Toxicity of manufactured copper nanoparticles - A review

Marzie Hejazy[1]*;Mohammad Kazem Koohi[2]; Adele Bassiri Mohamad Pour[3]; Davod Najafi[4]

[1] *Department of Basic Sciences, Faculty of Veterinary Medicine, University of Tabriz, Tabriz, Iran*

[2] *Basic Science Department, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran*

[3] *Faculty of Veterinary Medicine, Islamic Azad University of Tabriz, Tabriz, Iran*

[4] *Faculty of Veterinary Medicine, University of Tabriz, Tabriz, Iran*

ARTICLE INFO

ABSTRACT

The health effects of nanoparticles (NPs) are raising considerable and growing concerns from the public and government around the world. Nano-metals are new forms of metal with special properties, but are characterized by having a particle size of less than 100 nm. Copper is an essential trace element and its deficiency leads to different diseases in humans. In general, copper NPs have been reported among the most toxic nanomaterials in mammals. The toxicity of nano-copper depends on sex. The increase in the production of reactive oxygen species (ROS) and reactive nitrogen species (RNS) plays an important role in copper-induced organic dysfunction.

In this review, we will not only summarize the tissues toxicology induced by nano-copper, but also we will discuss the functions of ROS and RNS in the pathogenesis and toxicity in different organs, DNA damage as a result of oxidative stress, changes in Blood biochemical indexes, and the mechanism of the nano-copper induced apoptosis.

INTRODUCTION

The health effects of nanoparticles (NPs) are raising important and growing concerns from the public and government around the world. However, most nano-toxicity investigations focused on respiratory exposure to assess the effects of NP [1]. The rapid growth of nanotechnology suggests that it will soon find widespread application in everyday consumer products and in new pharmaceutical, electronic and other industries. However, to date there is still no information on the implications for human health and the environment of the nanomaterials produced [2]. Nano-metals are new forms of metal with special properties but are characterized by having a particle size less than 100 nm [3]. However, several studies have demonstrated that metal NPs can cause histological damage, biochemical alterations and developmental problems in fish [4].

Copper is a necessary traceable element and its lack is resolved in various diseases in humans. On the other hand, it acts as a catalytic cofactor in some redox enzymes required in a broad spectrum of metabolic processes. When copper intake exceeds the tolerable limit, it shows toxic effects that lead to cell death [5]. Nano-copper has shown great promises as drugs for the treatment of osteoporosis, antibacterial agents, additives in cattle and poultry and the intrauterine contraceptive device [6].

At present, biosecurity and the nano-copper research mechanism have launched a preliminary investigation and the result showed that there was potential toxicity in both human and ecological systems [7, 8]. In fact, copper is maintained in the homeostasis of the human body, but copper overload in vivo can cause some toxicological activities. Compared to common and micro

* Corresponding Author Email: *mhejazy@ut.ac.ir*

copper, nano copper can cause serious toxicological effects and the kidney and liver are target organs of copper NPs [8, 9]. Recently, copper NPs are used as additives in lubricants, polymers / plastics, coatings and metal inks, etc. Thanks to the excellent repairing effect of NP copper, the lubricating oil is added as an additive to effectively reduce friction and wear or to repair a worn surface.

Copper NPs are deposited evenly on the graphite surface to significantly improve the loading structure load, coulomb efficiency, cycle characteristics and high-frequency performance as anodic lithium ion material [6]. Nano-copper compounds have a range of industrial applications [4]. Some studies have shown that environmental copper is toxic to *Cyprinus carpio* and causes a response to stress, sodium loss, tissue damage of the liver, immunomodulation, oxidative stress and metabolic disorders [10, 11]. In general, copper NPs have been documented as one of the most toxic nanomaterials in mammals, as indicated by inflammation in mice exposed sub-acute [12]. The nanocomposite copper fluoropolymer is used as a bioactive coating that can inhibit the growth of specific microorganisms such as Saccharomyces cerevisiae, Listeria, Escherichia coli and Staphylococcus aureus. Therefore, copper NPs, like other nanomaterials, can enter the environment and the human body through different routes, such as effluents, spills during shipping and administration, consumer products and disposal, and so on [13]. Although we have identified the potential risks of copper NP in human health, its subacute toxicity has not been described.

