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Ultrahigh reactivity provokes nanotoxicity: Explanation of oral toxicity of nano-copper particles

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Abstract

Recently, studies on the biological effects of nanomaterials show signs that some of the manufactured nanoparticles exhibit unexpected toxicity to living organisms. It has previously been reported that the copper particles possess size-depended toxicity. In this paper, we propose that the ultrahigh chemical reactivity of nano-copper results in the specific nanotoxicity which is fully proved by *in vitro* and *in vivo* experiment. Using chemical kinetics study (*in vitro*) and blood gas and plasma electrolytes analysis (*in vivo*), we found that high reactivity cause the big toxicological difference between small size (23.5 nm) and big size (17 μm). The result is also consistent with biochemistry assay, pathological examination and copper content measurement in renal tissue *in vivo*. For chemical reactive nanoparticles, metallic nano-copper for instance, both the particles themselves and the resulting product (copper ion) should be fully explored. The nano-copper particles may not compromise the mice directly, however, they lead to the accumulation of excessive alkalescent substance and heavy metal ions (copper ions) culminating the metabolic alkalosis and copper ion overload.

Keywords: Nanotoxicity; Ultrahigh reactivity; Copper; Metabolic alkalosis; Copper ion overload

1. Introduction

Recently, many studies focus on the safety issue of manufactured nanomaterials to minimize or eliminate their nanotoxicity even before they are wildly used in industry (Lam et al., 2004; Hoet et al., 2004; Oberdörster et al., 2005; Wiesner et al., 2006). Compared to classical substance (in micro scale), the nanoparticles, due to their nanoscale and huge surface area, may interact with biological systems by more efficient approaches, sometime beneficial but sometime producing grave toxicity. Because the nanosize/surface area of the nanosubstance is directly correlated to many essential characteristics like surface property, chemical reactivity, physical absorption ability, etc., all these factors strongly dominate nanotoxicological behavior *in vivo* (Zhao et al., 2007). However, it usually does not take the effects of physical size and sur-

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face area into consideration in traditional toxicological research.

Though the nanoparticles may primarily target at respiratory organs, however, other organs, e.g., gastrointestinal tract, also need to be considered. Because nanoparticles could get into gastrointestinal tract by many ways, such as indirectly via mucociliary movement, directly via oral intake of water, food, cosmetics, drugs and drug delivery system in nanoscale (Hoet et al., 2004; Oberdörster et al., 2005). Our previous study indicates the acute oral toxicity of copper particles, has shown a significant correlation with its size distribution (Chen et al., 2006). With the particle size reducing from 17 μm (particle number: 44 per μg; surface area 3.99×10^2 cm^2/g) to 23.5 nm (particle number: 1.7×10^{10} per μg; surface area 2.95×10^5 cm^2/g), LD_{50} of copper particle sharply increase from >5000 mg/kg (non-toxic) to 413 mg/kg (moderately toxic) based on the Hodge and Sterner scale (Fig. 1). For identical chemical composition, why do the nano-copper particles possess unique biobehavior (nanotoxicity) *in vivo* comparing to those in bigger size (in micro scale)? Looking for answer to this question may provide an insight on nanotoxicity reducing or elimination. We hypotheses that the oral toxicity of nano-copper particles is directly generated from their ultrahigh chemical reactivity *in vivo*. To supply reliable evidence to this hypothesis, a series of experiments were carried out *in vitro* and *in vivo*. In this study, the ultrahigh reactivity of nano-copper particles is confirmed by *in vitro*, and the specific mechanism of its nanotoxicity *in vivo* is discussed. We found the nano-copper nanoparticles may not compromise the mice directly, however, they lead to the accumulation of excessive alkalescent substance and heavy metal ions (copper ions) culminating the metabolic alkalosis and copper ion overload.

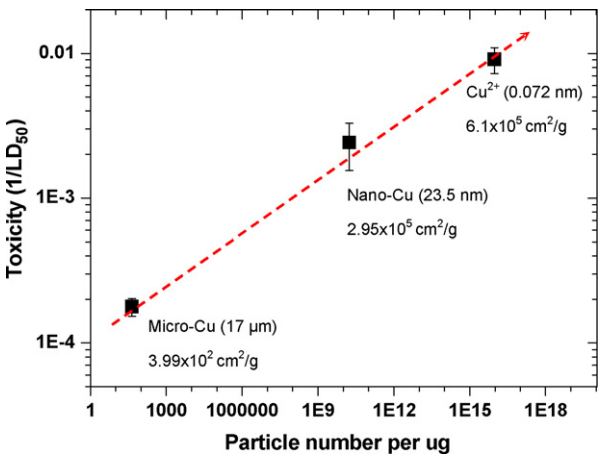


Fig. 1. The function of toxicity of copper and particle number on same mass. On same mass, copper toxicity strongly correlates to particle number. The number of nano-copper particles per μg (size: 23.5 nm; surface area 2.95×10^5 cm^2/g) is 3.8×10^8-folds to micro-copper (size: 17 μm; surface area 3.99×10^5 cm^2/g), and the LD_{50} of copper particles sharply increase from >5000 mg/kg to 413 mg/kg.

