Review

Pathogenesis of Osteomalacia in Itai-itai Disease

Takashi Umemura¹ and Yumi Wako²

¹Laboratory of Comparative Pathology, Hokkaido University Graduate School of Veterinary Medicine, N18 W9, Sapporo 060–0818, Japan
²Kashima Laboratory of Mitsubishi Chemical Safety Institute Ltd., Kamisu-shi, Ibaraki 314–0255, Japan

Abstract: Itai-itai disease (IID) is the most severe form of chronic cadmium (Cd) intoxication of human. The patients of IID suffer from renal anemia, tubular nephropathy and osteopenic osteomalacia, and 90% of the patients are post-menopausal women. Many efforts have been paid for reproducing the bone lesions using various animal species, but the pathogenesis of them is still controversial and conflicting among the reports. Two hypotheses have been proposed for the pathogenesis of the bone lesion: direct and indirect effects of Cd on bone. The former includes toxic effects of Cd on osteoblasts and impediment of calcification at the ossification front by Cd or Fe. The latter indicates nephrogenic osteopenia via hypocalcemia, hypophosphatemia and hyperparathyroidism. Each hypothesis secures solid scientific basis and may not be alternative. Our animal experiments using rats and monkeys demonstrated that hypoestrogenism induced by ovariectomy enhanced Cd toxicity, and osteopenic osteomalacia and tubular nephropathy distinctive to IID could be reproduced by Cd treatment without involvement of malnutrition such as hypovitaminosis D. Vitamin D has been prescribed to IID patients for bone lesions. However, the efficacy of the treatment is unpredictable depending on patients. Our experiments suggest concurrent administration of estradiol helps the vitamin D therapy for IID patients and the removal of Fe at the mineralization front of bone is important for the recovery to normal bone remodeling. (J Toxicol Pathol 2006; 19: 69–74)

Key words: cadmium, estradiol, iron, itai-itai disease, osteomalacia, osteoporosis

Introduction

Osteoporosis, osteopenia in other words, is metabolic bone disease characterized by loss of skeletal mass due to enhanced bone resorption relative to formation, and the remaining bone exhibits a normal ratio of mineralized to unmineralized (osteoid) matrix. Whereas osteomalacia is an excess of unmineralized bone matrix due to inadequate mineralization of newly formed bone matrix. Some of the causes, such as hypovitaminosis D and hypocalcemia are overlapped for both diseases and the severe osteoporosis accompanies osteomalacic change, i.e. formation of osteoid.

Cadmium (Cd) is a heavy metal that exists ubiquitously in the environment and major by-product at mines for lead and zinc. Recently, the surveillance of human exposure to the metal due to dietary intake, occupational exposure or smoking habit were carried out in many countries, and the correlation of Cd body burden and renal dysfunction or osteoporosis were evidenced among not only cadmium workers or inhabitants in heavily polluted areas but also general population in Japan, Sweden, Belgium, Poland and China.¹⁻⁹. Itai-itai disease (IID) is the most severe form of chronic Cd intoxication¹⁰. The disease was first recognized in the Jinzu river, Toyama prefecture, Japan, shortly after World War II and a total of 187 inhabitants to date around the river received an official recognition as the patients of IID. The patients of IID suffer from renal anemia, tubular nephropathy and osteopenic osteomalacia, and 90% of the patients are post-menopausal women.

Many efforts have been paid for reproducing the Cd-induced bone lesions using various animal species. Some workers have succeeded in reproducing osteomalacia in rats by long-term Cd treatment¹¹⁻¹⁶, while the others have failed due to the difference in the species of experimental animals, dose levels, routes of Cd burden and durations of Cd exposure¹⁷⁻¹⁹. The pathogenesis of the bone lesions is still controversial and conflicting among the reports: some researchers insist that Cd act secondarily on bone through abnormal Ca homeostasis caused by renal and/or intestinal damage²⁰,²¹, while the others report a direct action of Cd on bone²²,²³. In this review article, we give the overview on the pathogenesis of bone lesions in IID.

Direct Effects of Cd on Bone

Yoshiki et al.¹² could reproduce the bone lesions in
female rats fed a diet containing cadmium (0, 10, 30, 100, 300 ppm) for 5, 7, 9, and 12 weeks. Disappearance of metaphyseal trabeculae and shortened epiphyseal cartilage were observed before the occurrence of kidney lesions. The osteal changes were osteoporosis, and not osteomalacia. They suggested that Cd might act primarily on bone rather than secondarily through disturbances of the renal functions.

Furuta\textsuperscript{13} could reproduce the Cd-induced bone lesions in male rats by a single and daily subcutaneous injection of CdCl\textsubscript{2} for 3 weeks. Microradiographical studies in his report showed that the cadmium caused osteoporotic and not osteomalacic changes. Disappearance of metaphyseal trabeculae and shortened epiphyseal cartilage were also observed.

Noda et al.\textsuperscript{24} investigated 62 human autopsy cases of IID by quantitative histology. Their results suggested that an impairment of osteoblastic function and mineralization occurred in IID patients and Cd was a possible etiological factor.

