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The influence of nanodiamond particles on rat health status

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Abstract: *The influence of nanodiamond particles on rat health status.* The objective of the present investigation was to evaluate the effect of nanodiamond (ND) particles on rats health status. 1 mg/kg b.w. of nanodiamond particles was administrated intravenously and intraperitoneum. The presence of an adverse impact was examined. The results show significant changes in biochemical (glucose and total protein level decrease) and hematological (elevated platelets count) parameters, only in case of intravenous injection.

Key words: nanodiamond particles, health status, biochemical and hematological parameters, rats

INTRODUCTION

The recent large interest in carbon nanomaterials (fullerenes, nanotubes, nanodiamonds) is a consequence of their unique mechanical, electrical and thermical features. Numerous biological applications suggested by several investigators include their potential application as the drug delivery agents controlling the release of genes, proteins or other molecules (Bondar and Puzyr 2004). Nanodiamonds present the high effectiveness in drug delivering chemotherapy, presenting the low negative effects associated with the known drug-delivery agents. It was proposed to apply nanodiamonds as a biochip or biosensor for a medical use (Puzyr et al. 2007). As the biofunctional agent, nanodiamond should reveal the biocompatible characteristics. It should exibit the lowest toxic properties and it should remain neutral for the organism parameters (Bakowicz-Mitura et al. 2007). Accoridng to the recent studies, nanodiamonds present the high biological tolerance and they exhibit biocompatibility with the blood components (Mitura et al. 2006). However, we observe the insufficiency of the *in vivo* studies characterizing the systemic organism response to diamond nanoparticles. The hematologic and biochemical parameters of rats are published by Exotic Animal Companion Medicine Handbook for Veterinarians (Johnson-Delaney 1996).

Therefore, the objective of the study was to evaluate the effect of intravenous and intraperitoneal administration of nanodiamond on biochemical and hematological parameters. Properly interpreted results of the blood chemistry values may provide the precise picture of the animal health status at the time of its sampling (i.e., nutritional status, disease condition and stress after environmental changes). Therefore, using these data for diagnostic purposes we compared the obtained results with reference or normal values.

MATERIAL AND METHODS

Diamond nanoparticles (ND) was produced by the method described by Danilenko (2003) with modification of ampoule-free synthesis in the explosion chamber instead of ampoule synthesis. Graphite was placed directly into a cylindrical charge consisting of a TNT--hexogen mixture TG40. The charge was enveloped in a water jacket to suppress graphitization and reduce the unloading rate of the synthesized diamond. Shape and size of ultrananocrystalline diamond (2–10 nm) were inspected by transmission (TEM) and scanning (SEM) electron microscope (Bakowicz 2003, Czerniak--Reczulska et al. 2010). Figure 1 shows that the grains of nanoparticles forming conglomerates (HR TEM). Figure 2 shows nanodiamond clusters (SEM).

ND at the concentration of 500 mg/l was suspended in deionized water then the mixture was sterilized and sonificated. 21 male Wistar rats were divided into three equal groups and kept in individual cages for 10 days under standard conditions: temperature 22°C, humidity 50–70%, light/dark cycle 12/12h. The animals had free access to water and feed. Diet was formulated in compliance with NRC requirements (1995). Rats were administrated by intravenous (group 1) and intraperitoneal injection (group 2) of 0.5 ml of ND colloid (1 mg/ /kg body weight of ND particles). Clear deionized water was administrated by intravenously into the animals of control group (group 3). At the end of the experiment the rats were fasted for 12 h and then sedated by intramuscular ketamine (Ketamini hydrochloricum 5%, Narkamon, SPOFA, Praha, Czech Republic). Blood was sampled from the heart into heparinized tubes and cooled to 4°C. The animals were euthanized by ketamine overdoses. The samples were collected for further analysis. Blood morphology (red blood cell (RBC) count, hematocrit, mean cell volume (MCV), hemoglobin concentration, white blood cell (WBC), neutrophils, lymphocytes and platelets

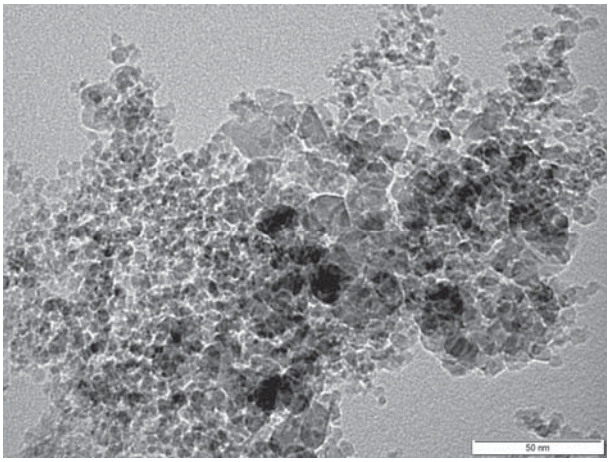

FIGURE 1. Nanodiamond particles manufactured by detonation method — HR TEM. Reprinted with permission from Bakowicz (2003)

