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In vitro toxicity of dimethyl phthalate to human erythrocytes: From the aspects of antioxidant and immune functions☆

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ABSTRACT

In the study, the effects of dimethyl phthalate (DMP) on the antioxidant defense capacity and immune functions of human erythrocytes were experimentally explored. DMP affected the activities of superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX) and the contents of glutathione (GSH) and malondialdehyde (MDA) in erythrocytes, thus impairing the function of antioxidant defense system of erythrocytes. When DMP concentration increased from 0 to 28 μmol L^{-1}, the SOD and GPX activities were increased firstly and then gradually decreased. When DMP concentration was below 20 μmol L^{-1}, the relative activity of SOD was enhanced by DMP and the effect was known as hormesis. The relative activity of GPX was also increased when the concentration of DMP was below 12 μmol L^{-1}. The CAT activity was more significantly inhibited by DMP than the activities of SOD and GPX, whereas the relative GSH content was increased by DMP. MDA levels were significantly changed after the exposure to DMP (0–24 μmol L^{-1}). The experimental results of the activity of SOD and CAT, and the content of MDA also suggested that DMP could inhibit the immune functions of red blood cells (RBCs), which were further proved by the decrease of two indicators (RBC-C_3b and RBC-IC) due to the destruction of C_3b receptor with immune adherence function on erythrocyte membrane. The study provides a deep understanding of the toxicity of DMP on erythrocytes.

1. Introduction

Phthalate esters (Lu et al., 2009; Ventrice et al., 2013) (PAEs) are one kind of the most important chemicals due to their extensive application in plastics, coatings, synthetic fibers and cosmetics industries as polymer plasticizers and additives to increase flexibility and toughness. There is no chemical bond between PAEs and plastic molecules, which are connected by hydrogen or van der Waals forces (Abdel daiem et al., 2012), thus leading to their wide distribution in various environmental media such as water (Peijnenburg and Struijs, 2006), atmosphere (Teil et al., 2006) and soil (Wang et al., 2013a). PAEs are lipophilic organic compounds with high log values of the octanol-water partition coefficient (K_{OW}) and lower water solubility (Julinová and Slavík, 2012). PAEs tend to accumulate in organisms with bioaccumulation, bioamplification, and biomagnification behaviors (Lien et al., 2015). The damage of PAEs to organisms is mainly realized through three major pathways including inhalation, dietary intake and dermal absorption (Rael et al., 2009; Wang et al., 2013b), which are also the essential routes of PAEs to enter the blood. PAEs have been found in human body fluids, such as blood, amniotic fluid, umbilical cord blood and urine (Johns et al., 2015). PAEs are a kind of endocrine disrupting chemicals (EDCs) and can interfere with the hormonal system and affect the intact physiology of organisms. The toxicological characteristics and mechanism of PAEs are widely concerned.

Dimethyl phthalate (DMP) is the simplest member of the PAEs group and one of the most frequently occurring PAEs in diverse environmental samples (Xu et al., 2009; Zhang et al., 2016). Increasing evidences show that DMP, the lower molecular weight PAEs, and their metabolites have toxic effects on development and reduction systems by disrupting endogenous hormones and their receptors (Cheung et al., 2007). DMP at high doses had carcinogenic, teratogenic and mutagenic effects, which remained to be explored (Matsumoto et al., 2008). More and more researchers

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have begun to focus on the DMP toxicity these years.

As the main blood cells, erythrocytes (Zhao et al., 2017) are the key targets of toxic pollution and play important roles in key physiological functions such as gas transport (oxygen and carbon dioxide), blood pH control, antioxidant defense capacity (Maćczak et al., 2017; Ratn et al., 2017) and body immunity (Garratty, 2010). PAEs, detected in blood at μg L^{-1} level (Chen et al., 2008), produce toxicity during transportation and diffusion, and impair the physiological functions of erythrocytes. Some studies (Liang et al., 2009; Shao et al., 2012; Zhang et al., 2018) have established methods to determine DMP in human serum and provided the content range of DMP, 0.33–2347.2 μg L^{-1}, in human serum samples. For the workers exposed to high levels of PAEs, the PAEs concentrations in the blood were about 5–100 times higher than that of the general population (Chi et al., 2017b). Sicińska (2018) reported that DBP, butylbenzyl phthalate (BBP) and their metabolites could induce haemolysis and eryptosis in human erythrocytes. But there are few studies on toxicity effect of DMP on erythrocytes, especially from the aspects of antioxidant and immune functions.