2. Methodology

2.1. Tested chemicals

The size distribution of nano-copper particles (23.5 nm, Shenzhenjunye Nano Material Co., Ltd., 99.9%) and micro-copper (17 μm, Beijinghaoyun Co., Ltd, 99.9%) was confirmed by Transmission Electron Microscopy (TEM, Hitichi H-700) and Atomic Force Microscopy (AFM, Nano III a SPM, Digital Instruments Inc. USA 3A) before usage. The impurities were detected by X-Ray Fluorescence technique (XRF). All the results are consistent with the labeling information. To insure non-aggregation, both copper particles were dispersed in 1% (w/v) HPMC solution (Hydroxypropylmethylcellulose K4M, Colorcon Coating Technology Ltd.) via ultrasonic treatment in argon atmosphere. The preparation of suspending solution is accordance with our previous method (Chen et al., 2006).

2.2. Chemical kinetics study in vitro

37.5 mg nano-copper particles were suspended into 1.5 ml HPMC of 1% (w/v) solution, following by adding 60 ml artificial acid juice (pH 1.75) into the systems. The temperature of the suspending solution was kept at 37 °C and slowly stirred by magnetic rotator. Simultaneously, the pH values of the solution were recorded by real-time pH detector from 0 to 60 min. For micro-copper particles, the same procedure was performed under the equivalent condition. Similarly, the real-time pH detection was performed in neutral biological fluids (PBS solution, pH7.4).

2.3. Animals

Male ICR mice (Weitonglihua experimental animal Co., Ltd.), aged 8 weeks and weighting 25 ± 1 g, were used in the experiments. The animals were housed and maintained on commercial animal food, given deionized water *ad libitum* and kept in the cage in a 20–22 °C, 60% relative humidity room with a 12 h light/dark cycle. All the animals were quarantined and acclimated in the controlled environment for five days prior to the

study. Before oral administration, the mice would be fasted overnight. All animal experiments are performed in compliance with the local ethics committee.

2.4. Blood gas and plasma electrolytes analysis

To compare the change of blood gas and plasma electrolyte, 48 male mice were divided into four groups (12 mice in each group). Before the animal experiment, nano-, micro- and ion-copper were dispersed into the 1% w/v HPMC solution inside the glove box which was filled with dry argon gas to protect from oxidation. Then the mice were respectively exposed by oral gavage to nano- and micro-copper at dose of 70 mg/kg body weight, as well as $CuCl_2$ solution at 147.6 mg/kg (equivalent 70 mg/kg on copper mass). The mice were treated with 1% HPMC as control. 24 h after the exposure, random select six mice in each group were anesthetized by i.p. 20% urethane, and the arterial blood samples (about 0.3–0.5 ml) were immediately collected from arteria carotis, and placed in air-free anticoagulated tubes. Blood gas and plasma electrolytes analysis was performed (GEMPremier3000, Hongkong) within 2 h after the blood collection. The arterial blood pH, partial pressure of CO_2 in arterial blood ($PaCO_2$), partial pressure of PO_2 in arterial blood (PaO_2), concentration of bicarbonate (HCO_3^-), blood sodium (Na^+) and blood potassium (K^+) were detected. The arterial blood samples of the rest mice were collected at 72 h after the oral gavage, and the parameters were measured by the same method.

2.5. Autopsy and pathological examination

Autopsy and pathological examination were carried out following blood gas and plasma electrolytes analysis. The left kidney was stripped out and fixed in 10% formalin immediately for further histopathological examination; the right kidney was collected for inductively coupled plasma mass spectrometry (ICP-MS) measurement. After hematoxylineosin (HE) staining, the slides are observed and photos are taken using optical microscope (Olympus X71, Japan). All the identity and analysis of the pathology slides are blind to the pathologist.

