Toxicology Reports 5 (2018) 383–389

Contents lists available at ScienceDirect

Toxicology Reports

journal homepage: www.elsevier.com/locate/toxrep

Varied dose exposures to ultrafine particles in the motorcycle smoke cause kidney cell damages in male mice

Arinto Y.P. Wardoyo*, Unggul P. Juswono, Johan A.E. Noor

Department of Physics, Brawijaya University, Malang Indonesia Jl. Veteran, Malang, East Java, 65145, Indonesia

ARTICLE INFO

Keywords:
Ultrafine particles exposure
Motorcycle exhaust emission
Mice
Kidney cell damages

ABSTRACT

Ultrafine particles (UFPs) are one of motorcycle exhaust emissions which can penetrate the lung alveoli and deposit in the kidney. This study was aimed to investigate mice kidney cell physical damage (deformation) due to motorcycle exhaust emission exposures. The motorcycle exhaust emissions were sucked from the muffler with the rate of 33 cm^3/s and passed through an ultrafine particle filter system before introduced into the mice exposure chamber. The dose concentration of the exhaust emissions was varied by setting the injected time of the 20s, 40s, 60s, 80s, and 100s. The mice were exposed to the smoke in the chamber for 100 s twice a day. The impact of the ultrafine particles on the kidney was observed by identifying the histological image of the kidney cell deformation using a microscope. The exposure was conducted for 10 days. The kidney observations were carried out on day 11. The results showed that there was a significant linear correlation between the total concentration of ultrafine particles deposited in the kidneys and the physical damage percentages. The increased concentrations of ultrafine particles caused larger cell deformation to the kidneys.

1. Introduction

The number of motor vehicles in the world has been increased from year to year. In Ho Chi Minh City, Vietnam, the road was dominated by light gasoline vehicles, such as motorcycles (92%), cars (3.46%), and light trucks (2.8%) [1]. Motor vehicle emissions have become significant contributors to air pollution that need considerable attention due to their influences on air quality and human's health [2]. As the implication of the increased motor vehicles, a number of their emissions in ambient air will be increased too. The emissions have been identified as NO_2, particulate matters, polycyclic aromatic hydrocarbons, black elemental carbons, hopanes, steranes [3], nonvolatile particles [4], SO_2, and CH_4 [1]. The elements of volatile organic compounds, such as isopentane, toluene, and o-xylene can be found in the motorcycle exhaust emission during real-world driving [5].

Previous studies have examined the health effects of ambient air ultrafine particles. They have found the association between ambient ultrafine particles and relevant organ health disorders due to their composition and toxicity. They might be able to penetrate alveoli and deposit to the alveolar surface area [6]. They follow the bloodstream, and harm lung [7], brains [8], and erythrocytes [9]. The ultrafine particle impacts on health are still needed to be investigated deeply. Because of their size, ultrafine particles induce oxidative stress and steatosis in hepatocytes [10], and cause postnatal immunological dysfunction [11]. However, there are limited studies of the impacts of ultrafine particles emitted by motor vehicles, and no available information of the effect of the ultrafine particles on kidney damage. This study was aimed to investigate the effects of the motorcycle exhaust emissions exposure to male mice to get the correlation between the ultrafine particles dose concentration and the kidney cell physical damage (cell deformation).

2. Materials and methods

2.1. Vehicle sample

Five automatic transmission motorcycles (engine capacities: 125 cm^3) were selected based on the popularity at Malang (Indonesia). They were classified into M1 (model year: 2009), M2 (model year: 2011), M3 (model year: 2012), M4 (model year: 2013), and M5 (model year: 2015).

2.2. Experimental animals and treatments

All experimental animal treatments were in accordance with the international ethics guidelines and approved by the Animal Care And Use Committee of Brawijaya University Malang, Indonesia (Ethical Clearance No:541-KEP-UB). There were 31 male mice (mean body

* Corresponding author.
E-mail addresses: a.wardoyo@ub.ac.id (A.Y.P. Wardoyo), unggul-pj@ub.ac.id (U.P. Juswono), jnoor@ub.ac.id (J.A.E. Noor).

https://doi.org/10.1016/j.toxrep.2018.02.014
Received 5 July 2017; Received in revised form 27 February 2018; Accepted 27 February 2018
Available online 06 March 2018

weight: 22.7 ± 0.1 g) with the age of 10–12 weeks old. The mice were treated humanely and with regarding the suffering alleviation by providing food and water ad libitum. They were acclimated to the experiment environment (exposure chamber 20 × 20 × 30 cm^3) for 3 days [9]. The temperature of housing facility was maintained at 24–27 °C with a relative humidity of 75–78% and a 12 h light/dark cycle. After the acclimatization process, they were randomly divided into the control group (n = 6) and the treatment groups (M1; M2; M3; M4; M5; n = 5/group) and housed separately in the acrylic cages.