The oxidative stress after the exposure to various PAEs including DMP, DBP and diethyl phthalate (DEP) in *Cucumis sativus* L (Zhang et al., 2016), zebrafish embryos (Xu et al., 2013), freshwater fish *Cyprinus carpio* (Poopal et al., 2017), and Phaseolus radiatus L (Liu et al., 2014) had been extensively explored. However, little is known about the toxicity of DMP on the anti-oxidative defense system of erythrocytes. Although the effects of some toxic chemicals on the erythrocyte immune function had been reported, that of DMP is still unclear. In the study, how DMP affect the two important functions (antioxidant defense capacity and immune function) of erythrocytes were explored. The results provide the basis for the DMP toxicity study.

2. Materials and methods

2.1. Reagents and apparatus

EDTA-K2-stabilized blood samples were obtained from Weihai Central Blood Station (Weihai, China). The isolation of RBCs from donor blood were previously finished by the Weihai Central Blood Station. The retrieved RBCs were further washed and diluted with normal saline, which could protect RBCs in case of hemolysis. Normal saline (0.9% mass concentration) and EDTA-K2 of analytical grade were obtained from Tianjin Kermel Chemical Reagent Co., Ltd.

Normal saline was also used to prepare DMP solution. DMP (Sinopharm Chemical Reagent Beijing Co., Ltd, AR) was diluted to prepare 4.0×10^{-5} M DMP solution as the stock solution. Phosphate buffer (0.1 M, pH = 7.4) prepared with $Na_2HPO_4 \bullet 12H_2O$ and $NaH_2PO_4 \bullet 2H_2O$ (Sinopharm Chemical Reagent Beijing Co., Ltd, AR) was used to control the pH.

Angel Low Sugar Instant Dry Yeast (500 g) and serum solution were prepared to determine the immune adherence function. The serum solution was prepared with normal guinea pig serum (Taizhou Puxike Biological Technology Co., Ltd.) and ultrapure water according to the dilution ratio of 1:10–1:15.

In this study, all the UV-visible absorbance values were measured on a TU-1810 UV-Vis Spectrophotometer (Beijing Purkinje General Instrument Co., Ltd.). HF-3800 Fully-Auto Blood Cell Analyzer (Han Fang Medical Instrument Co., Ltd.) was used to measure the concentration of RBCs of the prepared blood samples.

Ethics statement: The study was approved by the Ethics Committee of Weihai Central Blood Station (Weihai, China).

2.2. Assessment of the function of erythrocyte antioxidant defense systems

The antioxidant defense capacity of the RBCs involved several hRBC-related biomolecules including SOD (superoxide dismutase), CAT (catalase), GPX (glutathione peroxidase), GSH (glutathione) and MDA (malondialdehyde). The relative activity or content of these biomolecules were measured with the corresponding assay kits (Jiancheng Bioengineering Institute, Nanjing, China).

The methods to measure the activities of antioxidant enzymes are as following: (1) The xanthine and xanthine oxidase system can generate the superoxide anion radical ($O^{2-}\cdot$), which can oxidize hydroxylamine to nitrite, causing absorbance at 550 nm. The SOD in samples can cause the inhibition of $O^{2-}\cdot$, lead to the reduction of nitrite, and reduce the absorbance. (2) The CAT can catalyze the decomposition reaction of H_2O_2. Adding ammonium molybdate could suspend this reaction rapidly. Then the rest of H_2O_2 could reacts with ammonium molybdate to produce a yellow complex, which could be measured at 405 nm. (3) The GPX can be the catalyst for the reaction of H_2O_2 and GSH to produce H_2O and oxidized glutathione (GSSG). The activities of GPX can be expressed by the rate of this enzymatic reaction.

