Letter to the Editor

The Mechanism of Acute Lung Injury Induced by Nickel Carbonyl in Rats*

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Nickel carbonyl is a highly toxic metal compound produced from the reaction that occurs between nickel and carbon monoxide under pressure. As previously reported, nickel carbonyl can cause acute aspiration pneumonia, and animal experiments showed it was toxic to animal lung, liver, brain, and other vital organs[1]. However, few studies have investigated nickel carbonyl poisoning in humans[2]. Current research has mainly focused on the carcinogenicity of nickel carbonyl and therapy by nickel carbonyl inhibition at the cytochrome level[3]. Nevertheless, there is still a lack of studies on the mechanism of nickel carbonyl poisoning and its toxic effects on gene expression and gene regulation.

Several reports showed that pro-apoptotic toxicants increase the concentration of Ca^{2+}, which results in the fragmentation of mitochondrial membranes. When the total anti-oxidation capability (T-AOC) decreases, the antioxidant capacity is reduced. Cell metabolism is decreased because of increased malonaldehyde (MDA) resulting in cellular dysfunction and potential cell death. The anti-superoxide anion free radical (ASAFR) describes a cellular anti-stress mechanism in response to oxidative damage[4]. In this study, we attempted to determine oxidative stress and damage in rats due to exposure to acute nickel carbonyl poisoning by investigating T-AOC, ASAFR, and MDA in lung tissue homogenates of rats. After DNA damage, Chk1 is activated and functions as a kinase by phosphorylating Cdc25 family members and other downstream substrates[1]. To explore the mechanisms of damage and apoptosis due to acute nickel carbonyl poisoning, Chk1 and Cdc2 expression were measured to study the cell cycle from the G2 phase to the checkpoint of M phase and to determine DNA damage of target cells.

We also determined dose-response and time-response of Chk1, Cdc2 expression and the nickel carbonyl acute poisoning starting at cellular and molecular injury, which may have important significance for the treatment of acute nickel carbonyl poisoning.

Eight-week-old Sprague-Dawley rats (weight 180-200 g, *n*=200, 100 male and 100 female) were maintained in specific pathogen free conditions and were randomly divided in a class 10 000 clean-level animal room, and fed with specific feed. The experiments were approved by the Ethics Committees of Lanzhou University. Attempts were made to minimize animal suffering. Nickel carbonyl dosage was fixed at three different doses by 5% median lethal dose (LC_{50}), 33.8% LC_{50} and 62.5% LC_{50} (LC_{50} was 400 mg/m^3 body surface area)[5]. The rats were divided into five groups as follows: three nickel carbonyl groups, A, B and C receiving 5%, 33.8%, and 62.5% LC_{50}, and group D receiving 250 mg Cl_2/m^3 body surface. Rats were placed in a dynamic inhalation chamber and gaseous nickel carbonyl was pumped into the interior of the cabinet for 30 min. Rats were anesthetized with Enflurane for 5 to 15 s and euthanized. The abdominal aorta was exposed immediately by Caesarean section and lung tissue was excised. Lung tissue was removed from each group of rats at 48 h, 72 h, and 7 d after nickel carbonyl exposure and 2-3 pieces were cut and immersed in Glutaraldehyde solution (2.5%) for conventional electron microscope studies (EMS), and sliced by Ultrathin microtome, fixed, dehydrated, and observed with transmission electron microscopy (TEM) after staining with Pbac (lead acetate). T-AOC, MDA, ASAFR, and Ca^{2+}-Mg^{2+}-ATPase activity were measured by enzyme-linked immunosorbent assay (ELISA) in accordance with the manufacturer's instructions. All kits were obtained from the Nanjing Jiancheng Bioengineering Institute.

Analysis of cell cycle distribution of the lung tissue and the content and ratio of G_0/G_1, S, G_2/M

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phase was performed by flow cytometry (FCM). The expression of Chk1 and Cdc2 in the lung tissue of rats was detected by quantification of density scanning with gel imaging analysis system (Tanon GIS-2010) by measuring the area and density.

All the data were statistically evaluated with SPSS/13.0 software. Hypothesis testing methods included one-way analysis of variance (ANOVA) and Spearman rank correlation analysis. Values of $P<0.05$ were considered statistically significant. All the results were expressed as mean±standard deviation (SD).

The group of lower dosage shows the following phenomena: karyopyknosis appeared in most of type II pneumocyte, the mitochondria was swelled, ridges decreased and dissolved, the lamellar body was emptied; The group of medium dosage shows that: the lamellar body of type II pneumocyte was emptied, the cytoplasm vacuoles increased, macrophage in alveolar spaces increased; The group of heavier dosage shows that the phenomena same as the medium group, the organelle swelled and the collagen fiber in the alveolar mediastinum increased; The group exposed to Cl_2 shows that: the mitochondria of of type II pneumocyte was swelled, the part becamed vacuoles, alveolar wall thickening was not appeared, but the collagen fiber in the alveolar mediastinum increased, showed in Figure 1.