Brzoska and Moniuszko-Jakoniuk\textsuperscript{25–27} suggested that the mechanisms of Cd action were associated with the effect on the rate of bone turnover, which depended on the stage of the skeletal development and disorders in Ca metabolism. Cd lead to low bone turnover at the young stage and induced high bone turnover due to enhanced resorption at the mature stage. The primary mechanism of toxic effect of Cd on bone involved the direct action on the osteoblastic and osteoclastic activities, while the secondary mechanism was associated with the indirect enhancement of bone resorption due to a lack of minerals, mainly Ca. Their suggestions are supported with higher Cd accumulation in young bone than in adult or aged bone\textsuperscript{28}, and an inhibitory effect of Cd on hydroxypatite formation and competition of Cd\textsuperscript{2+} with Ca\textsuperscript{2+} for incorporation into bone have been demonstrated by in vivo study\textsuperscript{29}.

**Indirect Effects of Cd on Bone**

Itokawa et al.\textsuperscript{11,30} reported the renal and skeletal lesions of rats fed on the diets containing Cd for 60 or 120 days. Thinning of bone cortex, disappearance of trabecular bone, decreases in the number of osteocytes and acid mucopolysaccharides in epiphyseal cartilage were observed with renal lesions. In addition to these changes, osteoid was formed as an indication of osteomalacia. The biochemical changes such as a decrease in urinary excretion of Ca and P, increases in serum Ca, urea and alkaline phosphatase, decrease in serum P suggested that the bone disease in Cd poisoning were secondary to the effect on renal function.

The distributions of \textsuperscript{45}Ca has been investigated to study the fate of Ca in bones after chronic oral administration of Cd\textsuperscript{21,23,25–27,31}. The fecal and urinary excretions of \textsuperscript{45}Ca of Cd-exposed animals were increased, and the increased release of \textsuperscript{45}Ca from bone occurred in the Cd-exposed animals. Therefore, the constant plasma Ca concentration in Cd treated animals may be explained by the remarkable increase of Ca resorption from bones.

Metallothionein (MT) is well known as protein that binds Cd with high affinity and protect against Cd toxicity\textsuperscript{32,33}. Although the liver is the most responsive organ to MT induction, MT is also found in other organs\textsuperscript{15,34,35}. The MT mRNA levels in the bone tissue of rats were increased by Cd administration. Immunohistochemically, MT positive cells were time-dependently increased, and the positive cells were osteocytes. MT, induced in osteocytes, may protect against Cd-induced bone injury\textsuperscript{36}. Recently, MT-I/II knockout (Mt-null) mice were used to investigate the role of MT in Cd toxicosis\textsuperscript{37–39}. Cd-induced kidney lesions were observed in both MT-null and background-matched wild-type (WT) mice. Histopathology showed proximal convoluted tubule degeneration and atrophy, chronic inflammation, intestinal fibrosis, and dilated collecting tubules. These lesions were more severe in MT-null mice than in the WT mice. On the other hand, tissue Cd concentrations in MT-null mice were only about one-fifth of that in WT mice. Even though the renal Cd concentrations were much lower in the MT-null mice, they were more sensitive than WT mice to Cd-induced renal injury\textsuperscript{39}. There was no difference in bone Cd content between WT and MT-null mice, but the loss of bone and the decrease in bone density were more marked in MT-null mice than WT mice. Histopathology showed dilatation of haversian canals with increased osteoid seams, rounded osteocytes with expanded pericellular space, and hyperplastic bone marrow. These lesions were more marked in MT-null mice. This study demonstrates that deficiency in MT renders animals more susceptible to Cd-induced bone injury and MT plays a protective role to Cd-induced osteopenic osteomalacia\textsuperscript{38}, and suggests the significance of indirect effects of Cd on bone via nephropathy.

Aoshima et al. investigated 53 postmenopausal IID patients and suggested that the bone turnover, in particular the bone formation, was influenced by renal tubular function assessed by the levels of glomerular filtration rates\textsuperscript{40}. Not only high exposure to Cd, but also low-level Cd exposure was associated with an increased risk factor\textsuperscript{4,19}.

**Intravenous Administration of Cd to Ovariectomized Animals**

Ninety percent of IID patients is postmenopausal women and absorption rate of per orally administrated Cd in rats through the intestine is 0.4–0.5%. In our experiments for the experimental reproduction of the bone lesions of IID, we injected Cd intravenously (iv) instead of per orally to ovariectomized (OX) animals. Prior to the experiments, we found that the LD\textsubscript{50} in rats was 5.2 mg/kg in acute toxic study.