Different volumes of 4.0×10^{-5} M DMP solution (0.1, 0.2, 0.3, 0.4, 0.5, 0.6, and 0.7 mL) were added to the diluted blood samples (0.2 mL), which was then diluted to a volume of 1 mL with normal saline, followed by 3 h of mixing containing shaking once every half hour. Finally, the blood sample exposed to DMP was obtained. The supernatant of erythrocyte samples was obtained by centrifugation at 2000 rpm for 5 min and the sample was refilled with pure water to a volume of 1 mL to achieve hemolysis for the measurement. Then the obtained hemolysate was used to measure the activities of the SOD, CAT and GPX and the contents of the GSH and MDA.

2.3. Determination of immune function of erythrocytes

The RBC-complement 3b (RBC-C_3b) rosette forming assay and RBC-immune complement (RBC-IC) rosette forming assay invented by Guo (1982), were designed to detect the immune function of erythrocytes. The erythrocyte C_3b receptor adheres to C_3b on the surface of several complement-sensitized yeast polysaccharides to form a rosette. The rosette rate can reflect the immune adherence function of erythrocytes. The complement-sensitized pathogens in blood system adhere to RBC-C_3b receptor in erythrocytes to form the RBC-IC. At this point, the complement fragment on the surface of the pathogen adhered by RBCs can adhere to the yeast polysaccharide on the surface of the yeast again to form an RBC-IC rosette. Thus, RBC-IC rosette rate can explain how many pathogens RBCs adhere to, indirectly indicating the degree of inhibition of the immune adhesion function of RBCs.

This method has been widely used to detect erythrocyte immune function. However, the method has some shortcomings, which affect the formation rate of rosette. In the study, the improved method proposed by Yuan et al. (1993) were adopted. By optimizing the testing steps and choosing a new dyeing method, the improved method can reduce the nonspecific binding rate, reduce the destruction of the rosette, and allow the more reliable results.

2.3.1. Determination of RBC-IC rosette forming rate

The complement non-sensitized yeast polysaccharide reagents were prepared as follows. Firstly, a small amount of yeasts were added into normal saline to prepare 10 mL of 10–20 g L^{-1} yeast suspension and then boiled for 20–30 min. Then, 2×10^8 mL^{-1}

yeast polysaccharide suspensions were mixed with phosphate buffer (Shen et al., 2008) to expose yeast polysaccharide. The obtained suspension could be used as yeast polysaccharide stock solution for the RBC-IC rosette forming assay. The number of yeasts was determined with blood counting chambers.

The diluted blood samples (0.2 mL) were respectively added into 4.0×10^{-5} M DMP solution (0.1, 0.2, 0.3, 0.4, 0.5, 0.6, and 0.7 mL), diluted to a volume of 1 mL with normal saline, followed by 3 h of mixing containing shaking every 0.5 h. The number of diluted red blood cells was 1.25×10^{7} mL^{-1}.

RBC-IC rosette forming assay was performed as follows. Firstly, 0.075 mL of the diluted yeast polysaccharide suspension was dropped into the tube and mixed with the prepared RBCs sample. Then the solution was shaken and then put into water bath at 37 °C for 30 min. Then 0.05 mL of saline was added into the test tube. The mixture was mixed with 0.05 mL of 0.25% glutaraldehyde. The solution was smeared, dried, fixed with 95% anhydrous ethanol, stained with ammonium oxalate crystal violet diluted by 5 times for 1 min, and rinsed with water gently. After the slides were naturally dried, 100 or 200 red cells were read under a high magnification microscope. A red cell combined with 2 or more yeast strains was counted as 1 rosette, and the percentage of rosette formation (Hu et al., 2011) was calculated. C_3b adheres on RBC-C_3b surface of Yeast Polysaccharide sensitized by complement. More than two yeasts adhere to the surface of each red cell membrane, thus forming a "rosette-like" structure. RBC-IC adhered to the exposed yeast polysaccharides to form the same rosette.

2.3.2. Determination of RBC-C_3b rosette forming rate

Complement-sensitized yeast polysaccharides were prepared as follows. Firstly, 0.5 mL of serum solution was added to 0.5 mL of the prepared yeast polysaccharide suspension and put into water bath at 37 °C for 15 min. After PBS addition and centrifugation, 1×10^{8} mL^{-1} complement-sensitized yeast polysaccharide suspension was prepared. Yeast polysaccharides had been adhered to the complement in guinea pig serum and could be used in the RBC-C_3b rosette forming assay.

