Tumor Necrosis Factor-Alpha Release from Rat Pulmonary Leukocytes Exposed to Ultrafine Cobalt: *in vivo* and *in vitro* Studies

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Abstract

Ultrafine cobalt (Uf-Co), one of the new category of ultrafine particles, is generated in some industrial situations and it also exists in environmental particles. The aim of this study was to investigate the ability of rat pulmonary leukocytes to release tumor necrosis factor alpha (TNF-alpha) after exposure to Uf-Co *in vivo* and *in vitro*. Rats were intratracheally instilled with 1 mg of Uf-Co, and then wet lung weight and bronchoalveolar lavage fluid (BALF) profile were analysed 1, 3, 7, 15, and 30 days later. The effects of Uf-Co on indices that can be presumed to reflect epithelial injury and permeability (lactate dehydrogenase (LDH) and total protein (TP)) were increased throughout the 30 day post-exposure period. Furthermore, at 3 days after exposure, leukocytes were collected by bronchoalveolar lavage (BAL). After 3, 6, 12, 24, 48, and 72 hours of incubation, TNF-alpha in supernatants were determined by ELISA method. The results showed that TNF-alpha secretion by activated leukocytes from rats instilled with Uf-Co was significantly higher than that of the controls. BAL leukocytes from the lung of exposed rats revealed time- and dose-related increases in TNF-alpha release. In conclusion, our results reveal, for the first time to our knowledge, that exposure to Uf-Co can stimulate leukocytes to secrete TNF-alpha. These data suggest that the TNF-alpha release from pulmonary leukocytes probably plays a role in the pathogenesis of "cobalt lung".

Key words: ultrafine cobalt, bronchoalveolar lavage, leukocyte, TNF-alpha, lung toxicity

Introduction

Occupational exposure to hard metal including cobalt is known to cause hard metal pneumoconiosis as well as asthma¹⁻³. Inhalation of cobalt as metal or oxide has produced fibrotic and allergic effects in animals⁴⁻⁶. Ultrafine metallic cobalt with a mean diameter of 20 nm is a new category of ultrafine particles, made by the process of vacuum vapor deposition. The characteristics of Uf-Co were described in detail previously⁷⁻⁸. Previous studies have revealed that ultrafine particles, e.g., ultrafine titanium dioxide (Uf-TiO₂) with a diameter of 20 nm, can induce a pulmonary inflammatory reaction of a greater magnitude than 250 nm TiO₂ at the same deposited mass⁹⁻¹³. Our studies also revealed that ultrafine nickel and ultrafine titanium dioxide (Uf-TiO₂) had more toxicity than standard nickel and titanium dioxide¹⁴⁻¹⁵. Furthermore, Kyono et al.⁷ showed that inhalation of Uf-Co induces macrophage damage, intracellular edema of the type I alveolar epithelium, interstitial edema, and proliferation of the type II alveolar epithelium. To date, however, there have been

no reports on the ability of pulmonary leukocytes to release pro-inflammatory mediators such as TNF-alpha after exposure to Uf-Co. The purpose of the present study was to study the TNF-alpha release from pulmonary leukocytes after *in vitro* and *in vivo* exposure to Uf-Co. The data obtained from this study have potential benefit for understanding the mechanism of lung disease development after exposure to Uf-Co.

Materials and Methods

Experimental animal

Wistar male rats (specific pathogen-free), weighing 180-200 g and 7-8 weeks of age were supplied by Clear Japan, INC (JCL). Rats were housed in the animal center, Fukui Medical University, Japan. They were fed a conventional laboratory diet and had free access to food and tap water.

Particles

Cobalt particles with a diameter of 20 nm on average, called ultra fine cobalt (Uf-Co) (Ultra fine powder, Lot No. 2237, INABTA and Co., LTD. Vacuum Metallurgical Co., LTD., Japan). The size distribution of Uf-Co was detailed previously⁸. The particles were suspended in physiological saline to a concentration of 1mg/ml. The particle suspensions were ultrasonicated for about 30 minutes and then sterilised at

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0.5kgf/cm² and 120°C temperature for 15 minutes.