Nano copper toxicity

The LD50 values of nano-copper (23.5 nm) are 413 mg / kg of body weight and are considered moderately toxic materials. Nano-copper toxicity may be related to in vivo ionization [1]. Therefore, compared to the particles of micro-copper, nano-copper (same mass), they are more likely to collide with the bio-substances in vivo. For nanometer-sized copper particles, a large surface leads to ultra-high reactivity. NP copper reacts drastically with H^+ in the gastric juice and can lead to massive formation of HCO3, which is extremely excreted by the kidney due to kidney disorders. The excess of $HCO3^-$ in vivo becomes the main cause of metabolic alkalosis, which becomes the origin of the symptom hypopnea and the electrolyte pathologies produce tremors observed in mice exposed to copper NP. In vivo homeostasis continues to be copper ions metabolized in the liver and in the evacuated kidney [1, 9, 14]. The toxic classes of NP copper are class 3 (moderately toxic). One study suggests that ionic copper and nano copper have different effects on fish. They enter the body of the fish probably with different shapes, generating different effects with different sizes in the plasma of copper, ceruloplasmin, iron and ions. Copper, sodium and iron chloride in plasma suggest that Gill is the main site for absorption of ionic copper, while nano-copper can be absorbed through the intestine [15]. Nano-copper NP can not directly involve mice, however, causing an excessive accumulation of alkalizing substances and heavy metal ions (copper ions), the culmination of metabolic alkalosis and overload of copper ions [16]. Furthermore, the toxicity of nano-copper depends on sex: male mice exhibit more severe toxic symptoms and suffer more from nano-copper than women after being exposed to the same mass of particles. [1] The reason for the difference in the distribution, penetration and tissue damage NP in several studies could be due to different methods of synthesis, which lead to a size, shape and other different physical and chemical properties of the NP. Therefore, the interaction and impact of NP on animal cells and tissues will vary [17]. Several studies support the fact that increased production of reactive oxygen species (ROS) and reactive nitrogen species (RNA) plays an important role in copper-induced organic dysfunction. Exposure to NPs of copper caused altered levels of intracellular production of ROS and NO [18]. Changes in the structural physico-chemical properties of NPs can end up with changes in biological activity, including ROS generation. Oxidative stress induced by NP is the result of cellular factors, composition, size, particle surface and presence of metals, while cellular responses such as mitochondrial respiration, cell interaction and immune cell activation are responsible for the mediated damage from ROS. NP-induced oxidative stress responses have torches and other pathophysiological effects that include genotoxicity, inflammation, and fibrosis as evidenced by the activation of associated cell signaling pathways [18]. Exposure to NP is likely to occur with microbial agents. The defense of the innate host mediated by neutrophils and is fundamental in the control of bacterial elimination in the host [19]. It is of fundamental importance for host defense to maintain neutrophil concentrations

at appropriate physiological levels [20]. Recently, it has been reported that copper NP, compared to other NP metal oxides, is highly toxic under in vitro conditions [21]. Recent research on copper NP after subcutaneous injection has shown that the regulatory effects of NP on organisms depend on the dose used [22].

To investigate the mechanism of nanotoxicity of copper NPs it is possible to provide a detoxification solution. Timely and appropriate clinical therapy, containing detoxification due to heavy metal overload and correction of acid-base imbalance, is required when acute intoxication is verified by the gastrointestinal tract [23]. This strategy can be deduced from other nano-metallic particles due to the fact that most non-valas metallic particles (eg Nano Zn) are easy to oxidize to ionic acids in acidic solution even if they are inactive in micro-state.

Hepatotoxicity

At a mean dose of copper NP (341 mg / kg), steatosis was observed around the central veins of the liver tissue of experimental mice. The anatomo-pathological examination revealed that the kidneys, the liver and the spleen are the target organs for copper NPs. These were also shown by measurements of the biochemical blood index (BUN, Cr, ALP and TBA) which reflects the renal and hepatic function of experimental mice [1]. In one study, the effects of intraperitoneal injection of various doses (10, 100 and 300 mg / kg) of copper NP on the liver and the performance of liver enzymes in rats were investigated [24]. Histology of the lungs showed thickening of the sacral wall and intensified fibrous tissue in all groups. Hepatic histology also showed vascularization in the central veins and vessels of the portal triad, and the disappearance of the liver hexagon lobules in the three treatment groups receiving different doses of copper NP. Results of the biochemical analysis of liver enzymes showed that the mean SGOT enzymes, two days after the intervention, in the 10 mg / kg and 100 mg / kg group of copper NPs were significantly higher than those in the control group. On day 14 after surgery, a significant difference was observed between the groups receiving 300 and 10 mg / kg of copper NP. Furthermore, the level of SGPT was significantly higher in the group that received 10 mg / kg compared to that which received 100 mg / kg [24]. Recently it has been reported that 3 hours after the injection of copper NP (2 mg / kg) into the muscle, these NPs could

be observed in the vascular areas of the periportal hepatocytes and in the cytoplasm of the Kupffer cells of the liver in the treated rats. Furthermore, they disappeared within 3 days after treatment [25]. Apoptosis can be observed in the periportal epithelium of the hepatic tubule and kidney after 3 days and 3 hours, respectively, by 3 injections of copper NP [25].

Doudi *et al.* has indicated that all copper NP concentrations induce toxicity and changes in the histopathology of rat liver and pulmonary tissues [24]. Therefore, they can not be used by humans because of their toxicity. Exposure to nano-copper increased ROS production, one of the most frequently reported toxicities associated with NP. Studies on the mechanism of signal transduction showed that the exposure to the mitochondrial membrane potential modified with nano-copper and subsequently helped to release cytochrome c from the mitochondria to the cytosol. Nano copper can trigger both intrinsic and extrinsic apoptotic pathways in oxidative stress [26].

Splenic toxicity

Copper NPs causes severe atrophy and color variations in the spleen, and research indicates that copper is induced dramatic atrophy of the spleen. This suggestion is the one of the main target organs of copper NPs. On the other hand, collectors of some treated mice exposed to copper NP exhibited a blackish color at necropsy [1]. This NP induces the reduction of splenic units, the decrease of lymphocytes and splenic interstitial fibrosis [1].