RBC-C_3b receptor rosette forming assay was performed as follows. The treatment of erythrocyte suspension was the same as that in Section 2.3.1. RBC-C_3b receptor rosette forming assay was performed with the complement-sensitized yeast polysaccharide suspension according to the same method in Section 2.3.1.

2.4. Statistical analysis

Statistical analysis was performed in Statistical Package for the Social Sciences (SPSS) 10.0. All data were from at least three independent experiments and expressed as mean ± standard deviation (SD). The test method was *t*-test. A P-value less than 0.05 was considered to be statistically significant.

3. Results and discussion

3.1. Effects of DMP on antioxidant defense capacity of erythrocytes

Oxidation reaction is the basis of human life activities and its by-product, free radical, is the focus of disease prevention and control. Low doses of radicals may be beneficial to or even essential in key processes such as intracellular messaging, angiectasis and defense against micro-organisms and can increase phagocytic bactericidal activity (Mates et al., 1999). In contrast, a high concentration of radicals results in oxidative stress, which may be the vital contribution factor in metabolic malfunctions and chronic-inflammatory, vascular and neoplastic diseases (Roversi et al., 2006). In aerobic organisms, reactive oxygen species (ROS) are chemically reactive chemical species, including hydroxyl radicals (·OH), superoxide anions ($O^{2-}\cdot$), hydrogen peroxide (H_2O_2), lipid peroxide and nitric oxide (NO), and involved in plenty of human beings' diseases (Herken et al., 2000). ROS (Pan et al., 2018) are generated in response to both internal stimuli (normal cell metabolism) and external stimuli (smoking, radiation, environmental pollutants et al.), leading to lipid peroxidation and specific oxidation of some enzymes and increase DNA damage and apoptosis.

Erythrocytes in the circulation system have an antioxidant defense system for reducing the effects of excessive ROS and the resultant oxidative stress. The antioxidant defense system involves both enzymatic antioxidant defense and non-enzymatic low molecular weight antioxidants (Pan et al., 2018). The enzymatic antioxidant system contains three main functional enzymes: superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX), which largely determine the antioxidant defense capacity of human beings. The wide array of non-enzymatic antioxidant defense, including glutathione (GSH), ascorbic acid (vitamin C), and α-tocopherol (vitamin E) can also play a significant role in antioxidant defense capacity (Mates et al., 1999). The synergistic action between enzymatic antioxidant defense system and non-enzymatic antioxidant defense system prevent the oxidative stress and avoiding cell structural damage and dysfunction. In this section, we investigated the toxic effects of dimethyl phthalate (DMP) on the antioxidant defense capacity of erythrocytes including the activities of antioxidant enzymes (SOD, CAT, and GPX) and the contents of GSH and MDA.

The toxic effects of DMP on the SOD activity are shown in Fig. 1. When the concentration of DMP in the erythrocyte sample was increased from 0 to 28 μmol L^{-1}, the relative SOD activity was increased from 100% to the maximum value (135%) and then decreased to 95%. The maximum value of 135% was measured under the DMP concentration of 20 μmol L^{-1}. However, when DMP concentration increased from 0 to 28 μmol L^{-1}, the relative activity of CAT (Fig. 2) decreased from 100% (initial) to 68%. Effect of DMP on the activity of GPX (Fig. 3) showed the similar results to that of SOD. When DMP concentration increased from 0 to 12 μmol L^{-1}, erythrocytes gave rise to a slight increase in the activity of GPX; When DMP concentration increased from 12 to 28 μmol L^{-1}, the activity of GPX declined. The relative activity of GPX reached its maximum