2.6. Identification of copper content in renal tissue

To identify the content of copper elements in renal tissues, serum and urine, ICP-MS measurement was performed. After accurate weighting, each sample was rendered soluble by the addition of nitric acid (HNO_3, 67%, MOS grade) and hydrogen peroxide (H_2O_2, 30%, MOS grade) in a glass mini-backer. Two days later, each acid digestion was heated (80 °C) for evaporation, 2% nitric acid was added to 10 ml (copper measurement in renal tissue) or 1 ml (copper measurement in serum and urine) as the final volume. Calibration plots of standards of Cu^{2+} are obtained by injecting a series of standard solutions (10, 50, 100, 500 ng/ml in 2% HNO_3, flow rate 1.0 ml/min). Then the resulting solution is injected into ICP-MS system (Thermo Elemental X7). The accuracy and precision of our technique can be assessed from the measured concentrations and relative standard deviations for the content of indium ^{115}In salts.

2.7. Biochemistry assay

Another 48 mice were treated with nano-, micro-, ion-copper and 1% HPMC solution (same as the treatment described above) and housed in metabolism cage. The mice plasma was respectively collected at 24 h and 72 h by centrifuging whole blood at 2000 × *g* for 15 min at 4 °C. The serum copper (SC) and serum ceruloplasmin (CP) were measured by using ceruloplasmin (CP) (50T) kit (Nanjingjiancheng Bioengineering Institute, China) and inductively coupled plasma mass spectrometry (ICP-MS) measurement. The urine samples of the mice in nano-, micro-, ion-copper and control groups were respectively combined together during 0–24 h postexposure after the oral gavage, and further for urine copper (UC) detection by ICP-MS.

2.8. Statistical analysis

All of the results were calculated as mean ± standard deviation (S.D.). The statistical significance of the changes between tested groups and the control group were analyzed by the multiple comparison test method (*t*-test) using SAS 6.12 (SAS Institute, Inc., Cary, NC).

3. Results

3.1. Chemical kinetics study

pH of the solution rapidly increase (the line with square symbols in Fig. 2(a)) when nano-copper added, and its value ascend to ∼5.5 at 50 min, which indicate that a great many of H^+ has been consumed. Unlike to this, pH value of the solution added micro-copper remains stable even at the end of the experiment (the line with circle symbol in Fig. 2(a)). Fig. 2(b) shows the appearance of yield solution with and without centrifugation. After centrifugation treatment, the transparent

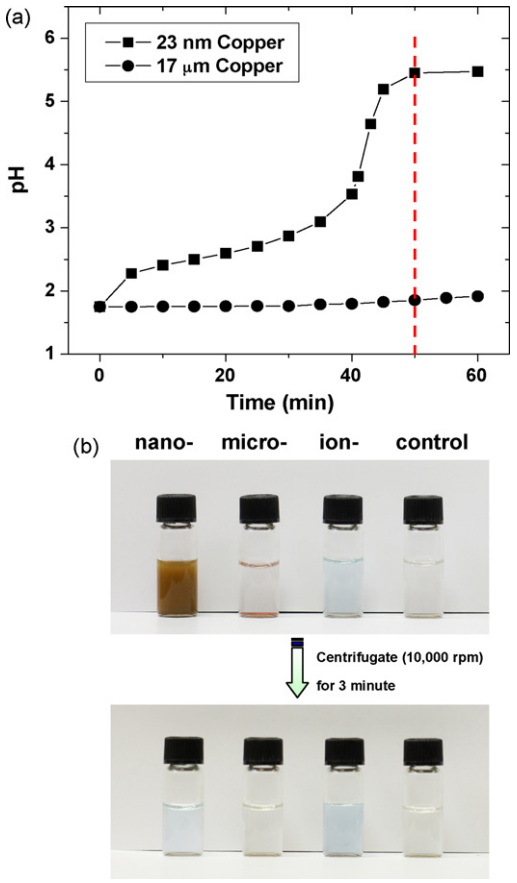


Fig. 2. (a). The pH values were performed as a time function in the artificial acidic juice when micro- or nano-sized copper particles were added into acid solution from 0 to 60 min. (b). Nano-, micro-, ion-copper and control (1% HPMC) react with artificial acidic juice for 60 min (the upper picture). The transparent solution appears to be slightly blue in nano-copper group after centrifugal treatment at 10,000 rpm for 3 min (the lower picture). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

solution appears identifiable blue color in nano-copper group which is further recognized as Cu^{2+}, by contrast, the solution in micro-copper group keep colorless (Fig. 2(b)). When we performed the real-time pH detection in neutral biological fluids, pH value keeps stable even at the end of the experiment in micro- and nano-group.