Nephrotoxicity

In one study, morphological changes of kidney in mice exposed to nano-copper (1080 mg / kg) show dramatic changes in color and transformed bronze [1]. Damage to proximal renal tubules was observed in mice exposed to copper NP. In the kidney, the glomerulus was reduced and swollen in the light of Bowman's capsules, indicating glomerulonephritis [1]. One investigation demonstrated both the toxicity and the kidney damage and the liver induced by nano-copper and verified that these signals were similar to those of their in vivo soluble copper equivalent [27]. Exposure with copper NP increased levels of malondialdehyde (MDA) and carbohydrilation of proteins in the renal tissue of experimental animals [18]. NP copper exposure in one study intensified glutathione disulfide (GSSG) levels, reduced glutathione (GSH) and

thus reduced the GSH / GSSG ratio in the renal tissue of experimental animals [18]. In addition, podocytes called visceral glomerular epithelial cells contributed to the glomerular filtration barrier and were the target of lesions in many glomerular diseases. Numerous studies have shown that oxidative stress is a significant mediator of podocyte lesions [28]. When the kidney was injured, the podocytes changed, including the retraction of the foot processes and also the loss of cells, and then gave rise to many glomerular diseases. In a study in which the podocytes were analyzed with nano-copper, the viable cells were reduced with the concentrations and the increased period. In this study, viability was 1.40% when the nano-copper level reached 100 μg / ml for 24 hours. Concentration of 100 μg / ml seemed too high and so toxic, so the concentration of 1, 10 and 30 μg / ml was selected to detect the cytotoxic effect of nano-copper, thereby increasing ROS was observed in podocytes of the nano -copper group and were in a positive part of the dependent concentration. Meanwhile, T-SOD products were significantly reduced when they were treated with nano-copper in podocytes [29]. Accumulated evidence suggests that oxidative stress also played an important role in the mechanism of podocyte injury. Podocyte lesions accompanied by reduction of nicotinamide-adenine dinucleotide phosphatase oxidase induced by increased renal oxidative stress [30]. ROS, including superoxide anions, hydrogen peroxide, hydroxyl radicals, etc., had a higher reactivity than molecular oxygen. If present at high levels, ROS may cause damage to nuclear DNA and alterations in proteins, lipids and carbohydrates. Superoxide dismutase (SOD), as one of ROS's enzymatic sequestrates, can combat the agglomeration of ROS and limit oxidative damage. In other words, the SOD activity level is related to the antioxidant capacity [29, 31, 32]. The researchers reported that SOD could suppress apoptosis and reduce SOD usually corresponds to an increase in cell death in apoptosis. The relationship between ROS and SOD levels observed in nano-copper-treated podocytes indicates that free radicals were generated by exposure to nano-copper. All of the above has shown oxidative-antioxidant stability of nano-copper and cytotoxicity in podocytes [29, 33, 34].

Current results showed that nano-copper MDA levels in podocytes markedly increased, causing peroxidation of lipid cell compositions and confirming that cellular damage induced by nano-copper by oxidative stress. In addition, when previously treated with N- (2-mercaptopropionyl) -glycine (N-MPG), a type of ROS sequestering, nano-copper-induced apoptosis induced podocyte [29]. These results demonstrated that increased oxidative stress was an important mechanism in nano-copper-induced podocyte damage.

Pulmonary toxicity

The NP that is eliminated through the respiratory tract from the mucociliary escalator can be ingested in the gastrointestinal tract [1]. Kim, J.S., *et al.* They recorded that PN Cu caused strong inflammatory reactions that iron oxides (Fe), titanium (Ti), silver (Ag) with improved total cells and neutrophils at the lungs recruitment, as well as increased total activity of proteins and lactate dehydrogenase (LDH) in fluid bronchoalveolar lavage (BAL) [35]. However, cytotoxicity and DNA damage has been demonstrated in the lung epithelial cells of type II A549 all the metal oxide particles analyzed (CuO, TiO_2, ZnO, Fe_2O_3 and Fe_3O_4) at 40 and 80 ug / ml [36]. Both inhalation exposure systems, such as instillation, have recognized limits. Several studies have shown that inhalation was more effective in assessing the inflammatory response, collagen deposition, oxidative stress and fibrosis [37, 38]. The important role of chemokines / cytokines and inflammatory cells for bacterial infections and pulmonary inflammation has been demonstrated in recent studies [39, 40]. Tumor necrosis factor [TNF] -α is an important early cytokine required for neutrophil recruitment. Monocyte chemoattractant protein [MCP] -1 may also amplify the recruitment of neutrophils and macrophages in the lung. These increases are consistent with a high number of macrophages and neutrophils in bronchoalveolar lavage (BAL) and histopathological evaluation of lung tissues of mice exposed to Cu (alveolitis and perivasculitis) [35, 41]. The key functions of neutrophil inflow against bacterial infection in the host defense system are controversial. Contents Recruitment of neutrophils causes a decrease in bacterial clearance, while an excessive flow of neutrophils can cause severe lung inflammation and neutrophil-mediated damage that could cause reduction of lung clearance [42]. Recent data also show that exposure to Cu NP through instillation and inhalation of attenuated bacteria removal of the lung, although there has been strong inflammation [35]. Therefore, exposure to Cu NP may increase the risk of lung infection affecting host defense against bacteria.