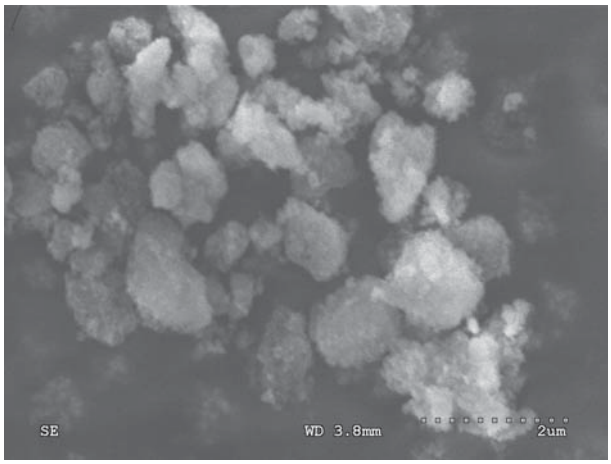

FIGURE 2. View SEM of nanodiamond powder manufactured by detonation method. Reprinted with permission from Czerniak-Reczulska et al. (2010)

count was determined using standard methods and a Danam-510 analyser (France). The following were assayed in serum: total protein, albumin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), glucose, triglycerides using a Vitros DTII analyzer (Johnson and Johnson).

RESULTS AND DISCUSSION

The haematological parameters indicate that hydrocolloid of detonation diamond nanoparticles, regardless of the route of administration results in the elevation in platelet number in the rats, compared to the control group. Other morphological indicatives remained unchanged (Table 1). Additionally, hydrocolloidal ND intravenously injection decreased glucose and total protein concentration in the animal serum. Other biochemical parameters did not differentiate the experimental groups and remained within the reference values (Table 2).

Platelets are assisting and modulating inflammatory reactions and immune responses. This is achieved by the regulated expression of the adhesive and immune receptors on the platelet surface and also by the release of a multitude of secretory products including inflammatory mediators and cytokines, which can mediate the interaction with leukocytes (Hundelshausen and Weber 2007). The primary regulator of platelet production is thrombopoietin (THPO). After binding to the cell receptor, it affects all stages of development of megakaryocytes. THPO is synthetized mainly in the liver and it is stimulated by IL-6 (Śliwińska-Staczyk 2005). Based on the presented results, we hypothesize that the ND stimulates immune system and it leads to the expression of inflammatory mediators (IL-6), which had consequences in the increased production of blood platelets. However non-specific immune and hematologic indicators (Niemiec et al. 2011), do not prove any inflammatory activity of ND. However, the presented blood cell

TABLE 1. Hematoloogical parameters in peripheral blood of control and experimental rats

Parameter	Reference values*	Control	Injection		SE pooled	*P*-value
			intravenous	intraperitoneal		
RBC** (10^{12} /l)	6.76–975	8.1	7.7	8.0	0.242	0.5561
Hematocrit (l/l)	37.6–50.6	41.8	41.9	42.6	0.99	0.207
MCV** (fl)	50–80	52.0	54.0	53.0	0.88	0.207
Hemoglobin (g/dl)	11.5–16.1	14.0	14.0	14.2	0.26	0.861
WBC** (10^9/l)	6.6–12.6	8.6	8.3	7.6	1.197	0.8245
Neutrophils(%)	3–42	18.0	12.0	11.0	3.42	0.276
Lymphocytes (%)	50–95	81.0	88.0	88.5	3.46	0.285
Platelets (10^9/l)	150–460	487.3 b	645.5 a	715.2 a	45.70	0.009

*Exotic Animal Companion Medicine Handbook for Veterinarians, Johnson-Delaney 1996.
**Abbreviations: RBC = red blood cells, MCV = mean corpuscular volume, WBC = white blood cells.
a, b – significant difference at $P < 0.05$.

TABLE 2. The level of blood serum parameter in control and experimental rats

Parameter	Reference values*	Control	Injection		SE pooled	*P*-value
			intravenous	intraperitoneal		
Glucose (mmol/l)	2.8–7.56	9.01 b	7.58 a	9.24 b	0.236	0.0002
Triglycerides (mmol/l)	0.35–1.4	1.1	0.97	1.39	0.119	0.0685
AST** (U/l)	39–111	124.6	119.4	112.0	8.33	0.573
ALT**(U/l)	20–61	57.7	54.28	51.42	5.414	0.7178
Albumin (g/l)	38–48	34.4	29.7	36.7	2.34	0.126
Total protein (g/l)	53–69	66.3 b	54.0 a	67.7 b	3.58	0.027