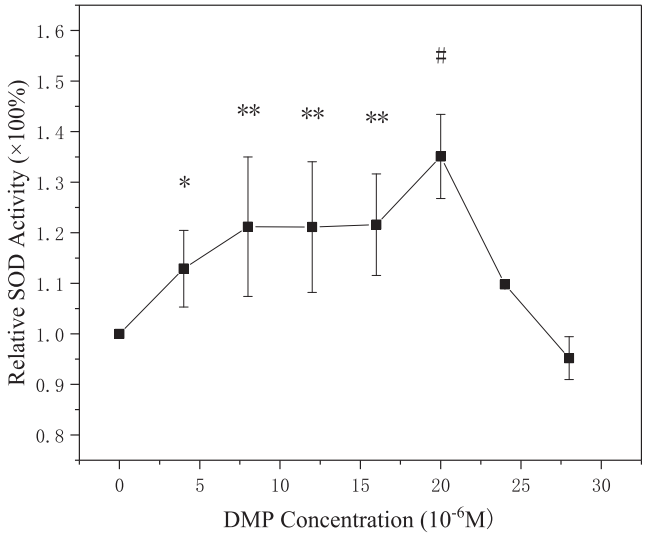

Fig. 1. Erythrocyte SOD activity under different DMP conditions. Conditions: RBCs = 2.0×10^{8} mL^{-1}; T = 298 K; pH = 7.2; *P < 0.05, **P < 0.01, #P < 0.001 vs normal control group.

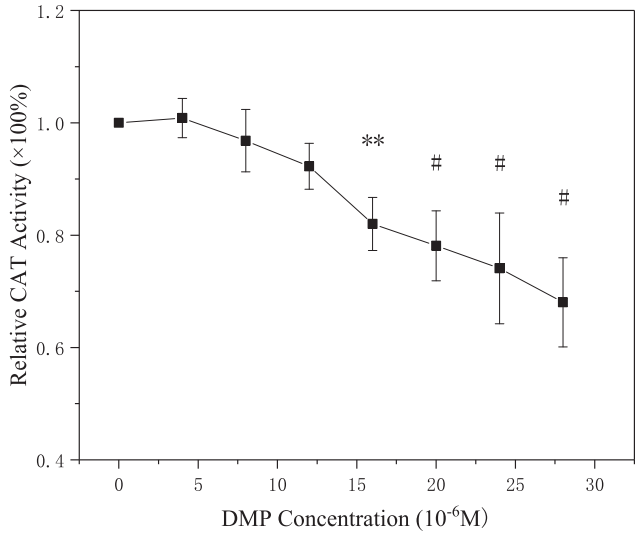

Fig. 2. Erythrocyte CAT activity under different DMP conditions. Conditions: RBCs = 8.0×10^7 mL^{-1}; T = 298 K; pH = 7.2; *P < 0.05, **P < 0.01, #P < 0.001 vs normal control group.

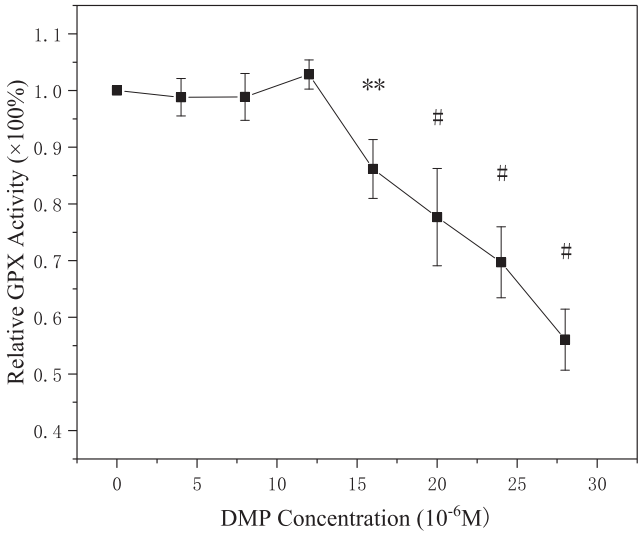

Fig. 3. Erythrocyte GPX activity under different DMP conditions. Conditions: RBCs = 2.0×10^8 mL^{-1}; T = 298 K; pH = 7.2; *P < 0.05, **P < 0.01, #P < 0.001 vs normal control group.

value (103%) at DMP concentration 12 μmol L^{-1}.