Gastrointestinal system

The gastrointestinal tract is also considered as a possible admission portal [1]. There are many ways in which NP can be ingested in the gastrointestinal tract. The absorption of particles of different sizes through the gastrointestinal tract can also end up in various toxic effects [43]. Although NP may contact the respiratory organs, however, other organs such as the gastrointestinal tract should be considered because NP can enter the gastrointestinal tract in many ways and indirectly through the mucosa or directly via the oral route. [44, 45]. There are few reports on the toxicological study of the gastrointestinal tract of nanomaterials. In a study in which only a few mice exhibited spectral symptoms, all mice treated with nano-copper clearly showed symptoms of food channel dysfunction, loss of appetite, diarrhea and vomiting. [1] Nano-copper can be translocated light intestinal tract containing lymphatic intestinal tissue (Peyer's plaques (PP)) and M cells (specialized phagocytosis enterocytes). Furthermore, it can promote phagocytosis in the gastrointestinal mucosa and produce immune responses mediated by antigens [46]. Consistency can be directly linked to the route of exposure and the physico-chemical properties of the nanosustancia (for example, type, size, structure, surface modification, crystalline phase). The oral route is a relatively simple toxicological process compared to the pulmonary routine [7]. Once introduced into the stomach, NP copper will react drastically with hydrogen ions (H +) of gastric juice and can quickly become ionic. This chemical process undoubtedly causes an ionic copper overload in vivo. Because the ultrafine NPs of copper nanoparticles are highly active in the biological system when they are in the stomach. Nano-particles cause the accumulation of copper highly alkaloscentes heavy substances and excessive copper ions concludes with metabolic alkalosis and copper overload [16]. When nano-copper reacts to the acidic substance in the stomach, many proton ions are consumed. Metabolic alkalosis, such as poisoned copper ions, ends with a higher mortality than microcirculation in the same dose [16].

In one study, the appearance of the stomach of mice exposed to nano-copper swelled and presented a cyan color. The result of nano-copper suggests that it may remain in the stomach longer, in other words, the lasting interaction with the acidic juice can cause the persistent production of heavy metal ions in vivo. [7, 9] Nano, micro, and copper ions show different biological characteristics in vivo through routine oral exposure. With regard to nano-copper particles, both metabolic alkalosis and copper overload contribute to its severe toxicity. Unlike this, micro-copper does not stagnate in the stomach and the ionization rate is much lower than that of NPs. After the particles have been pushed into the small intestine, the ionization reaction is prohibited due to the primary condition and finally it is expelled as faeces. For direct ingestion of copper ions, the temporary glomerulonephritis and the alimentary canal disturbance occur in experimental animals. These toxicological responses can be partially corrected within 72 hours [7].

Neurotoxicity, genotoxicity and carcinogenicity

In the first carcinogenicity and genotoxicity studies of copper-soluble copper compounds, such as copper sulfate, they were genotoxic, with functions including induction of chromosomal aberrations and micronuclei Leghorn white chickens and chromosomal aberrations in Swiss mice [47, 48].] It has now been documented that different NPs elicit different responses from different cell lines or biological systems [49]. Copper NPs have been shown to be extremely reactive in a simulated intracorporeal environment [50]. Studies have shown that copper NPs can interact with DNA. This was demonstrated by a study in which NP (4-5 nm) dependent degradation caused dose of isolated DNA molecules to generate singlet oxygen ($1O_2$) in 937 and HeLa [51] cells. NP copper and its compounds caused a variety of effects, including oxidative stress, cytotoxicity, neurotoxicity, DNA damage and DNA lesions in a variety of cell lines [49].

DNA damage has been documented as a result of oxidative stress, controlled by elevated 8-isoprostane levels and the percentage of glutathione disulfide (GSSG) total glutathione in respiratory epithelial cells in the human airway (Hep-2). High oxidative stress can cause damage to DNA, which in turn has the potential to be carcinogenic [26, 49]. In another study on A549 cells, copper oxide NPs were the most potent with respect to cytotoxicity and DNA damage [36]. Copper NPs (<100 nm) were reported to be more toxic to human A549 cells than copper particles and were also shown to induce sensory neuronal toxicity [52]. The size, surface chemistry, surface area, morphology and reactivity of particles in the soluble particle are key factors that must be

clarified to accurately assess the toxicity of NPs. Nano-copper material appears to be toxic not only for DRG neurons but also for glial cells. Recently, the results showed that at 10 and 20 μM, copper NPs did not show significant toxicity in DRG neurons. This could be due to differences in the properties of copper and NP pure copper NPs and could also be due to the nature of cells in different studies and the duration of exposure to NP [52]. A recent report showed dose-dependent toxicity (10-100 μM) in human H4 glial cells exerted by NP of cupric oxide [53]. These NPs of copper oxide within neurons would inhibit mitochondrial dehydrogenases and cause ROS generation. Several studies have demonstrated cytotoxicity resulting from the primary induction of lipid peroxidation of a mitochondrial membrane of a metal that can lead to the breakdown of electron transport, the decoupling of oxidative phosphorylation and decreased mitochondrial membrane potential [54, 55]. Said that the neurodegeneration associated with copper overload in Wilson disease can cause mitochondrial damage, increased ROS production and failure of antioxidant defense mechanisms [56]. Copper can also induce oxidative stress by reducing glutathione levels in neurons. Copper NPs enter the cell, so they can attack mitochondria and cause an increase in oxidative stress [57]. Prabhu, B. M., *et al.* have shown that exposure to copper NP has led to significant toxicity for DRG neurons grown at concentrations of 40-100 μM but not at 10-20 μM. However, exposure to NP of copper size 40 nm, 60 nm and 80 nm had toxic results in DRG neurons, 40 nm copper NP and 60 nm size had a higher toxic result of 80 nm particles nm. Therefore, the toxic effect seems to depend on concentration and size. The mechanism that correlates the toxicological effects followed with the exposure of DRG neurons to copper NPs may be oxidative stress [52]. Unresolved inflammation can cause aberrations and DNA abnormalities that may be mutagenic. Yang *et al.* Wistar male rats used to study the mechanisms of hepatotoxicity induced by copper NP with the identification of hepatic gene expression profiles that were phenotypically correlated with conventional toxicological outcomes [58].

