Individual Susceptibility to Cadmium Toxicity and Metallothionein Gene Polymorphisms: with References to Current Status of Occupational Cadmium Exposure

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Abstract: The incidence of serious poisoning caused by occupational cadmium exposure has declined over the past four decades due to improvements in the work environment. However, long-term low-level exposure to cadmium needs to be addressed. For workers in industries that handle cadmium, it is necessary to consider the daily cadmium intake from contaminated foods such as cereals and rice in addition to the occupational exposure, since workers might be exposed to higher levels of cadmium from a combination of these sources. Cadmium accumulates in the renal cortex by the long-term exposure along with increased concentrations of metallothionein, an important protein for protection from cadmium toxicity. However, some individuals have lower metallothionein levels despite increased cadmium accumulation in the kidneys. This article describes the strategy method for analyzing individual susceptibility to cadmium toxicity and genetic polymorphisms of metallothionein, with reference to the current status of occupational cadmium exposure.

Key words: Cadmium, Occupational exposure, Metallothionein, Polymorphism

Occupational exposure to cadmium remains an issue

Cadmium is used in various industrial products such as electrodes in nickel-cadmium batteries, pigments (cadmium-yellow), coatings for machinery parts, and alloys and control rods for nuclear reactors. During the early 20th century, cadmium poisoning due to occupational exposure and environmental pollution (e.g. itai-itai disease) was reported in industrialized countries. Improvements in workplace environments and soil decontamination have substantially decreased the prevalence of serious life-threatening cadmium poisoning. The European Union (EU) countries have already discontinued usage of cadmium-including pigments. The EU has officially announced the Restriction on Hazardous Substances (RoHS) that bans the inclusion of hazardous substances such as cadmium in electrical and electronic equipment, and has prohibited the import of appliances containing cadmium since July 2006. The Japanese Ministry of Health, Labour and Welfare has already restricted workplace concentrations of cadmium based on the “Ordinance on Prevention of Hazards Due to Specified Chemical Substances”. Actually, Friis et al. found that the cadmium concentrations in the kidney cortex of Swedish individuals aged <40 yr was about 40% of that in 19761), and they suggested that reduced cadmium contamination from industries and changes in dietary habits were responsible, because the reduction was similar between smokers and nonsmokers1). However, based on observations of urinary cadmium excretion by 889 Japanese workers in Toyama prefecture, Miyamoto et al. reported that concentrations of the excretion of urinary cadmium in both men and women between 1993 and 1998 were lower than in 1985, although no significant differences were found between 1998 and 1993; levels in 1998 still exceeded the tolerable limit levels in Japan (2 to 3 µg/d)2, 3). Further, Yassin et al. reported urinary cadmium concentrations
obtained from 11,228 workers in USA from 1988–1994, and metal industry workers showed higher cadmium levels than agricultural workers\(^6\). Therefore, some workers are still exposed to comparatively high levels of cadmium.

In fact, cadmium exposure and physical dysfunctions have continued to surface in many countries even during the past decade. Cadmium fume inhalation by workers in the silver jewelry industry has occurred in India\(^5\). Paint workers in Nigeria showed higher serum cadmium levels compared to non-paint factory workers, and their biochemical indices of renal function were slightly elevated\(^6\). In Thailand, blood and urinary cadmium levels of spray painters working in automobile body repair shops were significantly higher than among controls\(^7\). In China, workers employed in a smelter showed higher prevalence of renal dysfunction\(^8\). In UK, a significantly increased mortality for non-malignant diseases such as those of the respiratory and genitourinary systems was identified among workers employed in the nickel-cadmium battery industry\(^9\).

Unpredictable accidental exposure still occurs. For example, among emergency response workers during rescue and recovery activities at the World Trade Center after the September 11 attacks, a torch cutter was overexposed to cadmium\(^10, 11\). A fuel oil leak due to the accidental sinking of the oil tanker, Prestige, was cleaned up by French and Spanish troops and local personnel. Cytogenetic and endocrine effects were found in those exposed to the fuel oil including toxic metals such as cadmium, aluminium, nickel, etc\(^12\). Acute lung injury and respiratory distress syndrome were also identified among employees in Mumbai accidentally exposed to metal fumes including cadmium while working in local silver jewelry industries\(^13\), and in the galvanized steel industry in USA\(^14\).