SOD is a protective enzyme which can remove superoxide anion radical ($O^{2-}\cdot$) by catalyzing its dismutation to hydrogen peroxide (H_2O_2) and molecular oxygen (O_2) (Herken et al., 2000). CAT is a heme-containing redox enzyme which can catalyze the decomposition of H_2O_2 into water and molecular oxygen to protect the body from oxidative stress (free radical damage) (Qin and Liu, 2013). GPX is an enzyme with the peroxidase activity and can promote the reaction of H_2O_2 with GSH to produce H_2O and GSSG, thus protecting cell membrane structure and function from damage (Chahbouni et al., 2017). The change in SOD, CAT and GPX activities have an effect on the detoxification of ROS in organisms including animals, plants and bacteria. According to the results shown in Figs. 1 and 3, the relative activities of SOD and GPX were enhanced under low DMP concentrations (20 μmol L^{-1} for SOD and 12 μmol L^{-1} for GPX). However, the SOD and GPX activities in erythrocytes showed a decline trend under higher concentrations of DMP (20–28 μmol L^{-1} for SOD and 12–28 μmol L^{-1} for GPX). The effect was known as hormesis (Calabrese and Baldwin, 1997). The possible mechanisms of the phenomenon of hormesis were overcompensation, free radical scavenging, immune function enhancing and gene expression and regulation. In this study, the SOD activity showed significant increases under low dose DMP, enhancing the capability of removing $O^{2-}\cdot$, resulting in more H_2O_2. Thus, the activities of GPX that eliminate H_2O_2 are also expected to increase simultaneously. The activity of CAT decreased continuously with the increase in DMP concentration. The experimental results indicated that the CAT activity was inhibited by DMP more significantly than the activities of SOD and GPX.

Glutathione (GSH) (Kumar et al., 2011) is an antioxidant in plants, animals and some bacteria and contributes to thiol-redox control and preventing damage to cellular components caused by ROS including free radicals, lipid peroxides. In the study, the relative GSH content of erythrocytes (Fig. 4) increased with increasing DMP concentration from 0 to 28 μmol L^{-1}. The relative GSH content of erythrocytes was 124% when exposed to 28 μmol L^{-1} DMP.

When erythrocytes were exposed to 0–28 μmol L^{-1} DMP, the antioxidant capacity of erythrocyte was affected, as proved by the changes in the antioxidant activities of various enzymes including SOD, CAT and GPX and the content of non-enzymatic defenses such as GSH. In order to further confirm the mechanism, we investigated the influences of 0–28 μmol L^{-1} DMP on the content of MDA in erythrocytes (Fig. 5). MDA (Chi et al., 2017a) is a degradation product of lipid peroxidation induced by oxidative damage and can indicate the level of lipid peroxidation. When treated with DMP under three concentrations (8 μmol L^{-1}, 16 μmol L^{-1} and 24 μmol L^{-1}), the relative contents of MDA in erythrocytes increased significantly ($P < 0.05$, $P < 0.001$ and $P < 0.01$, respectively). These data demonstrated that the exposure of erythrocytes to DMP could lead to the oxidative stress, which might be one of the toxicity mechanisms of DMP in erythrocytes.

In conclusion, DMP can decrease the antioxidant defense capacity of erythrocytes, lead to the oxidative stress, which can cause damages to cell structures and functions.

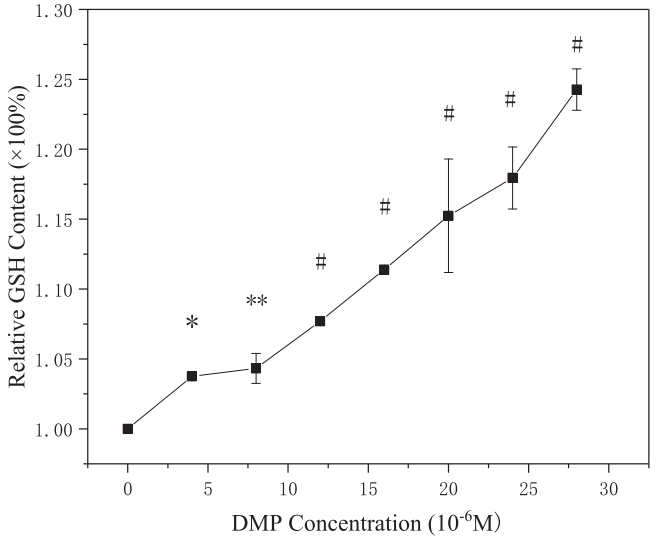

Fig. 4. Erythrocyte GSH content under different DMP conditions. Conditions: RBCs = 2.0×10^8 mL^{-1}; T = 298 K; pH = 7.2; *P < 0.05, **P < 0.01, #P < 0.001 vs normal control group.