Treatment

Sixty rats were divided randomly into ten groups of 6 rats. Each experimental rat was injected intratracheally with 1mg of Uf-Co in suspensions in 1ml of physiological saline. Hereafter, rats injected with physiological saline are called the control group. Groups of 5-6 rats were killed at 1, 3, 7, 15 and 30 days after injection.

Bronchoalveolar lavage

The rats were killed by an overdose injection of Phenobarbital solution (50mg/ml) into the abdominal cavity, and the lung and trachea were removed en bloc. The wet lung weight was measured after removing the heart and the mediastinal lymphoid and adipose tissue. The lung: the body weight ratio (lung indices) was calculated. Bronchoalveolar lavage (BAL) was accomplished by 4 × 10ml saline at 37°C, with massage. The recovery of bronchoalveolar lavage fluid (BALF) for each rat was measured, and the BALF recovery rate was calculated. The BALF was then centrifuged (1500rpm, 10mins). The first lavage was kept separate, and after centrifugation the first lavage supernatant was used to measure the activity of the LDH and total concentration of protein and TNF-alpha. The numbers of total cells, macrophages, neutrophils and lymphocytes were evaluated for BALF pooled from each rat.

Ex-in vivo exposure

12 rats were divided randomly into two groups of 6 rats. Experimental rats were injected intratracheally with 1mg of Uf-Co in suspensions in 1ml of physiological saline. Hereafter, rats injected with physiological saline are called the control group. Three days after injection, the rats were killed and BAL was done as explained above. The cells obtained by centrifugation from BALF, were adjusted to a concentration of 2×10^5 /ml with RPMI-1640. After culture for 3, 6, 12, 24, 48, 72 hrs, the culture supernatants were collected and centrifuged (1500rpm, 10mins).

In vitro exposure

Cells obtained by centrifugation from BALF, were adjusted to a concentration of 2×10^5 /ml with RPMI-1640, and then added 2ml of cells to the Lab-Tek chamber slide, respectively. Cells were cultured for 3 hrs in a humidified atmosphere of 5% CO₂/95% air at 37°C to allow macrophages to adhere. After then, nonadherent cells were decanted and the attached cells were washed three times with warm RPMI-1640 medium. Uf-Co was added to a final concentration of 2.5, 5, 10, 20 and 40 µg/ml according to requirement. For the time-course experiment, macrophages were exposed to 40 µg/ml Uf-Co, then cultured for 12, 24, 48 hrs, and then the culture supernatants were collected and centrifuged.

Biochemical and cytological evaluation of BALF and biochemical evaluation in supernatant

The LDH activity in the first tube of BALF was measured using an LDH C II-test kit (WAKO Pure Chemical Industries' Ltd) by the lactate matrix method¹⁶. The concentration of total protein in BALF was assessed by the Lowry method¹⁷. Cells were obtained by centrifugation from all the BALF, and counted in a hemocytometer by the conventional method, and cell differentiation was done by the Wright-Giemsa method. TNF-

alpha in cell supernatants was quantified by enzyme-linked immunosorbent assay (ELISA) kit (Biosource International). The assay utilized a standard, 96-well quantitative immunometric 'sandwich' enzyme technique according to the manufacture's recommended protocol.

Statistical analysis

Results were expressed as the mean and standard error. Students t-test was adopted for statistical testing of differences between means of the effect indices.

Results

In vivo Experiments

1. Rate of recovery of BALF

The rate of recovery of BALF did not differ significantly among the experimental groups and controls. Data are not shown.

2. Lung wet weight

The absolute and relative wet lung weights of rats instilled with 1mg Uf-Co were significantly higher than those of the controls at 1,3,7, 15 and 30 days after instillation (Table 1).

3. Cellular and biochemical constituents in BALF

Table 1 Lung wet weight and relative lung wet weight of rats after instillation Uf-Co at difference times

Days	Groups	Lung wet weight (g)	Relative lung wet weight (mg/g)
1	Control	1.36 ± 0.04	5.44 ± 0.19
1	Uf-Co	2.48 ± 0.27*	8.63 ± 0.86*
3	Control	1.49 ± 0.03	5.91 ± 0.14
3	Uf-Co	1.88 ± 0.06*	8.39 ± 0.33*
7	Control	1.48 ± 0.06	5.08 ± 0.20
7	Uf-Co	2.30 ± 0.16*	9.23 ± 0.95
15	Control	1.72 ± 0.05	5.30 ± 0.13
15	Uf-Co	2.31 ± 0.11*	7.64 ± 0.55*
30	Control	1.86 ± 0.02	4.77 ± 0.27
30	Uf-Co	2.27 ± 0.13*	5.97 ± 0.3*

Note. Values are mean ± SE of 5-6 rats.