2.3. Exposure dose measurements

The motorcycle samples were operated for 60 s for the engine warming up. The muffler was connected to an ultrafine particle filtering system containing a cylindrical tube (diameter: 2.54 cm), an N95 filter paper (3 M 8210 Particulate Respirator), and a sucking pump. The exhaust emissions from the motorcycles were filtered using the filtering system. The system lets the particles with the diameter less than 0.1 μm (ultrafine particles). The exposures of motorcycle exhaust emission doses were adjusted by setting of the sucking time for 20, 40, 60, 80, and 100 s to get the varied concentration of C1, C2, C3, C4, and C5. After passing through the filtering system, the exhaust emissions were introduced into the exposure chamber with the rate of 33 cm^3/s. After sucking the exhaust emissions, the pump was turned off and disconnected from the exposure chamber using a valve. The ultrafine particle concentration was measured using a P-Trak UPC (TSI, model 8525) (Fig. 1) [9].

The total concentration of ultrafine particles (C_T) was calculated by summing the measured concentrations (C_t) [12].

$$C_T = \sum_{t}^{n=0} C_t \quad (1)$$

2.4. Mice exposures

The mice were exposed to the ultrafine particles with the concentration of C1, C2, C3, C4, and C5 for 100 s. Every group of mice was exposed to the filtered exhaust emission with the concentration of C1, C2, C3, C4, and C5 for 100 s for the ultrafine particle inhalation regarding their tidal volumes and respiratory rates [13]. Our previous research gave us information that the mice collapsed for the exposure more than 100 s. The control mice ($n = 6$) were not exposed to the filtered exhaust emissions. After the exposures, they have been released to their origin cages. All mice were sacrificed in the day 11 [9].

2.5. Preparation and histopathologic examination

The mice from each group (n = 1/group) were sacrificed by a cervical dislocation [14]. Their kidneys were fixed in a 10% buffered formalin [15] for a histopathologic examination as long as seven days. After that, they were dehydrated through upgraded ethanol series, trimmed, and processed to paraffinization. The sections were cut using microtome (4 μm thickness) and stained routinely with hematoxylin and eosin. The observations were performed using a computer microscope (Olympus, BX-31). The histopathological examination was performed by scoring the kidney cell deformation level (physical parameter evaluation) of five random fields taken from the 400× magnification of each section [16,17]. In each field, the number of normal and abnormal tubular epithelial cells were counted, while the level of Bowman's space was measured (Fig. 2) [18]. Normal tubular epithelial cells had no edema and vacuolation [16]. A normal glomerulus indicated a normal organize appearance of glomerulus with a regular Bowman's space. The abnormal glomerulus indicated a loss of integrity, atrophy, and increasing of Bowman's space [19,20]. The deformation level of the glomerulus was focused on the capsular space and was classified into 20%, 40%, 60%, 80%, and 100% wider than the control. Eq. (2) was used to convey the physical damage percentage of the kidney cells.

$$\text{Physical Damage (\%)} = [(\Sigma\text{abnormal tubules}/\Sigma\text{tubules} \times 100\%) + \%\ \text{glomerulus deformation}]/2 \quad (2)$$

2.6. Statistical analysis

Values are reported as means ± standard deviations (SD), as indicated. The correlation between the ultrafine particle exposures and the mice kidney damages was evaluated by regression models using the Microsoft Excel 2016 software [21]. For all analyses, $R^2 > 0.80$ was considered statistically significant [9].

3. Results

3.1. Particle concentrations

Fig. 3 shows the example graphs of the measured ultrafine particle concentrations for M2 (as the representative of the whole samples). Particle concentration measurements were performed three times for each sample (1st measurement-3rd measurement).

The blue dots show the 1st measurement results. The red dots present the 2nd measurement. Finally, the green dots indicate the 3rd measurement. By using Eq. (1), we calculate the total concentration. All graphs have the same trend, where the higher dose contains a higher concentration of ultrafine particles. In order to get a better understanding, Table 1 presents the total concentrations of ultrafine particles from M1-M5 calculated for a single exposure. From this table, we found that different motor sample produced ultrafine particles with the different concentration. The oldest model emits the highest concentration. The highest exposure concentration of C5 is 4.00 × 10^5 particles/cm^3 emitted by the sample of M1. The lowest one is 3.32 × 10^5 particles/cm^3 obtained from the sample of M5. As expected before, it was estimated that the older model of the engine emitted the highest concentration. The highest value of C1 exposure is 2.52 × 10^5 particles/cm^3 obtained from M2. The lowest one is 1.63 × 10^5 particles/cm^3 obtained from M4.

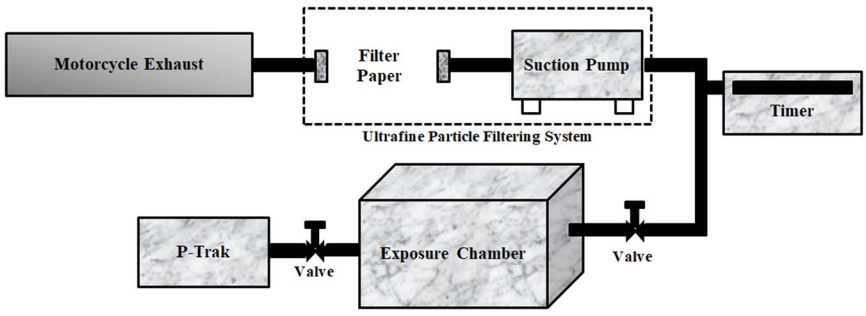

Fig. 1. Experiment set up of the ultrafine particles measurement.