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Comparison of the effect of dietary copper nanoparticles and one copper (II) salt on the metabolic and immune status in a rat model

Ewelina Cholewińska[a], Jerzy Juśkiewicz[b], Katarzyna Ognik[a,*]

[a] Department of Biochemistry and Toxicology, Faculty of Biology, Animal Sciences and Bioeconomy. University of Life Science in Lublin, Akademicka 13, 20-950 Lublin, Poland

[b] Institute of Animal Reproduction and Food Research of the Polish Academy of Sciences, Division of Food Science, Olsztyn, Poland

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ABSTRACT

The aim of the study was to evaluate the effects of a diet containing different levels of Cu in two different chemical forms (carbonate and nanoparticles) on metabolic, immune and antioxidant status in a rat model. Five experimental treatments (8 rats in each) were used to test different dosages of Cu added to the diet (standard −6.5 mg/kg, half the standard dosage −3.25 mg/kg, and no added Cu as a negative control) and two sources of added copper (standard −$CuCO_3$ and copper nanoparticles −CuNPs). Blood and urine samples were collected from all the animals after four weeks of treatment. Metabolic and immune parameters were determined in blood and urine samples. The study has shown that a dietary Cu deficiency (negative control) decreases rat's plasma levels of Cu, Fe, CREAT, BIL and IL-6, whereas reducing the level of Cu from the recommended 6.5 mg/kg to 3.25 mg/kg decreases only the plasma concentration of TG, IgE and IL-6. Replacing $CuCO_3$ with CuNPs in rat diets affects their metabolism, as indicated by decreased Ca, CREAT, BIL, ALB and IL-6 plasma levels. To sum up, CuNP added to a diet of rats have a more beneficial effect on metabolic indices (indicative of kidney and liver function) and inhibit inflammatory processes more effectively than $CuCO_3$.

1. Introduction

Together with iron and zinc, copper is one of the most important microelements regulating the growth and functioning of the body. As an electron donor or acceptor, copper is an essential cofactor and/or structural component of many important metalloenzymes involved in biological processes such as energy metabolism (cytochrome c oxidase), antioxidant defence (Zn,Cu-containing superoxide dismutases) or synthesis of neurotransmitters (dopamine beta-monooxygenase) and neuropeptides (peptidylglycine alpha-amidating enzyme) [1]. In addition, copper regulates assimilation of Fe and its distribution to the erythrocytes, where haemoglobin is synthesized [2]. Furthermore, by inducing arachidonic acid conversion and prostaglandin synthesis, Cu plays an active role in inhibition of systemic inflammation [3].

Both a deficiency and surplus of Cu can lead to metabolic disorders and also reduce immunity in animals. Copper deficiency in the body contributes to anaemia, bone development disorders, depigmentation and abnormal growth of the hair coat, growth and reproductive disorders, and the development of heart failure and gastrointestinal disorders [4]. An excess of this element, on the other hand, can lead to cirrhosis, lipid profile abnormalities, oxidative stress, renal dysfunction, and gastrointestinal mucosal irritation [5]. Therefore, it is accepted that the dose of Cu in the diet of rats should be in the range of 5–6.5 mg Cu/kg diet [6–9].

Copper is most often introduced to rat diets in an inorganic form, such as $CuCO_3$. However, it is relatively poorly assimilated [10]. As a result, the search continues for new solutions enabling better absorption of this microelement in the body. Research is increasingly carried out on replacement of standard Cu forms with nanoparticles (CuNPs) in animal feed. Due to their very small size and specific surface area, it is assumed that, they may be differently utilized by the body than their macro counterparts [11]. This suggests that they may be better absorbed in the body, so that the recommended dose of Cu in the diet can be reduced, and with it its excretion into the environment [12]. The use of CuNPs in the diet of rats, however, involves some risk. There are reports that they may adversely affect the body, exerting a negative effect on metabolic parameters and causing functional disturbances in vital organs such as the kidneys and liver [5,13,14]. Therefore the aim of the study was to evaluate the effects of a diet containing different levels of Cu in two different chemical forms (carbonate and nanoparticles) on metabolic, immune status in a rat model. It was hypothesized that the applied two forms of additional dietary copper

* Corresponding author at: Department of Biochemistry and Toxicology, University of Life Sciences in Lublin, Akademicka 13, 20-950 Lublin, Poland.
E-mail address: kasiaognik@poczta.fm (K. Ognik).