*Veterinary Reference Guide, Clinical Diagnostic Division Eastman Kodak Company.
**Abbreviations: AST = aspartate aminotransferase, ALT = alanine aminotransferase.
a, b – significant difference at $P < 0.05$.

parameters did not differ groups in the experiment. Puzyr et al. (2002) showed that ND damages blood cells including erythrocytes by following mechanisms: (1) direct binding of NDs to the cell membrane proteins, which may cause irreversible inhibition of their functions and (2) a imbalance of electrolytic and osmotic equilibrium caused by adsorption of blood plasma protein components to ND particles (Puzyr et al. 2002). Degradation of blood cells depends on the concentration and ND surface properties. In our study we demonstrated no significant changes in the quantity of white and red blood cells. Histological studies also did not confirm the negative impact of ND on kidneys and liver cell structures (Niemiec et al. 2010). According to Puzyr et al. (2007), the modified diamond nanocolloid administered for 6 months, did not affect the mortality of the tested mice. There were no abnormalities observed in the process of organ growth and weight, however the increased the level of leukocytes in the blood was shown. ND

administered subcutaneously did not cause any inflammation nor damage of the cells adjacent to applied nanoparticles. On one hand, we expect certain level of degradation of the treated cells after diamond nanocolloid applied *in vitro* versus *in vivo*. This may be related to the numerous of extracellular factors (protein, lipoprotein, sugars) involved in the nanoparticles interactions. On the other hand, diamond nanoparticles can affect the large number of the cells after *in vivo* injection. Puzyr et al. (2007) observed no changes in the blood morphological parameters after intravenous injection of ND. However, the results of that work were not significant and indicate the increase in the number of platelets after nanocolloid injection.

The values of biochemical markers of health in all experimental groups remained within the reference ranges. Glucose and total protein concentration was significantly lower in animals after intravenous injection of ND, compared to the control group. Puzyr et al. (2007) has documented that diamond nanoparticles modulate concentration of biomarker of liver injury (bilirubin/transaminases) and lipid metabolism (cholesterol, low density lipoprotein, triglycerides) indicators in the rabbit serum. We assumed that ND affects the excretion of urine glucose and protein by mechanical damage of renal corpuscles. Nephrotoxic compounds were shown to be a reason for pathological changes such as proteinuria and glycosuria (Ascioglu et al. 2000). However, Niemiec et al. (2008) has reported that histopathological examination of kidney and liver showed normal architecture, suggesting no morphological disturbances in rats treated by intravenously injection of nanodiamond particles. However, the histological assessment cannot rule out the presence of microdamages in the examined organs. This was confirmed by Yu et al. (2005) who presented the cytotoxicity of the diamond powder on the kidney cells.

CONCLUSIONS

The nanoscale diamond particles have become an interesting innovative pharmaceutical material. Due to particular size and surface structure, nanoparticles are well-suited for different biological applications. Most of the toxicology investigations carried out on *in vitro* models reveals positive results. The recent study presents wide biological benefits of nanodiamond particles obtained by detonation methods such as low cytotoxicity and biocompatibile (Bondar et al. 2005, Schrand et al. 2006, Bakowicz-Mitura et al. 2007). We have recently showed that intravenous and intraperitoneal injection of 1 mg/kg b.w. detonation diamond powder increased platelet counts on rats. Additionally, the intravenous injection of nanoparticles decreased serum level of glucose and total protein. It is hypothesized that ND stimulates the TPO synthesis pathway through the activation of pro-inflammatory cytokines. We also speculate that exposure to diamond nanoparticles may lead to the nephrotoxicity characterized by hematuria and proteinuria together with the reduced serum total protein and glucose level. Our results show the necessity of the determination of the minimum toxic dose and the evaluation of the ND biological properties, especially in the experiments conducted *in vivo.*

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Streszczenie: *Wpływ koloidu nanodiamentu na parametry stanu zdrowia szczurów*. Celem badań była ocena wpływu pozajelitowego wlewu hydrokoloidu nanodiamentu otrzymanego metodą detonacyjną na parametry hematologiczne i biochemiczne krwi u szczurów. Uzyskane wyniki wskaźników hematologicznych wskazują, że hydrokoloid nanodiamentowy, niezależnie od drogi podania, wpłynął na zwiększenie się liczby płytek krwi u szczurów w porównaniu z grupą kontrolną.

Ponadto wlew dożylny hydrokoloidu ND wpłynął na zmniejszenie koncentracji glukozy i białka całkowitego we krwi badanych zwierząt. Analiza danych doświadczenia dowodzi pilnej potrzeby ustalenia minimalnej dawki toksycznej nanocząstek ND oraz szczegółowej oceny biologicznych właściwości nanodiamentów otrzymywanych metodą detonacyjną w badaniach na zwierzętach.

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