID). All other chemicals and solvents were an analytical grade.

2.2. Ethical approval
Experimental protocol was approved by the ethics committee of the Faculty of Medicine, Universitas Indonesia (Indonesia).

2.3. Animal preparation
The present study was conducted on 8 weeks old healthy Sprague-Dawley rats and housed in cages. The animals were first acclimatized for 21 days to adapt to the new environments and gained the appropriate weight (130–200 grams). Animals were provided with standard feed in the cage and allowed water ad libitum throughout the experimental period. Rats were divided into three groups, group A is a control group without chemical treatment, group B is a group with BPA exposure, and group C is a group with the chemical mixture exposure of BPA and Cr(VI).

2.4. Formulation
BPA was dissolved into dimethyl sulfoxide and water. BPA solution was administered orally in Group B with dose volume of 10 mL/kg [8]. The daily administration was continued for 28 days. The mixture of BPA and potassium dichromate were administrated orally in Group C with dose volume of 5 mL/kg respectively [9]. The daily administration was continued for 28 days.

2.5. Experimental design
Experimental groups consist of three groups. Each group has 5 male rats. Rats of Group A were kept as negative control and were given water orally. Rats of group B were given BPA orally; rats of group C were given the mixture of BPA and potassium dichromate. The dose for group B was 2 mg/kg body weight for 28 days. The dose of BPA and potassium dichromate for group C was 4 mg/kg and 1.28 µg/kg body weight, respectively, for 28 days. The doses in this experiment were based on sub-chronic toxicity study.

2.6. Sample collection and preparation
Rats urine samples were collected using metabolic cages and frozen immediately for analysis. The samples were thawed at room temperature. Before analysis, 5 mL of methanol and 5 mL of 20 mM ammonium acetate pH 4.0 were used to precondition the SPE cartridge. The mixture of 20 mM ammonium acetate pH 4.0 and acetonitrile (97:3) (v/v) were used to wash the SPE cartridge after loading 500 µL of urine sample, and 8-OHdG was eluted into the mixture of 20 mM ammonium acetate and acetonitrile (80:20)(v/v).

2.7. Calibration curves, LOD and LOQ
Calibration curve was obtained using 8-OHdG standard solutions in the 1–50 ng/mL. The limit of detection (LOD) and limit of quantification (LOQ) were determined statistically using the linear regression equation from the calibration curves. The measurement value will be equal to the value of b on the linear regression equation of y = a+bx, whereas the standard deviation of the blank is equal to the standard deviation of residual S(y/x).

2.8. Instrumental analysis
Samples were inspected in a 20A liquid chromatography system (Shimadzu, Japan) coupled with an AB Sciex Q-Trap 3200 mass spectrometer (LC-MS/MS). All analytes were separated in a Hypersil Gold C, column (50 x 2.1 mm, 5 µm, Thermo Scientific). Acetonitrile and 20 mM ammonium acetate pH 4.0 were used as mobile phase. The gradient elution program is displayed in table 1. The following conditions were applied to the instrument, 0.5mL/min of flow rate, 40 °C of column temperature, and 10µL of injection volume.

The initial temperature was fixed at 550 °C, and the ionization voltage was 5500 V. The analyte was analyzed in multiple reactions monitoring (MRM) mode with a dwell time of 300 ms. Both
Table 1. The gradient mobile phases for 8-OHdG analysis

| Time (min) | Ammonium acetate (%) | Acetonitrile (%) |
|------------|----------------------|-----------------|
| 0.5        | 95                   | 5               |
| 1.0        | 5                    | 95              |
| 2.0        | 5                    | 95              |
| 2.5        | 95                   | 5               |
| 3.0        | Stop                 |                 |

Table 2. Optimization of MS/MS parameters for 8-OHdG analysis

| Precursor ion (Q1) | Product ion (Q3) | Retention time (min) | DP (eV) | EP (eV) | CE (eV) | CXP (eV) |
|--------------------|------------------|----------------------|---------|---------|---------|----------|
| 284.1              | 168.2            | 0.738                | 43.0    | 4.0     | 17.0    | 3.0      |
| 140.1              |                  | 0.738                | 43.0    | 4.0     | 40.0    | 3.0      |

Figure 1. Chromatogram Standard of 8-OHdG 100 ppb LC-MS/MS

nebulizer gas (GS1) pressure and the turbo heater gas (GS2) pressure were 55 psi. The curtain gas (CUR) was 20 psi and the collision gas (CAD) pressure was medium, respectively. The MS/MS parameters, containing parent ion (Q1), product ion (Q3), collision energies (CE), declustering potential (DP), entrance potential (EP) and collision exit potential (CXP) were optimized. The optimized parameters are listed in table 2.

3. Results and discussion

3.1. Calibration curves, LOD and LOQ

Figure 1 shows chromatogram of standard solution of 8-OHdG. The calibration curves for the standard compound was linear with correlation coefficient (≥ 0.999) in the range of 1–50 ng/mL, which covers the lowest concentration of 8-OHdG in rats urine, and the slope was 34.321. The limit of detection and limit of quantification was 1.350 ng/mL and 4.499 ng/mL, respectively, according to the calculation of the linear regression equation from the calibration curves.

3.2. Detection of 8-OHdG in urine samples

3.2.1. The effect of BPA exposure on the formation of 8-OHdG

In this experiment, in vivo study was conducted to see the formation of DNA adduct 8-OHdG in the urine sample due to BPA exposure for 28 days with a dose of 2 mg/kg body weight. BPA exposure in rats could generate reactive oxygen species such as •OH radicals and leads to the formation of DNA adduct 8-OHdG, as shown in figure 2. The lowest and highest of 8-OHdG concentrations are equal to 27.597 ng/mL and 31.683 ng/mL, respectively. The mean of 8-OHdG concentrations due to BPA exposure is 29.258 ng/mL.
3.2.2. The effect of BPA and Cr(VI) exposure on the formation of 8-OHdG. This experiment was conducted to see the synergistic effect of BPA and Cr(VI) exposure for 28 days with the dose of 4 mg/kg and 1.28 μg/kg body weight, respectively. The exposure of BPA and Cr(VI) metal of Group C may cause the formation of DNA adduct 8-OHdG and its concentration is higher than Group A, as shown in figure 3. From the data results, it is proved that the presence of Cr(VI) metal will enhance the production of reactive oxygen species such as •OH radicals.

The results of urine samples measurements in group C showed that Cr(VI) exposure had a synergistic effect on the formation of DNA adduct 8-OHdG as seen from the increase of 8-OHdG levels compared to group B, as shown in figure 4. Cr(VI) metal will undergo reduction process in the presence of NAD(P)H to a lower and more stable valence state, Cr(V), Cr(IV), and Cr(III), the
reaction will produce a large number of reactive oxygen species as shown in figure 5 (Fenton-like reaction) [6]. The lowest and highest of 8-OHdG concentrations is equal to 27.638 ng/mL and 51.290 ng/mL, respectively. The mean of 8-OHdG concentrations due to BPA and Cr(VI) exposure is 34.356 ng/mL.

4. Conclusions
BPA and Cr(VI) exposure to rats can lead to the formation of DNA adduct 8-OHdG. Due to BPA exposure for 28 days, the formation of urinary 8-OHdG reaches 31.683 ng/mL. The Cr(VI) exposure may increase the urinary 8-OHdG concentration due to its synergistic effect with BPA exposure. BPA and Cr(VI) exposure can lead to urinary 8-OHdG formation up to 27.638 ng/mL.

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