Copper NPs have also been shown to be neuropathological and neurotoxic. However, apart from the induction of other types of pathology, none of these studies reported carcinogenesis. In general, changes in apoptosis, gene expression, oxidative stress and persistent inflammation were

the main effects of copper-based NPs that may predispose to carcinogenicity.

Blood biochemical indexes

Pathological examinations and morphological changes indicate that the kidney and liver are two important target organs for copper NP through the oral route. Therefore, the biochemical parameters of blood (BUN, Cr, TBA and ALP) that reflect renal and hepatic function are more important. In one study of all mice exposed to NP, these four biochemical indices were significantly higher than the control. The anomaly of BUN and Cr is particularly obvious [1]. Increased triglycerides in serum, liver and renal tissues could be considered an important sensitive index reflecting lipidosis caused by nano-copper. To date, it is unclear whether nano-copper can enter the bloodstream through the entire gastrointestinal lining [27]. The increase in the pH of the blood causes a compensation: (a) the respiratory compensation is naturally caused by several minutes. However, respiratory compensation is limited, as high $PaCO_2$ and low PaO_2 should stimulate the apneustic center to prevent hypoxia. Therefore, $PaCO_2$ was only partially improved 24 hours after exposure; (b) in theory, renal clearance begins relatively late, but can be maintained for a long time [7, 59, 60].

Unlike micro-copper, nano-copper could cause a high level of serum copper (SC), an index of acute toxicosis. More importantly, nano-copper has a low elimination frequency in vivo, which can worsen heavy metal toxicosis. It maintains a high level of SC in the nano group even at 72 hours, suggesting that mice carry high persistent copper concentrations in the blood, possibly eventually ending up in a fatal copper overload [7, 61].

Nano-copper and apoptosis

Bcl-2 proteins are regulatory upstream of the mitochondrial membrane. From immunoblotting, it was observed that pro apoptotic Bax regulated protein increased nano-copper and anti-apoptotic Bcl-2 protein reduce mitochondrial membrane potential. Apoptotic cell death causes oxidative stress directly related to mitochondrial dysfunction. Alteration of mitochondrial membrane potential, cytochrome c release in the cytosol and possibly activation of caspase 3 are biomarkers related to cell death induced by oxidative stress via dependent mitochondrial pathway. The influence of Bcl-2 family proteins in mitochondria regulates mitochondrial
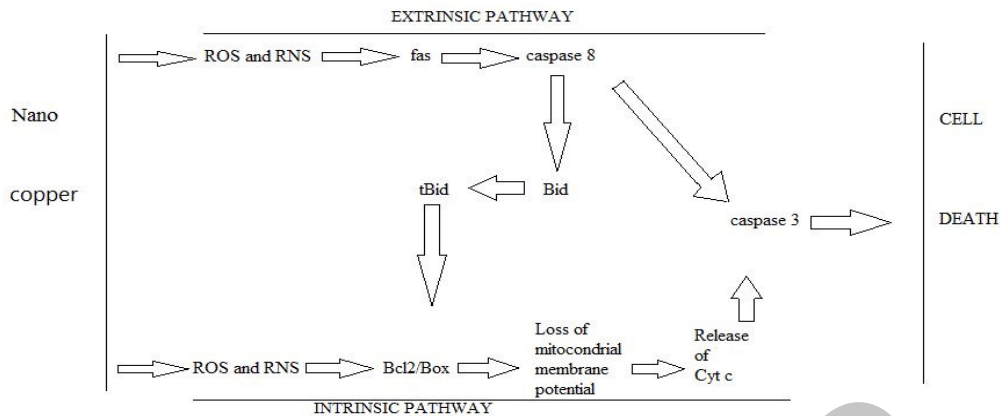
6

Fig. 1. Schematic diagram of the nano-copper induced apoptosis mechanism.

dependent cell death [62]. Immunoblot analysis showed that nano-copper poisoning leads to high levels of cytosolic cytochrome c, Apaf 1 caspase 9 and caspase 3 cleaved. New research has shown that nano-copper exposure significantly increases the cellular level of Fas (protein), caspase 8 (protein) and tBid (protein) [18].

The increase in the ROS level induces a cascade of pathways that in turn activate the transcription of several genes; those genes change the regulatory pathways of cell survival and eventually lead to apoptosis. Apoptosis could be mediated by dependent and independent mitochondrial pathways. Tests suggest that the variation of mitochondrial membrane potential can alter the cells involved in apoptotic death through cascades sensitive to oxidative stress signaling via mitochondrial dependent [63].

When decreasing the expression of Bcl-2 proteins and the Bax protein expression is improved, there will be a decrease in mitochondrial membrane potential due to rupture of the mitochondrial membrane (Fig. 1). This phenomenon helps to release cytochrome c from the mitochondria to the cytosol. When this happens, a cascade reaction involves the binding of cytochrome c with Apaf1, with consequent activation of the active caspase-9, creating an apoptosomal complex that triggers the activation of caspase-3 [18]. The extrinsic pathway is triggered by a committed death receptor (Fas, TNF, etc.), which initiates a signaling cascade mediated by the activation of caspase-8.

