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A study of the correlation between ultrafine particle emissions in motorcycle smoke and mice erythrocyte damages

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ABSTRACT

Sharply increasing of motor vehicles every year contributes to amounts of ultrafine particles (UFPs) in the air. Besides, the existence of UFPs in the blood may cause erythrocyte damages that subject to shape deformation. This study was aimed to investigate the influence of UFPs in the motorcycle smoke exposed to mice in different concentrations to the erythrocyte damages. The experiments were conducted by injecting the motorcycle smoke with the varied amounts in an experimental chamber (dimension of 30 × 20 × 20 cm^3) where the mice were put in advance for exposuring twice a day (100 s). Total numbers of UFPs in the smoke were calculated by measuring the total concentrations multiplied by the smoke debit. They were measured using a TSI 8525 P-Trak UPC. The effects of the smoke exposures in the mice's erythrocytes related to the UFPs in the smoke were observed by a binocular CX-31 Computer Microscope after the 2nd, 4th, 6th, 8th, and 10th exposure days. The erythrocyte damages were calculated from the total abnormal erythrocytes divided by the total erythrocytes. Our results showed that more UFPs exposed to mice resulted in more the erythrocytes damages. Longer exposures caused more damages of the mice erythrocytes. This study found significant correlations between the numbers of UFPs exposed to mice and the erythrocyte damages. Our finding gives important evidence that motorcycle emissions especially UFPs affect on health.

1. Introduction

Motor vehicles have significantly increased in the recent year. In Indonesia only, a number of motor vehicles in 2013 reached 104,118,969, consisted of 11,484,514 passenger cars, 2,286,309 buses, 5,615,494 trucks, and 84,732,652 motorcycles (SI, 2014). In 2011, vehicles in Ho Chi Minh City, Vietnam, was dominated by light gasoline vehicles, such as 92% of motorcycles, 3.46% of cars, 2.8% of light trucks, 0.1% of buses, and 1.1% of heavy truck diesel vehicles (Ho and Clappier, 2011).

Road transport becomes a significant contributor causing an air quality problems in many cities (Pandey and Venkataraman, 2014). Several studies revealed that motor vehicles identified as the source of air pollutants (Chiang et al., 2014; Vanhulsel et al., 2014; Zhou et al., 2014). The pollutants are in gaseous and particulate matters (Kampa and Castanas, 2008). The gasesous emissions were reported about 94% CO, 68% NMVOC, 61% SO_2, and 99% CH_4 (Ho and Clappier, 2011), meanwhile the particulate matters consisted of elementary carbon of 0.559% and organic carbon of 0.202% (Hu et al., 2015). In terms of particulate matters (PMs), a variation of concentration, composition, and size distribution were emitted from different vehicles (Morawska and Zhang, 2002). Particles with different particle size distribution have been measured, such as: ultrafine particles (UFPs), fine particles, and PM_{10}. Especially in UFPs, they have a diameter less than 0.1 μm (Madl and Pinkerton, 2009).

The impacts of UFPs emitted by motor vehicles on human health have been investigated in previous studies due to their composition and toxicity characteristics (Sioutas et al., 2005; Weichenthal et al., 2014). In terms of their size, UFPs might be able to penetrate lung until the ends of alveoli, then following through blood stream (Oberdörster et al., 2005). They may deposite in the human respiratory tract and have impacts on cardiovascular health (Delfino et al., 2005). UFPs effects on experimental animals have been observed, and the results showed that UFPs caused damages into their organ, such as in pulmonary (Yamamoto et al., 2006), brain (Allen et al., 2014), heart (Jia et al., 2012), and erythrocyte cells (Nemmar and Inuwa, 2008). The effects of UFPs on erythrocytes has been studied in terms of erythrocyte sedimentation, induced hemmagglutination, and hemolysis, deformation, agglutination, and membrane damage (Li et al., 2008). Due to the very important role of erythrocytes in transporting oxygen to tissues or

Abbreviations: UFP, ultrafine particles; PAHs, Polycyclic Aromatic Hydrocarbons; VOCs, Volatile Organic Compounds

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Table 1
Motorcycle samples.

Motorcycle	Model Year	Cylinder Volume (cm^3)
M1	2009	125
M2	2011	125
M3	2012	125
M4	2013	125
M5	2015	125

organs, it is necessary to investigate factor influencing the damages especially deformation. This study was aimed to investigate the correlation between UFPs in the motorcycle smokes and the erythrocyte damages to obtain a better understanding of the impacts on UFP emissions on erythrocytes. The special reasons for this study were because of the limitation data of the impacts of UFPs on erythrocytes, and unavailable data for impacts on UFP emissions of motorcycles on human health.

2. Material and methods

2.1. Motorcycle samples

Five motorcycles were preferred as samples of UFPs concentration sources due to their popularity in Indonesia. The motorcycles were produced by the same manufacturer with different model years as presented in Table 1. All samples were considered as the sources of UFPs.