Today, movements lobbying for the cessation of industrial cadmium usage have become powerful around the world, and this has minimized the likelihood of routine exposure to high cadmium concentrations except for the unexpected and accidental occupational exposure as mentioned above. Thus, the long-term effects on worker’s health of low-level cadmium exposure should be analyzed. Morris et al. postulated that trace amounts of cadmium could also result in overexposure\(^15\), because in the process of adding special high-grade (SHG) zinc to molten brass or bronze alloys, cadmium levels in employees exposed to as little as four pounds of SHG zinc containing 0.0004% cadmium exceeded both the action level (2.5 \(\mu g/m^3\)) and permissible exposure limit (5.0 \(\mu g/m^3\)) of the Occupational Safety and Health Administration (OSHA). Sişman et al. described possible renal dysfunction among Turkish workers employed at a tobacco leaf processing factory\(^16\). Both levels of blood cadmium and renal dysfunction were significantly increased, and they postulated that cadmium exposure affects kidney function even when below generally accepted toxic limits\(^16\).

In addition to occupational environments, humans can be exposed to cadmium in general environments, especially by consuming contaminated foodstuffs such as rice and wheat harvested from soil polluted with cadmium (see below). Industrial workers residing in cadmium-polluted areas (and of course, in non-polluted areas) should be carefully monitored because they may be exposed to additional cadmium via the consumption of contaminated rice and other foodstuffs. Jin et al. compared the renal damage levels among smelter workers who lived in a cadmium polluted-area (combination group), with others who never worked in the plant but lived in the same area (area group). The prevalence of renal damage was higher in the combination group, than in the area group, suggesting that both occupational and environmental cadmium exposure results in a higher prevalence of renal dysfunction\(^6\). D’Elia et al. reported that gardened and cultivated urban soil might be an occupational exposure source, although the levels of various pollutants including cadmium in both soil and leaves in Bologna were below the threshold limit of ACGIH during the period of their study\(^17\).

Cadmium usage in Japan remains rather high. Statistics generated by the Agency of Natural Resources and Energy show that the total annual use of cadmium in Japan (over 6,000 tons) was the highest in the world, accounting for 43.4% of all worldwide cadmium consumption during 1995. This import volume rapidly decreased to 3,500 tons in 1998 varying from 2,000 to 3,000 tons thereafter (up to 2004)\(^18\). Data published by the Ministry of the Environment shows that the nonferrous metal manufacturing industry was responsible for most of the emissions and movements of cadmium or cadmium products at 163 tons/yr in 2003. This value accounted for 63.8% of the total amount of emissions and movements. Metal mining and electromechanical device manufacturing consumed 32 tons (12.6%) and 24 tons (9.3%) of cadmium, respectively, and these three industrial categories accounted for about 86% of the total. Therefore, attention is warranted regarding cadmium exposure among workers employed in these industries. Furthermore, the report from the Ministry of the Environment in 1998 indicated that over 50 areas in Japan are polluted with cadmium, and the level of cadmium in Japanese soil is far higher than those in other countries due to historical mining and smelting, as well as a large amount of cadmium importation\(^19, 20\). Therefore, in Japan, rice is more contaminated with cadmium when harvested from these soils compared to other countries\(^21, 22\). Rice is the staple food
of the Japanese and is, therefore, a major source of cadmium intake. Even if cadmium concentrations in the industrial or general environments are restricted or improved, the possibility of workers being exposed to combined sources of cadmium must be carefully considered. Cadmium should remain an important issue for occupational health and welfare policies.

**Toxicity and disorders caused by cadmium exposure**

Workers are mainly exposed to cadmium via fume or dust inhalation in the workplace, that is, via the respiratory route. Cadmium can also be orally introduced by consuming contaminated foods or water, that is, via the gastrointestinal tract. About 50–80% of absorbed cadmium accumulates in the liver and kidneys, where the accumulation ratio depends on the administration route; nonoral (such as inhalation, subcutaneous, intraperitoneal or intravenous) exposure to cadmium initially results in liver accumulation, followed by a subsequent decrease and shift to the kidneys, whereas oral exposure to cadmium results in accumulation in the kidneys as well as the liver. Metallothioneins play a considerable role in the shift of accumulated cadmium from the liver to the kidneys. Metallothioneins are low molecular weight proteins, the synthesis of which is induced by various heavy metals such as cadmium, and they suppress toxicity of heavy metals by binding to these metals. Cadmium that is imported into cells, such as hepatocytes, induces the synthesis of metallothionein, to which over 80% of the cadmium binds. Cadmium-metallothionein complexes are released into the bloodstream, re-absorbed through the proximal tubules and accumulate in renal cells. Free cadmium ions released by cadmium-metallothionein degradation in lysosomes induce the synthesis of metallothionein protein, to which they bind again, and accumulate in the renal cells in a low-toxicity state. The target organ of cadmium accumulation also depends on the period of administration; most cadmium accumulates in the liver after short-term exposure, but in the kidneys in long-term exposure. The estimated half-life of cadmium in humans ranges from 10 to 30 yr, and very small amounts of accumulated cadmium in humans are excreted. Therefore, the amount of cadmium in the human kidneys increases with age, because the kidneys are the excretion route of cadmium.