We compared OX female rats and non-OX female rats after intravenous administration of CdCl\textsubscript{2} at doses of 2.0 and 3.0 mg/kg for 14 days. Hepatic and renal lesions, similar to IID, were more severe in OX rats than non-OX rats, however, skeletal lesions did not developed because of short-term administration\textsuperscript{41}. To investigate the effects of
long-term administration of Cd on the bone, OX rats were injected iv with CdCl₂ at doses of 1.0 and 2.0 mg/kg for 13 weeks. Mild osteomalacic and osteoporotic lesions with increased cancellous bone mass of the metaphysis, dilated midshaft haversian canals in the cortical bone accompanied by osteoid seams were reproduced in this experiment. These histopathological changes of the bone were similar to other reports.

To investigate more long-term effects of Cd, we injected iv in OX rats at doses of 0.05 and 0.5 mg/kg/day for 12, 25, 50 and 70 weeks. The rats treated with 0.5 mg/kg of Cd for 50 and 70 weeks developed severe tubular nephropathy with sclerosis, renal anemia and osteopenic osteomalacia (Figs. 1 and 2) compatible with those in human IID. In this experiment, we noticed Fe deposition lines on the mineralization fronts of bones after 50 weeks of Cd treatment (Fig. 3). Quantitative analysis of bones revealed Cd accumulated in the bones decreased and Fe deposited in inverse proportion after 25 weeks of Cd treatment (Fig. 5).

In the next experiment, we intravenously injected 1.0 and 2.5 mg/kg of Cd in OX monkeys for 13 to 15 months. The monkeys of both dose groups showed severe osteopenic osteomalacia, renal anemia and tubular nephropathy compatible with those of IID patients without involvement of malnutrition. Details of these results will be published elsewhere. In this experiments, the monkeys treated with Cd developed diabetes mellitus due to B cell destruction in the pancreas (Fig. 4)

Discussion
Pathogenesis of bone lesion is the keynote issue for the development of novel therapy of IID. The skeletal changes of IID have been reported as being severe osteopenic (osteoporotic) osteomalacia of high turnover type with multiple pathological fractures, irregularly increased osteoid seams and active osteoclasts. Two hypotheses have been proposed for the pathogenesis of the bone lesion: direct and indirect effects of Cd on bone. The former includes toxic effects of Cd on osteoblasts and impendiment of calcification at ossification front by Cd or Fe. The latter indicates nephrogenic osteopenia via hypocalcemia, hypophosphatemia and hyperparathyroidism. Each hypothesis secures solid scientific basis and may not be alternative. The osteopenic osteomalacia in IID patients may be caused by direct and indirect toxic effects of Cd.

Our animal experiments using rats and monkeys clearly demonstrated that hypostrogenism induced by ovariectomy enhanced Cd toxicity, osteopenic osteomalacia and tubular nephropathy distinctive to IID could be reproduced by Cd treatment without involvement of malnutrition such as hypovitaminosis D. Unexpectedly, Fe deposited on the mineralization front of those animals. Noda et al. detected Fe at the mineralization front of IID patients without clinical history of blood perfusion. In addition, osteomalacia was induced in rats fed a diet containing iron lactate, and iron-positive reaction was detected at the interface between osteoid and mineralized bone. It is interesting to know why Fe deposits at the mineralization fronts in replacement of Cd at chronic stage of Cd toxicosis. However, the mechanisms of how Cd and Fe were bound to the bone and how Cd deposited in the bone was replaced with Fe remain unknown.

Vitamin D₂ (Ergocalciferol, Calciferol) and active
metabolites of vitamin D$_3$ have been prescribed to IID patients for bone lesions. However, the efficacy of the vitamin D treatment is unpredictable depending on patients and number of published epidemiological studies concerned with the treatment is very limited. In our previous report\(^6\), the efficacy of calciferol pulse therapy was tested using OX and non-OX rats treated with Cd for 70 weeks. Osteoid volume in the cortical bone and Fe-deposition at mineralization front were decreased by the therapy in non-OX rats but not in the OX rats. The results suggest concurrent administration of estradiol helps the vitamin D therapy for IID patients and the removal of Fe at the mineralization front of bone is important for the recovery to normal bone remodeling.

### Conclusion

Two hypotheses have been proposed for the pathogenesis of the bone lesion of IID patients: direct and indirect effects of Cd on bone. The former includes toxic effects of Cd on osteoblasts and impediment of calcification at the ossification front by Cd or Fe. The latter indicates nephrogenic osteopenia via hypocalcemia, hypophosphatemia and hyperparathyroidism. Each hypothesis secures solid scientific basis and may not be alternative. Our animal experiments using rats and monkeys demonstrated that hypoestrogenism induced by ovariectomy enhanced Cd toxicity, and osteopenic osteomalacia and tubular nephropathy distinctive to IID could be reproduced by Cd treatment without involvement of malnutrition such as hypovitaminosis D. Vitamin D has been prescribed to IID patients for bone lesions. However, the efficacy of the treatment is unpredictable depending on patients. Our experiments suggest concurrent administration of estradiol helps the vitamin D therapy for IID patients and the removal of Fe at the mineralization front of bone is important for the recovery to normal bone remodeling.
References


Pathogenesis of Osteomalacia in Itai-itai Disease

1994.