3.2. Blood gas and plasma electrolytes analysis

The results of blood gas and plasma electrolytes analysis suggest that the consuming of a great many of H^+ directly influence the "acid–base" balance *in vivo* (Table 1). After the orally exposed to nano-copper for 24 h, the blood pH of the experimental animal tends to alkalescence (pH 7.46 ± 0.03, $p < 0.05$), and the level of plasma bicarbonate (HCO_3^-) and $PaCO_2$ are slightly elevated versus control group. In addition, PaO_2 is influenced, with lower levels of oxygen pressure noted in nano-copper group, but K^+ and Na^+ concentration in blood do not alter significantly. After 72 h, the $PaCO_2$ and PaO_2 tend to normal level, however, blood pH still keeps alkalosis and level of the HCO_3^- is significant higher than control. Comparing to this, no significant changes were observed in micro-copper groups.

3.3. Autopsy and pathological examination

Photos in Fig. 3 show morphological changes at 24 h of stomach of experimental mice exposed to nano-, micro-, ion-copper and control. The stomach appearance of nano-copper exposed mice swells up and presents cyan color. However, these mice of micro-copper and ion-copper exposed are almost same with the control. The result suggests that nano-copper may remain in stomach for longer time, in another word, the durative interaction with acid juice may lead to persistent heavy metal ions generation *in vivo*.

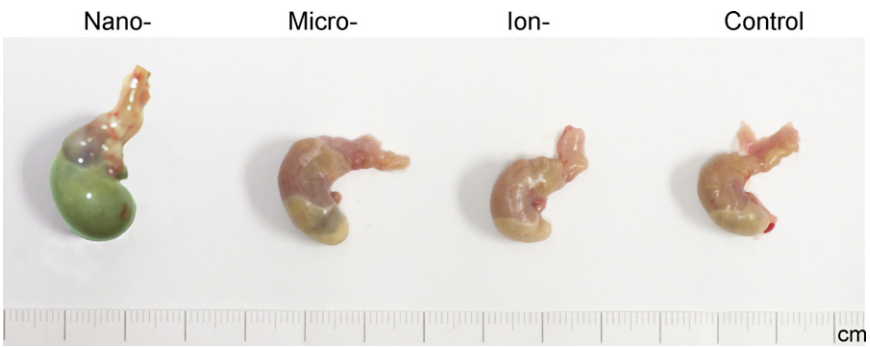


Fig. 3. The appearance of mice's stomach after single oral gavage for 24 h. The stomach is swelling up after nano-copper treatment and appearing significant changes in color. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

Table 1
Blood gas and plasma electrolytes analysis of the mice treated by nano-, micro- and ion-copper for 24 h and 72 h

Hours	Arterial blood (pH)	$PaCO_2$ (mmHg)	PaO_2 (mmHg)	HCO_3^- (mmol/L)	Na^+ (mmol/L)	K^+ (mmol/L)
Nano-copper						
24	7.46 ± 0.03*	45.2 ± 7.0	93.1 ± 9.2*	28.1 ± 1.6*	145 ± 1	6.3 ± 0.5
72	7.45 ± 0.04*	42.8 ± 4.1	105.4 ± 7.2	26.7 ± 1.9*	144 ± 2	6.8 ± 0.2
Micro-copper						
24	7.42 ± 0.03	43.2 ± 2.6	101.1 ± 5.7	16.3 ± 1.5	142 ± 2	6.7 ± 0.2
72	7.39 ± 0.02	39.2 ± 2.3	101.1 ± 5.7	14.7 ± 1.0	142 ± 1	6.9 ± 0.1
Ion-copper						
24	7.38 ± 0.03	40.5 ± 3.4	91.7 ± 8.0*	15.4 ± 2.0	140 ± 2	6.9 ± 0.2
72	7.40 ± 0.02	38.4 ± 1.3	109.6 ± 5.0	14.7 ± 1.5	144 ± 2	6.8 ± 0.2
Control						
24	7.41 ± 0.02	39.2 ± 2.7	109.0 ± 6.4	14.7 ± 1.7	145 ± 1	6.8 ± 0.1
72	7.41 ± 0.02	39.4 ± 1.7	113.8 ± 7.8	15.1 ± 2.4	145 ± 1	6.9 ± 0.2

* $p < 0.05$ vs. the control.