Long-term exposure to cadmium damages the kidneys and causes renal tubular dysfunction as assessed by increased urinary excretion of low molecular weight proteins such as α1-microglobulin (α1-MG) and β2-MG. Individuals exposed to high levels of cadmium develop severe renal dysfunction and bone damage characterized by osteoporosis, osteomalacia, bone mineral loss and anemia. One mechanism of bone damage is depletion of the bone calcium pool due to disrupted vitamin D metabolism in the kidneys, resulting from renal tubular dysfunction caused by cadmium and the continuous urinary excretion of calcium and phosphorus. Since the ionic radius of cadmium is similar to that of calcium, cellular cadmium uptake is thought to be mediated by calcium channels. Thus, cadmium competition for cellular calcium uptake also explains the calcium deficiency in individuals exposed to cadmium. Olfactory toxicity, male infertility, hypertension and cardiovascular diseases are also associated with cadmium poisoning.

Carcinogenesis caused by cadmium exposure represents another concern. Many experimental and epidemiological studies have examined cancers of various organs such as the liver, kidneys, lung, prostate, breast, brain and nervous system, testis and hematopoietic system. However, many human epidemiological surveillance investigations have not identified a relationship between cadmium exposure and cancer risk, although experimental animal studies have clearly demonstrated an association. Therefore, although the International Agency for Research on Cancer (IARC) has classified cadmium as Carcinogenic (Group 1) in 1993, debate about this association has continued.

Cadmium might act like an estrogen, mimicking the action of 17β-estradiol (E2) both in vivo and in vitro, by forming high-affinity complexes with estrogen receptors (ER) independently of E2, indicating that cadmium is a metalloestrogen. Jonson et al. reported that uterine weight increases and mammary glands develop in female rats exposed to environmental levels of cadmium. A role for cadmium in the development of human breast cancer has been proposed. Interestingly, the estrogen-like and ER-mediated activities of cadmium are inhibited by melatonin, a pineal gland hormone with antioxidant and antiestrogenic properties. Cadmium produces active hydroxy radicals that melatonin might scavenger. Furthermore, Ozan et al. reported that melatonin protects against damage to the human kidneys induced by cigarette smoke.

Metallothionein is the most important factor regulating the biological effects of cadmium. Treating mice with low doses of heavy metals (such as cadmium or zinc) induces metallothionein and obviously reduces the toxicity of subsequently administered lethal doses of cadmium. Transgenic mice that constantly overexpress metallothionein genes are also cadmium tolerant. In contrast, knockout mice with defective metallothionein genes are more sensitive to cadmium toxicity than wild-type mice. The findings of many similar studies support the notion that metallothionein is the main cellular deter-
maminant of the sensitivity of mammals and cultured mammalian cells to cadmium.

**Metallothionein is an important determinant of individual susceptibility to cadmium toxicity**

Since the excreted amount of absorbed cadmium is minimal, as described above, cadmium accumulates in human tissues, especially in the renal cortex. Cadmium concentrations in the renal cortex are higher among Japanese than US and Swedish individuals. Yoshida et al. reported that the cadmium levels in the renal cortex increased with age, along with the metallothionein levels, and the correlation coefficient between levels of cadmium and metallothionein was significant in the renal cortex (rho<0.001). However, they identified groups of individuals with (Group A), and without (Group B) an increase in metallothionein levels, even though their cadmium accumulation levels were similar (Fig. 1). Metallothionein levels at a cadmium concentration of 50–92 µg/g were 5–250 and 550–1,750 µg/g in Group B and Group A, respectively (according to Fig. 2-C of Yoshida's report). This indicates that some individuals have lower metallothionein levels regardless of increased cadmium accumulation.

We postulated that these individuals have abnormal metallothionein synthesis and might be sensitive to renal dysfunction due to cadmium exposure, because even those with cadmium levels in the renal cortex of 18.9–73.6 µg/g might show clinical and pathological findings of the itai-itai disease. Since cadmium induces metallothionein synthesis at the transcriptional level, we analyzed genetic polymorphisms of the region around the transcriptional start site (a known promoter region) of the metallothionein-2A gene, the main isoform of human metallothionein genes. We isolated DNA from blood samples provided by 119 Japanese individuals and used PCR to amplify a 222-base pair (bp) fragment located 202 bp upstream and 20 bp downstream of the transcription start site. We analyzed the amplified fragments by single-strand conformation polymorphism (SSCP) and found that about 17.6% of the 119 persons had a single nucleotide polymorphism (SNP) in this region. The genotypes of A/A, A/G, and G/G accounted for 98 (82.4%), 20 (16.8%), and 1 (0.8%) of them, respectively. We also confirmed that the A→G SNP significantly reduced cadmium-induced transcription of the metallothionein-2A gene, suggesting that although only one of our study participants had the G/G type, people with this genotype might be more sensitive to cadmium toxicity than those with the A/A type, because of a limited ability to induce metallothionein synthesis. Workers with the metallothionein-2A G/G genotype (perhaps also the A/G type) who work in industries that handle cadmium might be prone to developing various biological dysfunctions when exposed to cadmium, because cadmium affects various biological processes (see Discussion). Even if workers are exposed to cadmium concentrations that are below regulation values such as the current provisional tolerable weekly intake (PTWI) of 7 µg/kg, symptoms of long-term cadmium accumulation might gradually start to appear and should be carefully monitored.