*Significantly different from the control group $p < 0.01$.

Fig. 1 TNF-alpha released by leukocytes from the lungs of rats injected with Uf-Co after different times in culture.

Note. Values are mean ± SE of three experiments.

*Significantly different from the control group $p < 0.01$.

Table 2 Cellular and biochemical parameter in BALF up to 30 days after injection of Uf-Co

Days	Groups	Total cells ($\times 10^6$)	Macrophages (%)	Neutrophils (%)	Lymphocytes (%)	LDH activity (Wroblewski U)	Total protein (mg/L)	TNF-alpha (pg/ml)
1	Control	6.3 ± 0.4	97.5 ± 0.3	1.5 ± 0.4	1.0 ± 0.2	21.6 ± 0.8	107.9 ± 10.3	5.54 ± 0.24
1	Uf-Co	$30.0 \pm 2.5^*$	$63.0 \pm 5.1^*$	$36.3 \pm 4.9^*$	$0.7 \pm 0.3^*$	$331.3 \pm 114.5^*$	$413.2 \pm 85.2^*$	$33.67 \pm 1.2^*$
3	Control	6.1 ± 0.3	97.6 ± 0.2	1.2 ± 0.2	1.2 ± 0.2	21.3 ± 0.9	107.8 ± 10.3	5.79 ± 0.28
3	Uf-Co	$15.8 \pm 2.2^*$	$68.2 \pm 3.6^*$	$30.8 \pm 3.3^*$	$1.0 \pm 0.6^*$	$109.4 \pm 6.0^*$	$377.2 \pm 44.6^*$	$31.65 \pm 6.7^*$
7	Control	6.5 ± 0.2	97.7 ± 0.4	1.2 ± 0.4	1.1 ± 0.2	21.5 ± 1.1	111.4 ± 3.7	5.77 ± 0.31
7	Uf-Co	$11.6 \pm 1.8^*$	$69.5 \pm 1.7^*$	$28.5 \pm 1.1^*$	$2.0 \pm 1.0^*$	$48.3 \pm 5.5^*$	$293.4 \pm 34.3^*$	$23.53 \pm 2.14^*$
15	Control	6.1 ± 0.2	97.7 ± 0.4	1.4 ± 0.4	0.9 ± 0.1	21.0 ± 0.9	112.5 ± 3.1	5.47 ± 0.21
15	Uf-Co	$11.8 \pm 2.0^*$	$85.0 \pm 2.0^*$	$13.2 \pm 1.6^*$	$1.8 \pm 0.6^*$	$43.6 \pm 3.2^*$	$259.9 \pm 20.7^*$	$24.55 \pm 2.27^*$
30	Control	6.0 ± 0.3	97.6 ± 0.2	1.2 ± 0.2	1.2 ± 0.2	21.4 ± 0.5	115.9 ± 7.60	5.47 ± 0.45
30	Uf-Co	$9.8 \pm 1.1^*$	$88.2 \pm 1.9^*$	$9.3 \pm 1.1^*$	$2.5 \pm 0.9^*$	$40.0 \pm 2.2^*$	$226.1 \pm 17.0^*$	$23.23 \pm 2.23^*$

Note. Values are mean $\pm$ SE of 5-6 rats.

*Significantly different from the control group $P < 0.01$.

Fig. 2 Relative LDH activity in supernatant of macrophages from the rats after treatment with different dose of Uf-Co.

Note. Values are mean $\pm$ SE of three experiments.

*Significantly different from the control group $p < 0.01$.

Fig. 3 TNF-alpha in supernatant of macrophages from rats after treatment with different doses of Uf-Co.

Note. Values are mean $\pm$ SE of three experiments.

*Significantly different from the control group $p < 0.01$.