In pathological examination, proteinic liquid filled in renal tubules accompanying with renal tubule necrosis is seen, in which purple deposition is further found at 24 h (Fig. 4(a)) and 72 h (Fig. 4(b)) after nano-copper treatment. A pronounced lesion of glomerulonephritis in ion-copper group (Fig. 4(c)) 24 h after oral gavage, but the lesion could mostly recover 72 h after the exposure. No purple deposition was found in ion-copper group. We propose that the copper element, which is also proven by ICP-MS measurement, may easily deposit in renal tissue in form of insoluble salts which could not removed by organism due to the alkalescent microenvironment in nano-copper treatment group. No detectable pathological changes were found in micro-copper treated group.

3.4. Identification of copper content in renal tissue

We use ICP-MS to detect the copper content in renal tissue. These renal samples come from these mice after they were treated for 24 h and 72 h. The measurement shows that massive copper enriches in renal tissue 24 h after the mice exposed to nano- and ion-copper, the concentration rise up to 13.0 ± 4.1 ppm and 12.6 ± 2.2 ppm, respectively, which are equivalent to about three times of the control level (4.0 ± 0.8 ppm). The copper content in renal tissue drops from 12.6 ± 2.2 ppm to 6.5 ± 1.3 ppm in ion-copper group at 72 h, however, in nano-copper group, it still maintains high copper content level in kidney (11.5 ± 2.5 ppm). These imply that the rate of elimination of nano-copper is very low in

Table 2
Biochemistry analysis of the mice treated by nano-, micro- and ion-copper for 24 h and 72 h

Hours	Serum copper (SC, ppm)	Serum ceruloplasmin (CP, U/l)	Urine copper (μg/mouse in first 24 h)
Nano-copper			
24	3.20 ± 1.34*	24.3 ± 6.9	284.4[a]
72	4.02 ± 0.94**	27.4 ± 5.2	
Micro-copper			
24	1.13 ± 0.69	22.6 ± 4.4	11.9[a]
72	1.16 ± 0.35	26.5 ± 7.6	
Ion-copper			
24	4.61 ± 1.68**	27.4 ± 7.5	732.7[a]
72	4.41 ± 0.66**	32.9 ± 5.4*	
Control			
	0.97 ± 0.29	23.2 ± 5.7	14.2[a]

* $p < 0.05$ vs. the control.
** $p < 0.01$ vs. the control.
[a] Average urine copper content of six mice during 0–24 h postexposure. The value of urine copper is obtained by measurement of combination of urine samples of six mice.