Comparing with the control group, a significant decrease was observed in group A, B, C of exposed to nickel carbonyl at 1 d, 2 d, and 3 d, was observed in group D at 2 d and 3 d; And the statistical difference was observed in group B at 1 d and 7 d comparing with the group D. There was significant difference about the multiple comparisons among the activity of first three groups and the group C is lower than group A and B. Furthermore, an obvious difference was observed in the group C at all stages.

The statistical analysis shows that: the T-AOC was decreased after exposed in nickel carbonyl with different dosages, and no significant difference of the T-AOC among the different groups was observed ($P>0.05$).

The variation of MDA in the lung tissue at various stages: Table 1 shows the obvious decrease of MDA in the group B, and an obvious significant difference was observed between it and Cl_2 control and 20 mg/m^3 nickel carbonyl ($P<0.01$); between normal control and Cl_2 control, 20 mg/m^3 nickel carbonyl ($P<0.01$), too.

Table 2 shows the cell cycle distribution of the lung tissue was detected by FCM. A significant increase in S phase, a decrease in G_0/G_1 phase and G_2/M phase were observed in both nickel carbonyl exposure groups and Cl_2 exposure group.

The correlation between the express area of mRNA about Chk1, Cdc2 and the dosage was observed, it shows that a dose-effect relationship. The general regression equation of Chk1 is $Y=1185.788+2.779X$ ($r^2=0.64$; $F=17.829$, $P=0.002$), and the genaral regression equation of Cdc2 is $Y=1112.168+3.386X$ ($r^2=0.765$; $F=32.610$, $P=0.000$), both the statistical significances were observed of the line correlation of 1 d, 3 d, 7 d and the mean ($P<0.05$), the satisfied imitative effect judged according to the correlation.

Nickel carbonyl is a multi-organ toxic nickel compound that induces strong acute toxicity, which can impact a variety of important organs, leading to a variety of toxic effects. Acute lung injury is the most prominent injury in clinical cases of poisoning[1]. Nickel ions can also induce oxidative stress to produce superoxide anion radicals, leading to tissue damage in the body, and cell mutation that may lead to the formation of cancerous tumors[6].

Table 1. MDA Levels in Lung Tissue of Rats in Different Experimental Groups at Various Days after Exposure

Experimental Groups	Days after Exposure			
	1 d	2 d	3 d	7 d
A	59.86±2.78	72.23±8.81	72.98±6.57	70.848±8.62
B	55.07±3.22[a]	53.77±9.68[a]	44.18±3.75[a]	57.59±1.43[a]
C	69.90±8.91	63.71±5.00	50.80±3.99	57.60±6.39
D	62.28±2.32	59.01±5.74	56.20±9.49	74.27±5.38
E	63.10±7.33[b]	63.10±7.33[b]	63.10±7.33[b]	63.10±7.33[b]

Note. Values are given as means±SD for all groups. [a]$P<0.01$: group B *vs* group A and Cl_2 exposed group; [b]$P<0.01$: normal control *vs* group A and Cl_2 exposed group.

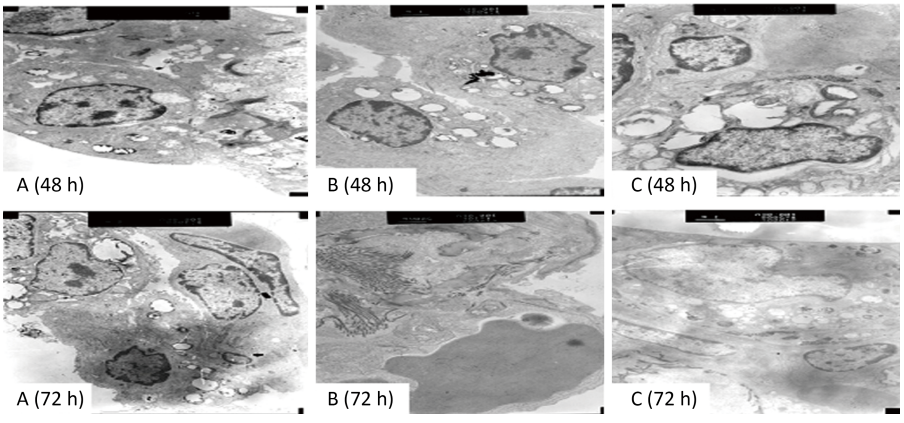

Figure 1. The ultrastructure of rats after exposure to nickel carbonyl viewed by TEM (magnification, ×6000). *Note.* Rats were exposed to nickel carbonyl at a dose of (A) 20, (B) 135, or (C) 250 mg/m^3. Figures demonstrate morphology from rats after 48 or 72 h of nickel carbonyl exposure.