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would cause different physiological response in the body of experimental rats.

2. Materials and methods

2.1. Animal protocol and dietary treatments

All animal care and experimental protocols were in compliance with current laws governing animal experimentation in the Republic of Poland and with guidelines established by an ethics committee, in accordance with the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes, Directive 2010/63/EU for animal experiments, and were approved by the appropriate Local Institutional Animal Care and Use Committee (Permit Number: 68/2017).

Forty, healthy, male, albino Wistar rats (Han IGS Rat [Crl:WI(Han)]) at the age of 5 weeks and a body weight of 135 ± 10 g were divided into 6 groups. The rats were housed randomly and individually in stainless steel cages at a stable temperature (21–22 °C), relative humidity 50 ± 10%, a 12-h light-dark cycle, and a ventilation rate of 20 air changes per hour. For 4 weeks, the rats had free access to tap water and semi-purified diets, which were prepared and then stored at 4 °C in hermetic containers until the end of the experiment (details in Table 1). The diets were modifications of a casein diet for laboratory rodents (AIN-93G) recommended by the American Institute of Nutrition. In the study, five experimental treatments were used to evaluate the effects of different levels of Cu added to the diet (standard dosage of 6.5 mg/kg diet, half the standard dosage, and no Cu supplement, as a negative control) and two Cu sources ($CuCO_3$, commonly used in rodent laboratory diets, and a preparation of Cu nanoparticles, 40 nm).

The rats were divided into following groups: CuD group − during all five weeks of feeding the rats were given a diet with mineral mixture (MX) without Cu ($CuCO_3$ excluded from MX) as a control deficient group (COND); CuS-H group − the rats were fed a diet with a standard MX resulting in 6.5 mg Cu (from $CuCO_3$ in MX) per kg of diet during 4 weeks of feeding; CuS-L group − the rats were given a diet with 3.25 mg/kg Cu (half the standard dosage) from $CuCO_3$ in MX during 4 weeks of feeding; CuNP-H group − the rats were given a diet containing the standard concentration of 6.5 mg/kg Cu per kg of diet from a preparation of Cu nanoparticles during 4 weeks of feeding; and the CuNP-L group − the rats were fed a diet with 3.25 mg/kg Cu (half the standard dosage) from a preparation of Cu nanoparticles during 4 weeks of feeding (see Table 1). Copper nanoparticles (CuNP, purity 99.9%, 40 ~ 60 nm, spherical, specific surface area 12 m^2/g, bulk density 0.2 g/cm^3) were purchased from SkySpring Nanomaterials (USA). For the safety of the individual preparing the experimental diets, the CuNP preparation was not added to the diet in a mineral mixture, but as an emulsion together with dietary rapeseed oil. The detailed composition of the mineral mixtures used in all experimental groups has been provided in Table 1. The experimental groups were additionally monitored for body weight gain and feed intake. Urine and blood samples were collected from each animal separately (n = 8 for each group). After a 10 d preliminary period urine was thoroughly collected for 5 d from all rats that were kept in balance cages (Tecniplast Spa, Buguggiate, Italy). After overnight fasting, blood samples for analysis were collected at the end of the study using standard venipuncture of the heart. The plasma was immediately separated by centrifugation and stored at −25 °C for further analysis. At the end of the experiment, the rats were fasted for 24 h and euthanized individually by carbon dioxide inhalation and dislocation of the spine.

Table 1
Composition of basal experimental diet fed to rats, %.

Basal diet		Chemical composition of main ingredients		
Ingredient	%		Casein	Maize starch
Casein	14.8	Water	8.0	8.8
DL-methionine	0.2	Crude protein	89.7	0.6
Cellulose[1]	8.0	Crude fat	0.3	0.9
Choline chloride	0.2	Crude ash	2.0	0.2
Rapeseed oil	8.0			
Cholesterol	0.3			
Vitamin mix[2]	1.0			
Mineral mix[3]	3.5			
Maize starch	64.0			