CONCLUSIONS

The rapid growth of nanotechnology suggests that it will soon find widespread application in everyday consumer products and in new pharmaceutical, electronic and other industries. When copper intake exceeds the tolerable limit, it exerts toxic effects that lead to cell death. The LD50 values of nano-copper (23.5 nm) are 413 mg / kg of body weight, respectively; it is considered moderately toxic material. The increase in the production of reactive oxygen species (ROS) and reactive nitrogen species (RNS) plays an important role in copper-induced organic dysfunction. All NP copper concentrations cause toxicity and alterations in the histopathology of the liver and pulmonary tissues of rats. The increase in oxidative stress is a fundamental mechanism in the damage of podocytes caused by nano-copper. Nano-copper can pass from the lumen of the intestinal tract through aggregations of intestinal lymphatic tissue (Peyer patches (PP)) containing M cells. When they are introduced into the stomach, the nano-copper particles react dramatically with hydrogen ions (H +) of the gastric juice and can quickly become ionic. The liver and kidneys are two target organs for exposure to NP of copper via the oral route. Compared to micro-copper, nano-copper could obviously induce more levels of serum copper (SC), a marker of acute toxicosis. New research has shown that nano-copper exposure significantly increases the cellular level of Fas (protein), caspase 8 (protein) and tBid (protein).

It will be important to continue the interpretation of laboratory data in the clinical context of the patient to use molecular and emerging technologies. At the same time, a growing understanding of the mechanisms that drive the toxicity of this NP will improve the classification, prognosis and treatment of patients with NP copper toxicity.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest regarding the publication of this manuscript.

REFERENCES

1. Chen Z, Meng H, Xing G, Chen C, Zhao Y, Jia G, et al. Acute toxicological effects of copper nanoparticles in vivo. Toxicology Letters. 2006;163(2):109-20.
2. Colvin VL. The potential environmental impact of engineered nanomaterials. Nature Biotechnology. 2003;21(10):1166-70.
3. Roco MC. Nanotechnology: convergence with modern biology and medicine. Current Opinion in Biotechnology. 2003;14(3):337-46.
4. Shaw BJ, Handy RD. Physiological effects of nanoparticles on fish: A comparison of nanometals versus metal ions. Environment International. 2011;37(6):1083-97.
5. G. Georgopoulos AR, MJ Yonone-Lioy, RE Opiekun, PJ Lioy, P. Environmental copper: its dynamics and human exposure issues. Journal of Toxicology and Environmental Health Part B: Critical Reviews, 2001;4 (4):341-394.
6. Liu G, Li X, Qin B, Xing D, Guo Y, Fan R. Investigation of the Mending Effect and Mechanism of Copper Nano-Particles on a Tribologically Stressed Surface. Tribology Letters. 2004;17(4):961-6.
7. Meng H, Chen Z, Xing G, Yuan H, Chen C, Zhao F, et al. Ultrahigh reactivity provokes nanotoxicity: Explanation of oral toxicity of nano-copper particles. Toxicology Letters. 2007;175(1-3):102-10.
8. Zietz B, Dieter H, Lakomek M, Schneider H, Keslergaedtke B, Dunkelberg H. Epidemiological investigation on chronic copper toxicity to children exposed via the public drinking water supply. The Science of The Total Environment. 2003;302(1-3):127-44.
9. Bertinato J, L'Abbé MR. Maintaining copper homeostasis: regulation of copper-trafficking proteins in response to copper deficiency or overload. The Journal of Nutritional Biochemistry. 2004;15(6):316-22.
10. Dautremepuits C, Betoulle S, Paris-Palacios S, Vernet G. Immunology-Related Perturbations Induced by Copper and Chitosan in Carp (Cyprinus carpio L.). Archives of Environmental Contamination and Toxicology. 2004;47(3).
11. Hoseini SM, Hosseini SA, Soudagar M. Dietary tryptophan changes serum stress markers, enzyme activity, and ions concentration of wild common carp Cyprinus carpio exposed to ambient copper. Fish Physiology and Biochemistry. 2012;38(5):1419-26.
12. Pettibone JM, Adamcakova-Dodd A, Thorne PS, O'Shaughnessy PT, Weydert JA, Grassian VH. Inflammatory response of mice following inhalation exposure to iron and copper nanoparticles. Nanotoxicology. 2008;2(4):189-204.
13. Cioffi N, Ditaranto N, Torsi L, Picca R, Sabbatini L, Valentini A, Novello L, Tantillo G, Bleve-Zacheo T, Zambonin P. Analytical characterization of bioactive fluoropolymer ultra-thin coatings modified by copper nanoparticles. Analytical and bioanalytical chemistry, 2005;381 (3):607-616.
14. Tao TY, Liu F, Klomp L, Wijmenga C, Gitlin JD. The Copper Toxicosis Gene Product Murr1 Directly Interacts with the Wilson Disease Protein. Journal of Biological Chemistry. 2003;278(43):41593-6.
15. Hedayati A, Hoseini SM, Hoseinifar SH. Response of plasma copper, ceruloplasmin, iron and ions in carp, Cyprinus carpio to waterborne copper ion and nanoparticle exposure. Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology. 2016;179:87-93.
16. Meng H, Chen Z, Xing G, Yuan H, Chen C, Zhao F, et al. Ultrahigh reactivity provokes nanotoxicity: Explanation of oral toxicity of nano-copper particles. Toxicology Letters. 2007;175(1-3):102-10.
17. Chaves SB, Lacava LM, Lacava ZGM, Silva O, Pelegrini F, Buske N, et al. Light microscopy and magnetic resonance characterization of a DMSA-coated magnetic fluid in mice. IEEE Transactions on Magnetics. 2002;38(5):3231-3.
18. Sarkar A, Das J, Manna P, Sil PC. Nano-copper induces oxidative stress and apoptosis in kidney via both extrinsic and intrinsic pathways. Toxicology. 2011;290(2-3):208-17.
19. Cai S, Batra S, Lira SA, Kolls JK, Jeyaseelan S. CXCL1 Regulates Pulmonary Host Defense to Klebsiella Infection via CXCL2, CXCL5, NF-B, and MAPKs. The Journal of Immunology. 2010;185(10):6214-25.
20. Li Y, Karlin A, Loike JD, Silverstein SC. A critical concentration of neutrophils is required for effective bacterial killing in suspension. Proceedings of the National Academy of Sciences. 2002;99(12):8289-94.
21. Karlsson HL, Gustafsson J, Cronholm P, Möller L. Size-dependent toxicity of metal oxide particles—A comparison between nano- and micrometer size. Toxicology Letters. 2009;188(2):112-8.
22. GLUSCHENKO N, Olkhovskaya I, Pleteneva T, Fatkullina L, Ershov YA, Fedorov YI. Biological Action of Fine Metal Powders. Izvestiya Akademii Nauk Sssr Seriya Biologicheskaya, 1989(3):415-421.
23. Wang B, Feng W, Wang M, Wang T, Gu Y, Zhu M, Ouyang H, Shi J, Zhang F, Zhao Y. Acute toxicological impact of nano- and submicro-scaled zinc oxide powder on healthy adult mice. Journal of Nanoparticle Research, 2008;10 (2):263-276.
24. Doudi M, Setorki M. Acute effect of nano-copper on liver tissue and function in rat. Nanomedicine Journal, 2014;1 (5).
25. Sizova E, Miroshnikov S, Poliakova V, Lebedev S, Glushchenko N. Copper nanoparticles as modulators of apoptosis and structural changes in some organs. Morfologiia (Saint Petersburg, Russia), 2012;144 (4):47-52.
26. Fahmy B, Cormier SA. Copper oxide nanoparticles induce oxidative stress and cytotoxicity in airway epithelial cells. Toxicology in Vitro. 2009;23(7):1365-71.
27. Lei R, Wu C, Yang B, Ma H, Shi C, Wang Q, et al. Integrated metabolomic analysis of the nano-sized copper particle-induced hepatotoxicity and nephrotoxicity in rats: A rapid in vivo screening method for nanotoxicity. Toxicology and Applied Pharmacology. 2008;232(2):292-301.
28. Wolf G, Chen S, Ziyadeh FN. From the Periphery of the Glomerular Capillary Wall Toward the Center of Disease: Podocyte Injury Comes of Age in Diabetic Nephropathy. Diabetes. 2005;54(6):1626-34.
29. Xu P, Xu J, Liu S, Yang Z. Nano copper induced apoptosis in podocytes via increasing oxidative stress. Journal of Hazardous Materials. 2012;241-242:279-86.
30. Shibata S, Nagase M, Yoshida S, Kawachi H, Fujita T. Podocyte as the Target for Aldosterone: Roles of Oxidative