Table 2. The Constitution Ratio of G_0/G_1 Phase, G_2/M Phase, and S Phase of Rats in Different Experimental Groups (%)

Experimental Groups	The Cell Cycle		
	G_0/G_1 Phase	G_2/M Phase	S Phase
A	61.0	13.5	25.5
B	92.9	5.2	1.9
C	79.8	5.1	15.1
D	73.5	3.3	23.2
E	85.9	7.9	6.2
x^2	461.40	508.28	43.76
P	0.000[a]	0.000[a]	0.000[a]

Note. [a]*P*<0.01:normal control *vs* nickel carbonyl and Cl_2 exposed groups.

Figure 1 shows that inhalation of different concentrations of nickel carbonyl induced damage of type II alveolar epithelial cells in rat lung tissue. Furthermore, pulmonary pathologic damage was aggravated with increasing doses of nickel carbonyl exposure, reflecting a dose-effect relationship. Willner et al. demonstrated that damage such as poisoning radiation could lead to the damage and dysfunction of type II alveolar epithelial cells[7]. Alveolar hypoxia can stimulate hyperplasia of fibroblasts and collagen, leading to the fibrosis of alveolar septa. Therefore, by adopting preventive treatment for early endothelial cell damage and blood circulation disorders, the development of pulmonary fibrosis can be prevented.

Compared with the control group, a significant decrease (*P*<0.05) in the activity of Ca^{2+}-Mg^{2+} -ATPase enzymes in lung tissue was observed in experimental groups exposed to nickel carbonyl on days 1, 2, and 3. Rat pulmonary alveoli were expanded because of the close relationship between the mitochondria[8], endoplasmic reticulum and cytosolic free Ca^{2+} concentrations, thus implying that as the intracellular Ca^{2+} concentration had changed, the Ca^{2+}-Mg^{2+}-ATPase enzyme activity was decreased.

O^-_2 is generated by single electron reduction in the cell allowing the plasma membrane system to induce lipid peroxidation, causing oxidative damage to carbohydrates, proteins, nucleic acids and lipids. In this study, statistical analysis demonstrated that the T-AOC was decreased after exposure to nickel carbonyl at different dosages, but that no significant difference of T-AOC was observed among the different groups (*P*>0.05). We observed that nickel carbonyl produced oxidative stress immediately upon entering rat lungs, causing the induction of anti-superoxide anion substances *in vivo*. The greatest increase of ASAFR occurred with a dose of 250 mg/m^3 nickel carbonyl, suggesting a dose-response between oxidative damage in lung tissue and nickel carbonyl. Thus, after nickel carbonyl enters the body it can cause serious organ oxidative stress in a short period, which can then further trigger oxidative damage of organs.

MDA is the end product of peroxidation where free-radicals act on lipids. Table 1 shows increased levels of MDA in lung tissues from group A, whilst a lower trend was observed in the high dose group. This may be related to the toxicity of nickel carbonyl itself. The exposure dose in group C caused pulmonary tissue edema and serious damage to the lung cells, suggesting that in the medium and high dose groups, the peroxidation of pulmonary tissue lipid decreases instead of rising.

Table 2 shows the cell cycle distribution of rat lung tissue measured by FCM. A significant increase in S phase and a decrease in G_0/G_1 phase and G_2/M phase were observed in both nickel carbonyl exposure groups and the Cl_2 exposure group. The amplitude of the changes with the cell cycle distribution of group D is higher than for group C.

Chk1 is expressed in both the cytoplasm and nucleus, but mainly plays a role in the nucleus. Chk1 mRNA and protein are mainly expressed in the S phase and G_2 phase of the cell cycle[9]. There was a significant correlation between the gene expression amounts of Chk1 and Cdc2 in the lung tissues of rats and the nickel carbonyl acute poisoning dose (P<0.05). This suggests that Chk1 and Cdc2 gene expression is important in damage repair and cycle regulation during nickel carbonyl acute poisoning. For instance, the Chk1 gene may be involved in the regulation of cell cycle S phase, and after exposure poisoning, increased Chk1 mRNA expression may cause S phase to be blocked, consistent with previous findings. Increased expression of the Chk1 gene may lead to increased phosphorylation of Cdc25A protein, leading to cell cycle arrest and to DNA damage repair[10].

To conclude, nickel carbonyl caused acute exudation and proliferative changes in lung tissue of rats, free radical-induced damage, disorder of the cell cycle, and altered expression of Chk1 and Cdc2, which confirm acute toxicity of nickel carbonyl in the lungs of rats and suggest a possible mechanism of acute nickel carbonyl poisoning. Nickel carbonyl causes strong oxidization that may disturb the metabolism of free-radicals. However, the product of lipid peroxidation, MDA, causes DNA damage and oxidative hyperirritability. Once damage is severe, it may cause cell senescence, mutations and even apoptosis. Finally, the body develops a range of symptoms induced by acute nickel carbonyl poisoning. Therefore, oxidative damage caused by disturbances in free radical metabolism plays an important role in the mechanism of injury in acute toxicity of nickel carbonyl in rats.

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