[1]α-Cellulose (SIGMA, Poznan, Poland).
[2]AIN-93G-VM [38], g/kg mix: 3.0 nicotinic acid, 1.6 Ca pantothenate, 0.7 pyridoxine-HCl, 0.6 thiamine-HCl, 0.6 riboflavin, 0.2 folic acid, 0.02 biotin, 2.5 vitamin B-12 (cyanocobalamin, 0.1% in mannitol), 15.0 vitamin E (all-rac-α-tocopheryl acetate, 500 IU/g), 0.8 vitamin A (all-*trans*-retinyl palmitate, 500,000 IU/g), 0.25 vitamin D-3 (cholecalciferol, 400,000 IU/g), 0.075 vitamin K-1 (phylloquinone), 974.655 powdered sucrose.
[3]AIN-93G-MX [38], g/kg mix: Calcium carbonate anhydrous $CaCO_3$ − 357, Potassium phosphate monobasic K_2HPO_4 − 196, Potassium citrate $C_6H_5K_3O_7$ − 70.78, NaCl − 74, K_2SO_4 − 46.6, MgO − 24, Microelement mixture.
* − 18, starch − 213.63.
*Microelement mixture g/kg mix: Ferric citrate (16,7% Fe) − 31, Zinc carbonate $ZnCO_3$ (56% Zn) − 4.5, Manganous carbonate $MnCO_3$ [44.4% Mn] − 23.4, Copper carbonate − different amounts and sources, depending on the experimental group#, Citric acid $C_6H_8O_7$ − to 100 g.
#Experimental groups: CuS-H − the rats were fed a diet with a standard mineral mixture (MX) resulting in 6.5 mg Cu (from CuCO3 in MX) per kg of diet during 4 weeks of feeding; CuS-L − the rats were fed a diet with half the standard dosage in the mineral mixture (MX), resulting in 3.75 mg Cu (from $CuCO_3$ in MX) per kg of diet during 4 weeks of feeding; CuNP-H − the rats were fed a diet containing 6.5 mg/kg Cu from a preparation of Cu nanoparticles per kg of diet during 4 weeks of feeding; CuNP-L − the rats were fed a diet with half the standard dosage (6.5 mg/kg) from a preparation of Cu nanoparticles per kg of diet during 4 weeks of feeding.
[3]administered to groups CuNP-H, CuNP-L (4 weeks of feeding); during 2–5 weeks of feeding, groups CuNP-H and CuNP-L were provided with the appropriate amount of Cu from the preparation of Cu nanoparticles as an emulsion together with dietary rapeseed oil.

2.2. Blood analyses

The concentrations of copper, zinc, iron, calcium, phosphorus and magnesium were determined by inductively coupled plasma optical emission spectrometry (ICP-OES). The Certified Reference Material NIST-1577C Bovine liver was used for quality control.

The content of uric acid (UA), urea (UREA), albumin (ALB), creatinine (CREAT), glucose (GLU), total protein (TP), bilirubin (BIL), total cholesterol (TC), HDL cholesterol, and triacylglycerols (TG) was measured using an automatic biochemical analyser (Plasma Diagnostic Instruments Horiba, Kyoto, Japan).

Haematological blood parameters, i.e. red blood cell count (RBC), haemoglobin (Hb), haematocrit (Ht), and white blood cell count (WBC), were analysed using an automatic haematology analyser (Abacus Junior Vet, Diatron, Hungary).

The level of rat immunoglobulins A, M and E and interleukin IL-6 in the blood plasma were determined on an ELISA reader using commercial kits from Elabscience Biotechnology Co., Ltd.

2.3. Statistical analysis

The model assumptions of normality and homogeneity of variance were examined by the Shapiro-Wilk and Levene tests, respectively. To compare the CuD (mineral mix deprived of copper) group versus each experimental one, the data were subjected to stundetized *t*-test procedure. In a model without the CuD group, the data were subjected to 2-way ANOVA to examine the main effects: F − Cu form effect (two types of Cu form: $CuCO_3$ in mineral mix and Cu nanoparticles; S and NP treatments, respectively), D − Cu dietary dose effect (3.75 and 6.5 mg Cu per one kg of a diet; L and H treatments, respectively), and the interaction between these two factors (F × D). If the analysis revealed a significant interaction ($P \leq 0.05$), the differences among the respective treatment groups (CuS-H, CuS-L, CuNP-H, CuNP-L) were then