Stress and Sgk1. Hypertension. 2007;49(2):355-64.

31. Martindale JL, Holbrook NJ. Cellular response to oxidative stress: Signaling for suicide and survival. Journal of Cellular Physiology. 2002;192(1):1-15.

32. Deres P, Halmosi R, Toth A, Kovacs K, Palfi A, Habon T, et al. Prevention of Doxorubicin-Induced Acute Cardiotoxicity by an Experimental Antioxidant Compound. Journal of Cardiovascular Pharmacology. 2005;45(1):36-43.

33. Rothstein JD, Bristol LA, Hosler B, Brown RH, Kuncl RW. Chronic inhibition of superoxide dismutase produces apoptotic death of spinal neurons. Proceedings of the National Academy of Sciences. 1994;91(10):4155-9.

34. Patel M. Inhibition of neuronal apoptosis by a metalloporphyrin superoxide dismutase mimic. Journal of neurochemistry, 1998;71 (3):1068-1074.

35. Kim JS, Adamcakova-Dodd A, O'Shaughnessy PT, Grassian VH, Thorne PS. Effects of copper nanoparticle exposure on host defense in a murine pulmonary infection model. Particle and Fibre Toxicology. 2011;8(1):29.

36. Karlsson HL, Cronholm P, Gustafsson J, Möller L. Copper Oxide Nanoparticles Are Highly Toxic: A Comparison between Metal Oxide Nanoparticles and Carbon Nanotubes. Chemical Research in Toxicology. 2008;21(9):1726-32.

37. Grassian VH, Adamcakova-Dodd A, Pettibone JM, O'Shaughnessy PI, Thorne PS. Inflammatory response of mice to manufactured titanium dioxide nanoparticles: Comparison of size effects through different exposure routes. Nanotoxicology. 2007;1(3):211-26.

38. Shvedova AA, Fabisiak JP, Kisin ER, Murray AR, Roberts JR, Tyurina YY, et al. Sequential Exposure to Carbon Nanotubes and Bacteria Enhances Pulmonary Inflammation and Infectivity. American Journal of Respiratory Cell and Molecular Biology. 2008;38(5):579-90.