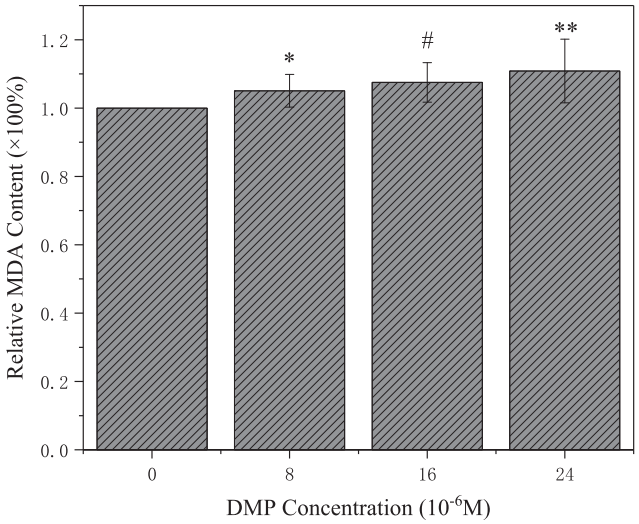

Fig. 5. Erythrocyte MDA content under different DMP conditions. Conditions: RBCs = 2.0×10^8 mL^{-1}; T = 298 K; pH = 7.2; *P < 0.05, **P < 0.01, #P < 0.001 vs normal control group.

3.2. Effects of DMP on the immune function of erythrocytes

Erythrocytes are important natural immune cells in blood circulation (Siegel et al., 1981). They have multiple effects such as identification, adhesion, concentration, and antigen killing. Lots of investigations indicated that the immune-related functions of erythrocytes were equally important as the functions of leukocytes on the immune system (Dai et al., 2017).

RBC-C_3b (Zhao et al., 2004; Janssen et al., 2006) receptors on cell membrane are intuitive immune indicators of erythrocyte immunity. RBC-C_3b plays an essential role in cleaning up the immune complexes (IC), identifying the stored antigen, and enhancing phagocytosis. RBC-IC are substances formed by the combination of antigens and corresponding antibodies. The IC of complement and other immune active substances are deposited on the vessel walls, thus leading to tissue damage and vasculitis and causing a series of diseases, such as systemic lupus erythematosus (SLE). Therefore, the erythrocyte immunity function was explored by detecting the rates of red blood cell C_3b (RBC-C_3b) receptor rosette and red blood cell immune complex (RBC-IC) rosette in the study.

Oxygen-derived free radicals (OFR) is constantly generated and cleared in organisms and can induce lipid peroxidation and impair the immune function (Su et al., 2004). SOD and CAT are the main indication enzymes of oxygen free radical metabolism and can scavenge OFR produced by phagocytic cells and resist the damage caused by OFR. The change in SOD activity reflects the ability to resist free radical damage and the decrease in SOD activity means the decrease in free radical scavenging ability. CAT is common redox enzyme in the body and can consume reactive oxygen species (ROS). The activities of the two antioxidant enzymes are closely related to the immune function of RBCs. In addition, MDA is the final product of lipid peroxidation damage.

In the section, we investigated the effect of DMP on the immune function of RBCs based on the changes in the activities of RBC-C_3b, RBC-IC, SOD, and CAT and the content of MDA in erythrocytes. The toxic effects of DMP on the RBC-C_3b rosette rate and RBC-IC rosette rate are given in Table 1. When the concentration of DMP in the RBC sample increased from 0 to 16 μmol L^{-1}, the RBC-IC rosette forming rate significantly decreased from 10.33% to 2.67% (P < 0.001). Compared with the control group (0 μmol L^{-1} DMP), RBC-IC rosette rate under 20 μmol L^{-1} DMP significantly decreased to 5.00% (P < 0.001), which was slightly higher than that under lower concentrations of DMP. RBC-IC rosette rate was then decreased when the concentration of DMP increased above 20 μmol L^{-1}. The RBC-IC rosette rate under 28 μmol L^{-1} DMP was significantly decreased to the minimum value (1%, P < 0.001). The RBC-C_3b rosette rate under lower concentrations of DMP (4 and 8 μmol L^{-1}) slightly increased. However, under higher concentrations of DMP (12–28 μmol L^{-1}), C_3b rosette rate decreased significantly. The rate decreased to the minimum value (6.00%, P < 0.01) under 28 μmol L^{-1} DMP. The experimental results indicated that the RBC-C_3b receptor rosette rate of erythrocytes exposed to DMP was much lower than that in the control group, suggesting that the CR1 activity in RBCs decreased, thus leading to the overactivation of the complement system (Peng et al., 2010). The RBC-IC rosette forming rate basically decreased when DMP concentration increased. The activity of RBC-C_3b receptors was decreased compared to that in the normal group, whereas the activity of RBC-IC was also decreased, indicating a primary lower of the erythrocyte immune function caused by the destruction of C_3b receptor on erythrocyte membrane.