Fig. 4 Relative LDH activity in supernatant of macrophages from the rats after treatment with Uf-Co at different times.

Note. Values are mean $\pm$ SE of three experiments.

*Significantly different from the control group $p < 0.01$.

Fig. 5 TNF-alpha in supernatant of macrophages from rats after treatment with Uf-Co at different times.

Note. Values are mean $\pm$ SE of three experiments.

*Significantly different from the control group $p < 0.01$.

Table 2 shows the cellular and biochemical constituents in BALF at 1, 3, 7, 15 and 30 days after instillation. Uf-Co injection caused an increase in the numbers of total cells, macrophages, neutrophils and lymphocytes in BALF. The total number of cells and neutrophils in BALF decreased throughout the experimental period. However, at 30 days after injection of Uf-Co, the total number of cells and neutrophils in BALF were still significantly higher than those in the control groups. The results also showed that LDH activity and the total protein and TNF-alpha in BALF decreased within the experimental period.

TNF- α release from alveolar macrophages from rats exposed with Uf-Co *in vivo* (Fig. 1) showed the release of TNF-alpha after different times in culture. The result showed TNF-alpha release from leukocytes were decreasing within the experimental period. The results also showed that the release of TNF-alpha by leukocytes from Uf-Co injected rats was consistently significantly higher than those from saline injected rats.

In vitro Experiments

Dose-response

Fig. 2 shows that the relative LDH activity increase in supernatant of macrophages after treatment with various doses of Uf-Co. Furthermore, the TNF-alpha released by leukocytes also increased in the supernatant of culture after treatment with various doses of Uf-Co (Fig. 3).

Time-effect

Fig. 4 shows the relative LDH activity in supernatants at 12, 24, 48 hours after treatment with Uf-Co. Treatment with Uf-Co caused mild macrophage damage which was reflected by the increase in the relative LDH activity in supernatant to only about twice the control levels. Fig. 5 shows the concentration of TNF-alpha in supernatant at 12, 24, and 48 hours after treatment with Uf-Co. Stimulation of alveolar macrophages with Uf-Co caused increased TNF production, and TNF production was sustained throughout the experimental period.

Discussion

Cobalt and its compounds have been shown to cause lung inflammation, fibrosis, emphysema, and alveolar proteinosis¹⁸⁻¹⁹. However, little is known of the pulmonary effects of Uf-Co although we have demonstrated that Uf-Co is inflammatorogenic in rat lungs⁸. Analysis of the cellular and biochemical profile in the BALF after exposure is a useful method to characterize the inflammatory response of the lung. This technique has been used to assess induction of lung injury by mineral dusts and metallic compounds^{8, 9-15, 20-22}. In the first part of the present study, we present results of a time-course study of the BALF profile following injection of a single dose of Uf-Co, aimed at assessing the general pulmonary toxicity of Uf-Co. The results showed that Uf-Co induced wet lung weight, cellular and biochemical increases in BALF up to 30 days after instillation with 1mg of Uf-Co. This kind of persistent inflammation is consistent with particles of high pathogenicity such as quartz^{8, 20-23} and ultrafine Ni⁸, whilst instillation of bolus doses of non-pathogenic particles such as ultrafine TiO₂ caused only transient inflammation that resolved in a few days post-instillation²³. The evidence that Uf-Co induces severe and persistent lung inflammation and

epithelial injury up to 30 days after instillation indicates that it may be a highly pathogenic particle type. Neutrophils primarily perform a defensive role in the lung but when they accumulate in large numbers in an activated state, they can release chemotactic factors, stimulate fibroblast proliferation and cause epithelial injury.²⁴ TNF is a proinflammatory cytokine produced by macrophages or monocytes in response to various stimuli²⁵⁻²⁶. Neutrophils are activated by TNF-alpha to a state where they are more likely to promote inflammation and cause injury to epithelial cells. Our results showed increased TNF-alpha production by leukocytes from the lungs of rats following Uf-Co treatment that was 3-4 times more than that of the saline control. These findings are consistent with a pulmonary inflammatory reaction with a highly activated neutrophil component capable of causing epithelial injury and prolonging the inflammation. We therefore suggest that high TNF-alpha levels in the lung is an important factor 'driving' the inflammatory response as has been suggested for other pathogenic particles²⁷.