2.2. Animals and treatment

In total, 130 adult male mice of the age of 10–11 weeks (23.2 ± 1.5 g BW (Jia et al., 2012)) were purchased and approved by the Brawijaya University ethical commission. They were housed under 12-h light/dark cycle (Allen et al., 2014) with controlled temperature 27.4 °C–28.1 °C and relative humidity 57.8%–58.8% in the exposure chambers as long as three days called acclimation treatments. These treatments were aimed to the mice be able to adapt into the exposure chamber. All mice were provided with water and mice's foods ad libitum and treated humanely with the regard for the suffering alleviation (Jia et al., 2012). All things needed for the study including of consumables, equipment, and substances used were a courtesy of mice used in accordance with the accepted standards of humane animal care and the guidelines by the Brawijaya University Ethical Commission (No. 541-KEP-UB).

The mice were arranged into six groups which each group consisted of five mice. One group was used as control animals, and the other groups were exposed by the UFPs contained in the motorcycle smoke (M1–M5). Prior to dosing, we consider to determine the injected times of the exposure treatment is below 100 s. Our pre-experiment gave us information that the mice collapsed for the given UFPs contained in the smoke by the injected time more than 100 s as the cut off (saturated) concentration. We decided the selected times in terms of exposure to 5 variations: 20, 40, 60, 80, and 100 s. Thus, the UFP concentrations were injected into the exposure chamber as long as 20, 40, 60, 80, and 100 s. After the UFP exposures, the mice were totally penned into the exposure chambers as long as 100 s for the inhalation procedures. The exposures were conducted twice a day in the morning and afternoon. After exposures, the mice were placed back to the housing chamber. The sacrifices were carried out on the day of 2nd, 4th, 6th, 8th, and 10th after the exposures.

2.3. UFP measurements

Fig. 1 shows the experimental setup for the UFPs measurements. The UFP concentrations of the smokes were measured after injecting the smokes into an exposure chamber (with the dimension of $30 \times 20 \times 20\ cm^3$) for 20 s. The measurements were repeated for the injecting time of 40, 60, 80, and 100 s. The measurement of UFP concentrations was conducted using P-Trak Ultrafine Particle Counter (TSI, model 8525).

The total ultrafine particles in the smoke were calculated using an equation as follows (Wardoyo et al., 2006, 2007) as a single dose.

$$C_{total} = Q_{total} \cdot \int_0^t C(t)dt \quad (1)$$

The total UFPs exposed to the mice were calculated for a different exposure day. The total ultrafine particles exposed for a day were calculated by the total particles every exposure times 2 in which the exposures were conducted twice a day. For 2nd days exposures, the total UFPs for one-day exposure were multiplied by 2. The same way was applied to calculate the total UFPs after the exposure day of 4, 6, 8, and 10.

2.4. Histopathological examination of erythrocyte

For the pathologic studies, all of the procedures were conducted using international laboratory standard. The blood smears were placed onto object glasses and dropped with methanol solution (70%). The blood smears were air-dried as long as 5 min. After Giemsa and buffer pro-Giemsa solution staining, the stains were rinsed using pure water. The results were observed under an optical microscope (Olympus CX-31) by a histopathologist (Yu et al., 2016).

The damage percentage was calculated from the normal and abnormal erythrocytes laying on the five random field of view (Brandenberger et al., 2015) using Eq. (2). The damage was considered from the difference between the damage percentages in the control and the treatment mice.

$$\text{Damage}\ (\%) = \frac{\text{total of abnormal erythrocytes}}{\text{total of erythrocytes}} \times 100\% \quad (2)$$

The criteria to define the normal and abnormal shapes of erythrocytes as the deformation changes (poikilocytosis) in the mice erythrocytes were resumed from many kinds of literature. The normal erythrocyte (normocytic) diameter is approximately between 6.7–8.2 μm. A sickle cell disease (SCD) or sickle-shaped (drepanocyte/eccentrocyte/schistocyte) is defined as the sickling of the red blood cells. Other shape variations of erythrocytes deformations are stomatocyte, tear-drop shaped (dacrocyte), helmet-shaped, saddle-shaped, and eliptocyte as seen in Fig. 2 (Almeida et al., 2015; Pretorius et al., 2016).

2.5. Statistical analysis

Statistical analysis was carried out by calculating the correlations between the total concentration of UFPs from each vehicle and the erythrocyte damage percentages. The data were presented as the mean values ± standard deviation/SD (Yu et al., 2016). The relationship between total UFPs and erythrocyte damage percentages was determined using R square value (Weichenthal et al., 2015).

3. Results

3.1. Total of UFPs

The total calculated UFPs are presented in Table 2 (as a single dose) for different injected times.

The adjusting of the smoke injecting times was meant to vary the ultrafine particle concentrations in the smokes that would be exposed to the mice. From Table 2, it can be seen that the numbers of UFPs

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