Efforts to use blood metallothionein gene transcription levels (mRNA) as a biomarker of metal exposure have been applied for almost two decades. We have also tried to establish an analytical method using expression profiles of human genes for over 10 metallothionein isoforms, that differ from those of other mammals, after exposure to various levels of metals (in preparation). Liu et al. recently found significantly lower metallothionein mRNA levels in both blood and buccal cells in Chinese patients with arsenicosis. Chang et al. also reported that the mRNA levels of metallothionein-1A (a human metallothionein isoform gene) in peripheral blood lymphocytes (PBLs) collected from workers exposed to cadmium were increased with increment of urinary cadmium levels and significantly correlated with renal dysfunction biomarkers such as urinary β2-MG and albumin. These findings indicated that metallothionein-1A mRNA levels in PBLs can function as a useful biomarker of renal dysfunction in occupational exposure to cadmium. The study of Liu using rats and mice also verified a positive correlation between blood metallothionein mRNA and hepatic and renal metallothionein mRNA as well as their protein levels. These results show that measuring met-

![Fig. 1. Schematic diagram of the relationship between renal cortical levels of cadmium and metallothionein.](image-url)
allothionein mRNA levels in PBLs can detect or screen for individuals who are sensitive to cadmium toxicity due to lower metallothionein levels; that is, those with limited ability for metallothionein synthesis such as the G/G type variant described above. This is important because performing biopsies of human tissues such as the kidneys can be quite difficult.

Discussion

Cadmium is a metal that should be addressed in occupational health even today, because cadmium exposure can lead to long-term effects on human health even at low concentrations. The estrogen-like effect of cadmium means that cadmium may act in the human body at considerably low concentration. The cadmium concentration increases in the renal cortex with age, and the mean value of the renal cadmium concentration in the general Japanese population aged 41–60 yr was reported to be 69.8 ± 39.5 µg/g tissue. The Joint FAO/WHO Expert Committee on Food Additives (JECFA) has estimated that the critical renal cadmium concentration is 200 µg/g tissue, meaning that exceeding this concentration might induce renal tubular dysfunction. The renal cadmium concentrations among middle-aged Japanese in general is about one-third of the JECFA critical value, therefore, the possibility that some workers employed in industries that handle cadmium might have already exceeded the JECFA value cannot be excluded. An important biological protection factor against cadmium toxicity is metallothionein which suppresses its toxicity by binding to cadmium in the renal cortex. If metallothionein synthesis is inhibited or disappears suddenly, then serious renal dysfunction might develop in individuals with high concentrations of cadmium. It is unlikely that an absence of metallothionein gene transcription could occur suddenly due to specific metallothionein gene knockout in adult humans. However, several reports indicate that metallothionein synthesis can be inhibited by DL-propargylglycine (PPG), an inhibitor of the cystathionine pathway (cystathionase inhibitor). If workers are exposed to "industrial materials" that participate in a inhibitory mechanism similar to that of PPG, then free cadmium ions released from cadmium-metallothionein will accumulate due to the absence (or a lower level) of metallothionein, resulting in serious renal dysfunction. Workers who are more susceptible to such "industrial materials" will develop more serious renal dysfunction.

Other factors that influence cadmium sensitivity might be decreased blood iron status, dietary composition and age. For example, cellular cadmium uptake is mediated by iron pathways, and a dietary iron deficiency promotes the intestinal absorption of cadmium in mice and humans. A recent Swedish study by Olsson et al. has shown that sensitive individuals also have low iron status. The authors also described that some individuals in their study population had renal dysfunction even at low levels of cadmium exposure. Levels of dietary iron and crude fibers are also associated with cadmium absorption, and deficiencies in these factors might increase intestinal cadmium absorption 5- to 8-fold. Therefore, individuals with low iron levels due to anemia might be cadmium-sensitive. Furthermore, Horiguchi et al. reported that age affects cadmium absorption rates from the intestine, and suggested that young populations (especially women) are at high risk of cadmium toxicity. Although these reports are examples related to cadmium absorption that differs from the cadmium toxicity described above, these factors should be considered in the setting of long-term exposure to cadmium among young workers, and workers who are susceptible to anemia, including women. Long-term exposure to lower cadmium concentrations should be considered a future occupational health issue because cadmium confounds many physiological processes.

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References