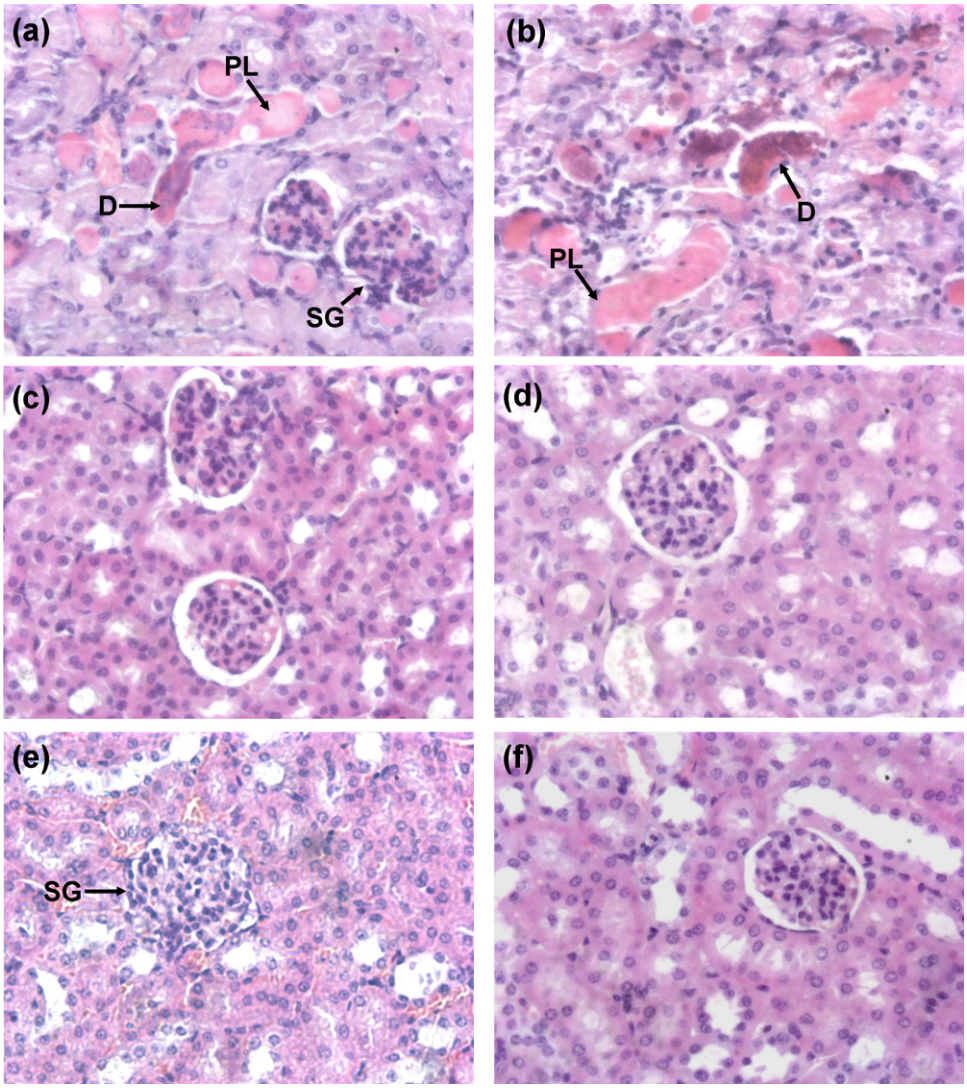


Fig. 4. The pathological examination of renal tissue when the mice were treated by nano-, micro-, ion-copper and control (1% HPMC) for 24 h and 72 h. (a). nano-copper group (24 h, 400×). (b). nano-copper group (72 h, 400×). (c). micro-copper group (24 h, 400×). (d). micro-copper group (72 h, 400×). (e). ion-copper group (24 h, 400×). (f). ion-copper group (72 h, 400×). "PL" indicates proteinic liquid, "D" denotes purple deposition, "SG" points to swollen glomerulus.(For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

kidney (Fig. 5), with only 1.5 ppm reduction within 48 h.

3.5. Biochemistry assay

To evaluate and compare toxicosis induced by copper particles in different size, biochemical parameters (SC, CP, UC) that reflect copper toxicosis were examined (Table 2). The nano-copper particle can be rapidly transformed into ion forms but micro-copper can not. Hence the SC level in nano-copper group is elevated in 24 h (3.20 ± 1.34 ppm) and remains at high level until 72 h (4.02 ± 0.94 ppm) versus control (0.97 ± 0.29 ppm). The ion-copper sharply enhances SC level at 24 h (4.61 ± 1.68 ppm) and begins to descend in 72 h (4.41 ± 0.66 ppm). Physiologically, excessive copper element is rapidly excreted via uropoiesis in kidney. UC level is evidently rising after nano- and ion-copper treatment, whereas, the elevation of copper in urine is not detected in micro-copper group. In addition, CP is an acute-phase reactant type protein, because it binds a large portion of serum copper. CP level tends to faintly raise 72 h after nano-copper exposure (but no statistical difference). No significant abnormalities are found

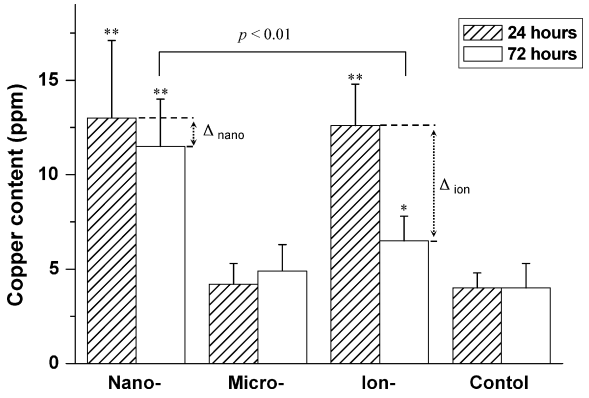


Fig. 5. ICP-MS was used to measure the content of Cu in the mice's renal tissues which are treated by nano-, micro- and ion-copper at the dose of 70 mg/kg. The asterisk indicates the copper content is significantly higher versus control (*$p < 0.05$; **$p < 0.01$). Both nano- and ion-copper lead to higher Cu content in renal tissue, by contrast, no difference is observed between micro-group versus control. In ion-copper group, the Cu content in kidney sharply declines 72 h after the oral gavage (Δ_{ion}), however, the Cu content remains stable when the mice were treated by nano-copper even 72 h after the exposure (Δ_{nano}).

in micro-copper treated mice, which suggest the mice do not suffer from heavy metal toxicosis in micro group (Table 2).