39. Marriott HM, Jackson LE, Wilkinson TS, Simpson AJ, Mitchell TJ, Buttle DJ, et al. Reactive Oxygen Species Regulate Neutrophil Recruitment and Survival in Pneumococcal Pneumonia. American Journal of Respiratory and Critical Care Medicine. 2008;177(8):887-95.

40. Yazdi AS, Guarda G, Riteau N, Drexler SK, Tardivel A, Couillin I, et al. Nanoparticles activate the NLR pyrin domain containing 3 (Nlrp3) inflammasome and cause pulmonary inflammation through release of IL-1 and IL-1 Proceedings of the National Academy of Sciences. 2010;107(45):19449-54.

41. Vanoirbeek JAJ, Rinaldi M, De Vooght V, Haenen S, Bobic S, Gayan-Ramirez G, et al. Noninvasive and Invasive Pulmonary Function in Mouse Models of Obstructive and Restrictive Respiratory Diseases. American Journal of Respiratory Cell and Molecular Biology. 2010;42(1):96-104.

42. Craig A, Mai J, Cai S, Jeyaseelan S. Neutrophil recruitment to the lungs during bacterial pneumonia. Infection and immunity, 2009;77 (2):568-575.

43. Böckmann J, Lahl H, Eckert T, Unterhalt B. Titan-Blutspiegel vor und nach Belastungsversuchen mit Titandioxid. Pharmazie, 2000;55 (2):140-143.

44. Oberdörster G, Oberdörster E, Oberdörster J. Nanotoxicology: an emerging discipline evolving from studies of ultrafine particles. Environ Health Perspect 113: 823–839. 2005.

45. Hoet PH, Brüske-Hohlfeld I, Salata OV. Nanoparticles–known and unknown health risks. Journal of

nanobiotechnology, 2004;2 (1):12.

46. Lomer MCE, Thompson RPH, Powell JJ. Fine and ultrafine particles of the diet: influence on the mucosal immune response and association with Crohn's disease. Proceedings of the Nutrition Society. 2002;61(01):123-30.

47. Bhunya SP, Jena GB. Clastogenic effects of copper sulphate in chick in vivo test system. Mutation Research/Genetic Toxicology. 1996;367(2):57-63.

48. Agarwal K, Sharma A, Talukder G. Clastogenic effects of copper sulphate on the bone marrow chromosomes of mice in vivo. Mutation Research Letters. 1990;243(1):1-6.

49. Magaye R, Zhao J, Bowman L, Ding MIN. Genotoxicity and carcinogenicity of cobalt-, nickel- and copper-based nanoparticles. Experimental and Therapeutic Medicine. 2012;4(4):551-61.

50. Meng H, Chen Z, Xing G, Yuan H, Chen C, Zhao F, et al. Ultrahigh reactivity and grave nanotoxicity of copper nanoparticles. Journal of Radioanalytical and Nuclear Chemistry. 2007;272(3):595-8.

51. Jose GP, Santra S, Mandal SK, Sengupta TK. Singlet oxygen mediated DNA degradation by copper nanoparticles: potential towards cytotoxic effect on cancer cells. Journal of Nanobiotechnology. 2011;9(1):9.

52. Prabhu BM, Ali SF, Murdock RC, Hussain SM, Srivatsan M. Copper nanoparticles exert size and concentration dependent toxicity on somatosensory neurons of rat. Nanotoxicology, 2010;4 (2):150-160.

53. Li F, Zhou X, Zhu J, Ma J, Huang X, Wong STC. High content image analysis for human H4 neuroglioma cells exposed to CuO nanoparticles. BMC Biotechnology. 2007;7(1):66.

54. Mattie MD, Freedman JH. Protective Effects of Aspirin and Vitamin E (α-Tocopherol) against Copper- and Cadmium-Induced Toxicity. Biochemical and Biophysical Research Communications. 2001;285(4):921-5.

55. Saris N-EL, Skulskii IA, Filardo G, Tiitta M, Sjöström M, Wold S, et al. Interaction of Cu+ with Mitochondria. Acta Chemica Scandinavica. 1991;45:1042-6.

56. Gaetke L. Copper toxicity, oxidative stress, and antioxidant nutrients. Toxicology. 2003;189(1-2):147-63.

57. Hultberg B. Copper ions differ from other thiol reactive metal ions in their effects on the concentration and redox status of thiols in HeLa cell cultures. Toxicology. 1997;117(2-3):89-97.

58. Yang B, Wang Q, Lei R, Wu C, Shi C, Wang Q, et al. Systems Toxicology Used in Nanotoxicology: Mechanistic Insights into the Hepatotoxicity of Nano-Copper Particles from Toxicogenomics. Journal of Nanoscience and Nanotechnology. 2010;10(12):8527-37.

59. Galla JH. Metabolic alkalosis. Journal of the American Society of Nephrology, 2000;11 (2):369-375.

60. Williams AJ. ABC of oxygen: Assessing and interpreting arterial blood gases and acid-base balance. BMJ. 1998;317(7167):1213-6.

61. Bremner I. Manifestations of copper excess. The American Journal of Clinical Nutrition. 1998;67(5):1069S-73S.

62. Verzola D, Bertolotto MB, Villaggio B, Ottonello L, Dallegri F, Frumento G, et al. Taurine Prevents Apoptosis Induced by High Ambient Glucose in Human Tubule Renal Cells. Journal of Investigative Medicine. 2002;50(6):443-51.

63. Keeble JA, Gilmore AP. Apoptosis commitment – translating survival signals into decisions on mitochondria. Cell Research. 2007;17(12):976-84.