Our *in vitro* study provides evidence that the alveolar macrophage is a likely source of the increased TNF-alpha since macrophages in culture released the cytokine in response to Uf-Co. LDH is a cytoplasmic enzyme that is only released extracellularly by damaged cells and, therefore, increased LDH activity in BALF reflects a degree of damage of lung cells and tissue^{8, 20, 22}. LDH data presented here suggest that Uf-Co is toxic to macrophages both *in vivo* and *in vitro* but that the effects were only severe in the early stages after instillation. In the later timepoints there was much less LDH, only about twice the control levels and this was also the magnitude of the increase seen *in vitro*. Cell death can 'drive' inflammation and so this may also be a factor in the inflammatory effects of Uf-Co at the acute time following instillation. At the 1 day timepoint, for instance, LDH was 15 times the control value but by day 30 it was only twice the control level. By contrast TNF was 6 times higher than the control levels at the 1 day timepoint and still more than 4 times higher than the control value at 30 days. Therefore cytotoxicity may be a major factor in driving inflammation at earlier timepoints but not at the later timepoints. We have previously reported that Uf-Co has a high level of associated free radical activity at its surface⁸ and this could promote both cell damage and cell stimulation³¹.

In the second part of the study, the aim was to examine the dose and time-course of pulmonary macrophage response to Uf-Co *in vitro* to better understand the mechanism of Uf-Co toxicity. Alveolar macrophages provide the principle defence against inhaled particulate materials in the lung and play a primary role in mediating the inflammatory response²³⁻²⁴. In our studies, alveolar macrophages exposed to Uf-Co *in vitro* release TNF, in a dose and time-responsive manner. Increased LDH release by alveolar macrophage exposed to Uf-Co also increased with time and dose but this increase was modest, being only up to 3 times background levels at the most. This study does not elucidate the mechanism how Uf-Co induces the production of TNF by leukocytes. However, our previous studies have shown Uf-Co to have free radical activity, and to induce lung lipid peroxidation reaction⁸. Stress arising in target cells due to free radicals is thought as the primary stimulant and is necessary for TNF production²⁵.

In summary, our results showed that pulmonary deposition of Uf-Co, one of the new category of ultrafine particles, causes a

sustained and substantial inflammation that is accompanied by an augmentation of production of TNF by pulmonary leukocytes. Increased TNF levels in the lungs following exposure is very likely a key initiating event in the pulmonary inflammatory effects of Uf-Co and may be generally important in the pathogenic effects of ultrafine particles.