4. Discussion

4.1. Ultrahigh reactivity provokes nanotoxicity

Chemical reaction between solid and liquid phase always initiates at the surface molecules of two phases, hence, the surface molecules can directly influence the chemical reactivity. The average specific surface area of the nano-copper (23.5 nm) used in this study is calculated as 2.95×10^5 cm^2/g. In accordance with the collision theory in chemistry, huge specific surface area must lead to a high probability of effective collision, which determined the ultrahigh reactivity during molecular interaction. Some chemical reactions are allowed in sense of chemical thermodynamics but could not happen in sense of reaction kinetics. However, when the particle size reduces to nano-scale, the huge specific surface area will sharply speed up chemical reaction and may eventually cause nanotoxicity that micro-scale substance do not have. Nano-copper paricles can quickly interact with H^+ in artificial gastric juice, and be converted into ionic states. Micro-copper particles (17 μm) have much smaller specific surface area as 3.99×10^5 cm^2/g, which is about 1/940 to the nano-copper. Relative to nanoparticles, the micro-copper appears chemically inert, because of lower specific surface area.

4.2. Ultrahigh reactivity leads to metabolic alkalosis and copper ion overload in vivo

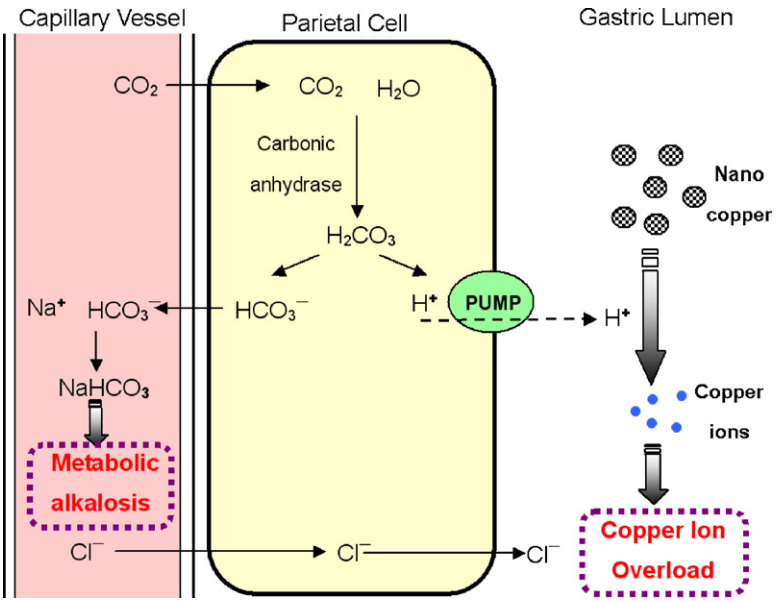
Due to the ultrafine nanosize, nano-copper particles are extremely active in biological system when they enter into stomach. The nano-copper particles may not compromise the mice directly, however they lead to the accumulation of excessive alkalescent substance and heavy metal ions (copper ions) culminating the metabolic alkalosis and copper ion overload (Scheme 1).

(1) Metabolic alkalosis

H^+ depletion leading imbalance of acid and base *in vivo* is proved by blood gas and plasma electrolytes analysis (Table 1). The nano-copper retained in gastric lumen can continuously react with the secreted acid juice (Fig. 3). The depletion of H^+ results in alkalosis because HCO_3^- is generated during production of gastric acid and returns to the circulation. The elevation of blood pH motivates a set of compensatory effects: (a) respiratory compensation is naturally provoked within several minutes. However, the respiratory compensation is limited, because high $PaCO_2$ and low PaO_2 must motivate apneustic center to prevent hypoxia (Galla, 2000). Hence, the $PaCO_2$ were only slightly enhanced 24 h after exposure; (b) theoretically, renal compensation starts relatively later but can sustain for a long time (several days) (Galla, 2000; Williams, 2005). However, a series of abnormalities were found in the pathological examination such as swollen glomerulus, dwindling in the lumen of Bowman's capsules, being signs of glomerulonephritis (Fig. 4(a) and (b)). The renal dysfunction may largely weaken renal compensation in nano-copper group and deteriorate the metabolic alkalosis.

