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Response of the antioxidant enzymes of the erythrocyte and alterations in the serum biomarkers in rats following oral administration of nanoparticles

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ABSTRACT

In this study, Al_2O_3, CuO and TiO_2 nanoparticles (NPs) were administered to mature female rats (*Rattus norvegicus* var. albinos) via oral gavage (0, 0.5, 5, 50 mg/kg b.w./day) for 14 days to investigate their effects on 14 serum biomarkers and 4 antioxidant enzyme (catalase, superoxide dismutase, glutathione peroxidase, glutathione S-transferase) activities in the erythrocyte. Data showed that Al_2O_3 did not cause any significant ($P > 0.05$) change in the parameters, except few cases, while CuO and TiO_2 caused significant alterations in antioxidant system parameters of the erythrocytes. Activities of catalase and superoxide dismutase significantly decreased in CuO and TiO_2 administered rats. Oppositely, glutathione peroxidase activity increased in CuO and TiO_2 administered rats. There were no significant alterations in the activity of glutathione S-transferase in the erythrocytes. Levels of glucose, cholesterol, bilirubin, triglyceride, triiodothyronine (T3), estradiol, prolactin and immunoglobulin M (IgM) in the serum altered after some of NP administrations, whereas cortisol, protein, creatinine, blood urea nitrogen (BUN), thyroxine (T4) and immunoglobulin G (IgG) levels in the serum did not change significantly after any of NP administration. There were outstanding increases in the levels of bilirubin and prolactin and decreases in the levels of triglyceride and estradiol. The present study demonstrated that the antioxidant enzymes in the erythrocyte were generally affected from copper and titanium NPs, while aluminium and copper NPs caused more significant alterations in serum biomarkers.

1. Introduction

Nano-sized (<100 nm) metal complexes have been used in different areas of technology, raising environmental concerns among environmentalists (Jeng and Swanson, 2006; Janrao et al., 2014). Although nanoparticles (NP) are used in medicine, textile and electronic industry, filters, toothpaste, suntan cream, toys, moisturizer, packing products, white goods and food industry ect., data concerning their effects in the environment and in human life are not enough to make a conclusion for their environmental fate. NPs have nano-sized reactive crystal materials with high surface to volume ratio, unique electronic properties and surface structure, functional groups, inorganic or organic coatings, shape and aggregation behavior. NPs are able to pass through cell membranes and their interactions with biological systems are relatively unknown (Jeng and Swanson, 2006). Therefore, NPs can potentially cause adverse effects on organ, tissue, cellular, subcellular and enzymes due to their unusual physicochemical properties (Schrand et al., 2010). Studies have shown that NPs have toxic effects on mammals depending on metal type, size, dose and administration route of these particles (Wang et al., 2013; Elle et al., 2013), altering several biomarker levels belonging to different metabolic systems in mammals after the oral route, inhalation or subcutaneous injection (Shrivastava et al., 2013; Lei et al., 2015; Hu et al., 2015).

Aerobic organisms face oxidants produced through their metabolisms or from man-made sources. An increase in oxidant levels and a decrease in antioxidant system elements may occur under certain conditions, causing oxidative stress due to changes in oxidative/antioxidative balance. Antioxidant system parameters include both enzymatic such as superoxide dismutase (SOD; converts superoxide anion radical into hydrogen peroxide), glutathione peroxidise (GPx; detoxifies hydrogen and organic peroxides), glutathione-S-Transferase (GST; catalyzes glutathione and xenobiotic conjugation) and catalases (CAT; eliminates hydrogen peroxide to water) and non-enzymatic (GSH; acts as a reductant in with xenobiotic conjugation glutathione) elements to eliminate

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oxidants present are crucial in the effort to counteract oxidative stress caused by toxicants (Pena-Llopis et al., 2001).

The serum of vertebrates is often used to determine the health status of animals as it gives crucial data. Therefore, researchers suggested that serum biochemical parameters can be useful as a diagnostic tool for animal health to identify their general status and target organs affected by contaminants. Endocrine system is crucial for the homeostasis in vertebrates and is known to be a target for xenobiotics. Glucose is a ubiquitous fuel and used as an energy source in all living organisms. When glucose levels are altered by any means, many psychological processes impair. The mammalian liver produces urea as a waste product of protein metabolism, helping to evaluate renal health. Bilirubin is excreted in bile and urine and elevated levels in the serum may indicate certain diseases. Cholesterol is the principal sterol synthesized by all animals and serves as a precursor for the biosynthesis of steroid hormones and is an important molecule for animal cell structure. Triglyceride is an ester derived from glycerol and three fatty acids, being main constituents of fat in mammals. The immune system comprises many biological structures and is a host defense system, detecting a wide variety of agents to function properly. Disorders of the immune system may result in autoimmune diseases, inflammatory and cancer. Above serum biomarkers were used in animal experiments to evaluate the effects of xenobiotics (Zha et al., 2009; Gao et al., 2012; De Jong et al., 2013; Yu et al., 2014; Atli et al., 2015).