Table 1
Comparison of erythrocyte immunity under different DMP concentrations ($\bar{x} \pm s$) in %.

DMP Concentration (μmol L^{-1})	RBC-IC (%)	RBC-C_3b (%)
0	10.33 ± 0.58	14.33 ± 1.15
4	7.00 ± 1.00**	14.33 ± 2.89*
8	6.33 ± 0.58#	15.67 ± 0.58**
12	4.33 ± 0.58#	12.33 ± 2.08
16	2.67 ± 1.53#	11.67 ± 2.52
20	5.00 ± 1.73#	7.00 ± 2.00*
24	2.33 ± 0.58#	6.00 ± 1.00**
28	1.00 ± 0.00#	6.00 ± 2.00**

Footnote: *P < 0.05, **P < 0.01, #P < 0.001 vs normal control group.

The relative activity of SOD was slightly increased under low dose DMP (below 20 μmol L^{-1}). The effect was known as hormesis. However, the activity of CAT decreased continuously. The CAT activity was inhibited by DMP more significantly than SOD activity, suggesting that DMP had an inhibitory effect on erythrocyte immune function. The relative content of MDA increased when erythrocytes were exposed to DMP (Fig. 5). MDA is the main decomposing product of unsaturated fatty acids in erythrocyte membrane lipid. Unsaturated fatty acids are oxidized by free radical to produce lipid peroxide (Wang and Liu, 2007). MDA can cross-link with proteins and phospholipids in the erythrocyte membrane and oxidize thiol groups of proteins, thus resulting in the changes in the composition, structure and function of the erythrocyte membrane. The changes in the membrane microenvironment inevitably affects the C_3b receptor on the erythrocyte membrane and reduces its activity and IC scavenging ability (Zheng et al., 2004). Therefore, the lipid peroxidation damage caused by the accumulation of oxidative free radicals may be one of the reasons for the impaired immune function of erythrocytes.

The decrease in RBC-C_3b and RBC-IC indicated the primary lower of the immune function of erythrocytes. The exposure of erythrocytes to DMP resulted in a declining trend of the activities of various enzymes including SOD and CAT and the decreased content of MDA, caused the lipid peroxidation and changed the erythrocyte membrane microenvironment. These results suggested that DMP could damage the immune functions of erythrocytes.

4. Conclusions

The study explored the effects of DMP on the antioxidant defense system and immune functions of erythrocytes by testing typical biomolecules (SOD, CAT, GPX, GSH and MDA) and immune

indicators (RBC-C_3b and RBC-IC) of RBCs. The experimental results indicated that DMP could decrease the antioxidant defense capacity, lead to the oxidative stress of erythrocytes, and damage the structure and function of cells. When the erythrocytes exposed to DMP, the C_3b receptor rosette forming rate and IC rosette forming rate were decrease, suggesting the primary lower of the immune function of erythrocytes caused by the destruction of C_3b receptor on erythrocyte membrane. Thus, erythrocytes exposed to DMP showed the impaired immune function, also being suggested by the experimental results of SOD and CAT activities. The decrease of MDA resulted in the changes of erythrocyte membrane microenvironment, thus affected the immune function of erythrocytes. The lipid peroxidation damage caused by the accumulation of oxidative free radicals might be one of the main reasons for the impaired immune function of erythrocytes. The established toxicity mechanism of DMP in erythrocytes can provide the basis for exploring the toxicity of PAEs in erythrocytes.

Conflicts of interest

The authors declared that they have no conflicts of interest in the authorship and publication of this contribution.

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