(2) Copper ions overload

In pathological examination, purple deposition is only found in nano-copper treated group, which is further testified to be copper deposition by ICP-MS (Fig. 4(a)(b), 5). Homeostasis of copper ions is maintained *in vivo* for normal body (Bertinato and L'Abbe, 2004). It is reported that the copper ions ingested are metabolized in liver and excreted via urine (Turnlund, 1998; Tao et al., 2004). Daily intake of copper in healthy adults varies between 0.07 mg/kg and 0.1 mg/kg body weight (WHO: IPCS Environmental Health Criteria: Copper. Geneva: World Health Organization, 1998). If the intake of copper exceeds the range of the tolerance, it would cause toxic effects to hepatic and renal tissues, which is consistent with our finding



Scheme 1. The scheme depicts why nano-copper particles possess grave nanotoxicity. When the nano-copper enter into mice, in one hand, they quickly react with acid substance (H^+), the resulting H^+ consumption could further leads to the alkalosis, in other hand, the copper ions whose toxicity is wildly studied give the organism grave lesion. The nano-copper nanoparticles may not compromise the mice directly, however they lead to the accumulation of alkalescent substance and heavy metal ions (copper ions) culminating the metabolic alkalosis and copper ion overload. H^+-K^+-ATPase is indicated by ellipse with "PUMP".

in pathological examination (Fig. 4) and biochemistry assay (Table 2). With respect to nano-copper, it may possess extremely high bioavailability, hence, the original safety limit may be modified to much lower level. The biological test is done in suspected copper toxicity in these mice when they are acutely exposed to nano-, micro- and ion-copper. Comparing to micro-copper, nano-copper could induce evidently high SC level, a sign of acute toxicosis. What is more important, the nano-copper exhibits lower elimination rate *in vivo*, which may worsen the heavy metal toxicosis. It retains an appearance of high level of SC in nano-group even at 72 h, suggesting that the mice is suffered from persistent high copper concentration in blood, maybe eventually cause fatal copper overload (Bremner, 1998).

Nano-, micro- and ion-copper exhibit different biological behavior *in vivo* via oral exposure routine. In terms of nano-copper particle, both copper overload and metabolic alkalosis contribute to their grave toxicity (Scheme 1). Unlike to this, the micro-copper does not stagnate in stomach, and the velocity of ionization is much slower than that of nanoparticles. After the particles propelled into small intestine by gastric emptying, the reaction of ionization is prohibited because of basic condition, and finally excreted as faeces. For direct intake of copper ion, transitory glomerulonephritis and alimentary canal disorder happens in experimental animals. These toxicological responses can be partly corrected within 72 h.

4.3. From in vitro to in vivo

It is urgent missions that people have to test and assess the healthy and environmental effects of any material as long as they are in a nanosize. To accelerate this process with high efficiency, low cost and high reliability, scientists attempt to extrapolate *in vitro* results to toxicological assessment *in vivo* (Sayes et al., 2007). Base on our results, for metallic nanoparticles (e.g. nano-copper) through oral pathway, whose toxicity is mainly determined by its reactivity, we obtained the consistent results *in vivo* and *in vitro*. The consistence may be directly related to exposure pathway and chemical–physical properties of nanosubstance (e.g. species, size, surface modification, structure, crystal, phase). The oral pathway is a relative simple toxicological process comparing to pulmonary routine. For chemical inert substance (e.g. nm-TiO_2), nanoparticles may pass the acid environment without change in chemical composition, however, for chemical reactive substance, metallic nanoparticles for instance, both the particles themselves and the resulting product (copper ions in our experiment) should be fully

explored. The difference of chemical reactivity proven by *in vitro* experiment rightly reflects the dominant factor of the grave nanotoxicity by oral intake.

4.4. Nanotoxicity reduction/elimination

To invest the mechanism of nanotoxicity of nano-copper particle may provide a solution of detoxification. Timely and appropriate clinic treatments are needed, including detoxification of heavy metal overload and correction of acid–base imbalance, when acute toxicosis through alimentary tract happened. This strategy can be extrapolated to other nano-sized metallic particles because most of nonvalent metallic particles (e.g. nano-Zn) are easy to be oxidated to ionic states in acid solution even though they are inactive in micro-sized (Wang et al., 2006).

5. Conclusion

The toxicity of copper particles highly correlated to the particle size/specific surface area because ultrahigh reactivity provokes nano-copper's toxicity *in vivo*. The nano-copper particles may not compromise the mice directly, however they lead to the accumulation of excessive alkalescent substance and heavy metal ions (copper ions) culminating the metabolic alkalosis and copper ion overload. When nano-copper reacts to the acid substance in the stomach, a great amount of proton ions are consumed. The metabolic alkalosis as well as the poisoning copper ions cause higher mortality than micro-copper on same dosage.

Acknowledgements

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