Al_2O_3, CuO and TiO_2 NPs are the most abundantly produced nanomaterial and have been used in diverse fields, including the medical, military, chemical industry, electronics, biomedicine, and cosmetics and food sectors (Klaine et al., 2008; Janrao et al., 2014). Once they are used for different purposes, these NPs will inevitably enter the environment and may cause adverse effects to aquatic or terrestrial organisms. The effects of Al_2O_3, CuO and TiO_2 NPs on the antioxidant system parameters in the erythrocyte of Wistar rats have not been studied to the best to our knowledge. Thus, the present study was undertaken to help understanding the potential effects of these NPs on mammals.

2. Materials and methods

2.1. Experimental protocols

Wistar Albino Sprague Dawley rats (*Rattus norvegicus* var. albinos) are reproduced in DETAUM of Cukurova University, with a photo-period of 12 h light at 22 ± 1.5 °C (moisture of 48 and 56%). All the experiments were carried out in this center, using mature female rats weighing between 190 and 220 g (size differences among groups were insignificant, $P > 0.05$). The rats were allocated into 10 cages and fed with the standard rat food during the experiments. Each experimental group consisted of 6 rats and a total of 60 rats were used in the experiments, including a control group. NPs were purchased from Sigma-Aldrich (Germany) or Nanografi (Turkey) companies. Characteristics of NPs were as follows; Al_2O_3 (~40 nm, >99% purity, >30 m^2/g surface area, 2.70 g/cm^3 density), CuO (~40 nm, >99% purity, >20 m^2/g surface area, 6.50 g/cm^3 density) and TiO_2 (~21 nm, >99% purity, >30 m^2/g surface area, 4.26 g/cm^3 density). Scanning electron microscope (Zeiss Supra 55VP, Germany) images of NPs were also taken to see their sizes and also compare the size data obtained from the producer. NP suspensions were mixed vigorously, sonicated (Bandelin HD2200, Germany) for 20 min on the ice and immediately applied to the related assay to minimize agglomeration.

NPs were administered to rats in 200 μl water via oral gavage (0, 0.5, 5, 50 mg/kg b.w./day). Controls received only the same amount of water. After 14 days, rats were killed with high doses of anesthesia (ketasol 10%, Harson Lab. India) and dissected carefully using sterile equipment. The bloods were taken out from the hearth using syringes and put into glass tubes. Blood samples were centrifuged at 3000*g* (Hettich Universal 30 RF, Germany) for 5 min (4 °C) to separate the serum and the cells. Serums were removed and the cells were washed three times with 0.09% NaCl.

2.2. Measurements of serum parameters

Analysis of the serum biomarkers was carried out in Central Laboratory of Balcali Hospital (Adana, Turkey) which was accredited by Joint Commission International for the third time, until January 2017. Levels of hormone and other serum parameters were analyzed in Beckman UniCel DXC 800 and DXI 800 Synchron (Beckman Coulter Inc., CA, USA) auto-analyzers. Beckman Coulter reagents were used to perform of serum parameters on the auto-analyzers. Immunoglobulin (IgG, IgM) levels in the serum were analyzed by nephelometric methods (Thomas, 1998; Winter et al., 2000), using a Siemens BN II nephelometer. Appropriate kits were supplied from Siemens Company (Germany).

2.3. Measurements of antioxidant system parameters

Activity of CAT was measured at 240 nm for 1 min by monitoring the H_2O_2 decrease. CAT activity was presented as μmol H_2O_2/mg prot./min. (Bergmeyer, 1974). Activity of SOD was measured by the indirect method involving the inhibition of cytochrome c reduction at 550 nm for 1 min using the method of McCord and Fridovich (1969). SOD activity was calculated as Unit/mg prot. Activity of GPX was measured by the NADPH decrease and GST activity was calculated by NADPH increase at 340 nm for 1 min. GPX and GST activities were given as μmol/mg prot./min. (Livingstone et al., 1992; Habig et al., 1974). Total, oxidized and reduced GSH levels were measured according to the method of Griffith (1980) at 412 nm for 1 min and given as μmol GSH/mg prot./min. The total protein levels were determined by the method of Lowry et al. (1951), using bovine serum albumin as a standard.

2.4. Statistical analysis

A SPSS statistical package program (SPSS 15, Chicago, IL, USA) was used for the analysis of data. Before the statistical analysis, homogeneity of variance was checked among different exposure periods to evaluate the distribution of data. Data were presented as mean and standard error of mean, presenting in figures. One way ANOVA test was applied to data if they are normally distributed, otherwise Kruskal-Wallis test was applied.

3. Results

There was no rat mortality and any apparent health problem in rats after oral NP administrations. Mean values and associated standard errors of antioxidant system parameters in the erythrocyte were given in Figs. 1–5 . Data showed that Al_2O_3 did not cause any significant ($P > 0.05$) change in the antioxidant system parameters, except a decrease in SOD activity and an increase in GSH level and GPX activity, while CuO and TiO_2 caused serious alterations. For example, catalase activity in the erythrocyte decreased significantly ($P < 0.05$) in CuO and TiO_2 administered rats. Likewise, SOD activity is severely affected from CuO and TiO_2, as its activity decreased after administration of both NPs. Oppositely, glutathione peroxidase activity increased significantly in CuO and TiO_2 administered rats. GSH levels were not affected from NPs, except two cases. GST activity was also not altered by any NP exposure. Table 1 shows mean and associated standard errors of serum biomarkers of female rats after NP administrations for 14 days. This table shows that the levels of glucose, cholesterol, bilirubin, triglyceride, T3, estradiol,