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Reference

- 1) Coates EO, Watson JHL. Diffuse interstitial lung disease in tungsten carbide worker. *Ann Intern Med* 1971; 75: 709-16.
- 2) Kitamura M, Kitamura H, Tozawa T, Kimura Y. Cemented tungsten carbide pneumoconiosis. *Acta Pathol Jpn* 1978; 28: 921-35.
- 3) Hartung M, Schaller KH, Brand E. On the question of the pathogenic importance of cobalt for hard metal fibrosis of lung. *Int Arch Occup Environ Health* 1982; 50: 53-7.
- 4) Camner P, Boman A, Johansson A, Lundborg M, Wahlberg JE. Inhalation of cobalt by sensitized guinea pigs: effects on the lungs. *Brit J Ind Med* 1993; 50: 753-57.
- 5) Kerfoot EJ, Fredrick WG, Domeier E. Cobalt metal inhalation on miniature swine. *Am Ind Hyg Assoc J* 1975; 36: 17-25.
- 6) Wehner AP, Busch RH, Olson RJ, Craig DK. Chronic inhalation of cobalt oxide and cigarette smoke by hamsters. *Am Ind Hyg Assoc J* 1977; 38: 338-46.
- 7) Kyono H, Kusaka Y, Homma K, Kubota H, Endo-Ichikawa Y. Reversible lung lesions in rats due to short-term exposure to ultrafine cobalt particles. *Ind Health* 1992; 30: 103-18.
- 8) Zhang Q, Kusaka Y, Sato K, Nakakuki K, Kohyama N, Donaldson K. Differences in the extent of inflammation caused by intratracheal exposure to 3 ultrafine metals: role of free radicals. *J Toxicol Environ Health* 1998; 58: 423-38.
- 9) Ferin J, Oberdöster G, Penney DP, Soderholm SC, Piper HC. Increased pulmonary toxicity of ultrafine particles? I. Particle clearance, translocation, morphology. *J Aerosol Sci* 1990; 21: 381-4.
- 10) Ferin J, Oberdöster G, Soderholm SC, and Gelein R. Pulmonary tissue access of ultrafine particles. *J Aerosol Sci* 1991; 4: 57-68.
- 11) Oberdöster G, Ferin J, Finkelstein G, Wade P, and Corson N. Increased toxicity of ultrafine particles? II. Lung lavage studies. *J Aerosol Sci* 1991; 21: 384-7.
- 12) Oberdöster G, Ferin J, Lehnert BE. Correlation between particle size, in vivo particle persistence, and lung injury. *Environ Health Persp.* 102(Suppl 3): 173-9.
- 13) Takenaka S, Dornhofer-Takenaka H, Muhle H. Alveolar distribution of fly ash and titanium dioxide after long-term inhalation by Wistar rats. *J Aerosol Sci* 1986; 17: 361-4.
- 14) Zhang Q, Kusaka Y, Sato K, Morita A, Iki M, Deguchi Y. The lung toxicity ultrafine. *Jpn J Hyg* 1996; 51(1): S349 (in Japanese).
- 15) Kusaka Y, Zhang Q, Sato K, Morita A. Mechanism for lung toxicity of suspending particulate particles: a study in rat model induced by ultrafine titanium dioxide powder. *Jpn J Hyg* 1997; 429(1): 95 (in Japanese).
- 16) Babson AL, and Phillips GE. A rapid calorimetric assay for serum lactic dehydrogenase. *Clin Chem Acta* 1965; 12: 210-5.
- 17) Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the folin phenol reagent. *J Bio Chem* 1951; 193: 265-75.
- 18) Kusaka Y, Yokoyama K, Sera Y, et al. Respiratory diseases in hard metals workers: an occupational hygiene study in a factory. *Br J Ind Med.* 1986; 43: 473-85.
- 19) Moulin JJ, Wild P, Mur JM, Fournier-Betz M, Mercier-Gallay M. A mortality study of cobalt production workers: an extension of the follow-up. *Am J Ind Med* 1993; 23: 281-8.
- 20) Zhang Q, Kusaka Y, Sato K, Mo Y, Fukuda M, Donaldson K. Toxicity of ultrafine nickel particles in lungs after intratracheal instillation. *J Occup Health* 1998; 40: 171-6.
- 21) Zhang Q, Kusaka Y, Sato K, Donaldson K. Differences in the inflammogenicity of standard and ultrafine nickel: the role of nitric oxide. *Advances in the Prevention of Occupational Respiratory Diseases.* Chiyotnni K, Hosoda Y, and Aizawa Y, editors. 1998 Elsevier Sciences 866-72.
- 22) Zhang Q, Kusaka Y, Zhang QF, He L, Zhang Z, Sato K. Dynamic changes of constituents in bronchoalveolar lavage fluid in experimental silicotic rats. *Ind Health* 1996; 34: 279-388.
- 23) Brown GM, Brown DM, Donaldson K. Inflammatory response to particles in the lung: secretion of acid and neutral proteinases by bronchoalveolar leukocytes. *Ann Occup Hyg* 1991; 35: 389-96.
- 24) Donaldson K, Brown GM, Brown DM, Slight J, Li XY. Epithelial and extracellular matrix injury in quartz-inflamed lung: role of the alveolar macrophage. *Environ Health Persp* 1992; 97: 221-4.
- 25) Kelley J. Cytokines of the lung. *Am Rev Respir Dis* 1990; 141: 765-88.
- 26) Matic M, Simon SR. Tumor necrosis factor release from lipopolysaccharide-stimulated human monocytes: lipopolysaccharide tolerance in vitro. *Cytokine* 1991; 3: 576-83.
- 27) Driscoll K, Carter JM, Hassenbein DG, and Howard B. Cytokines and particle-induced inflammatory cell recruitment. *Environ Health Persp* 1997; 105: 1159-64.