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Review

Paradigm Shift in Zinc: Metal Pathology

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Onosaka, S., Tetsuchikawahara, N. and Min, K.-S. *Paradigm Shift in Zinc: Metal Pathology.* Tohoku J. Exp. Med., 2002, **196**(1), 1–7 —— Zinc (Zn) is an essential, common metal in animal tissues. Zn levels were elevated in only four tissues after Zn administration, the highest increase being in the pancreas. Zn concentration was increased by metallothionein induction. Metallothionein-bound Zn significantly reduced the toxicity of the metals Cd, Cu and Hg. It should be noted that tissue Zn levels are different in experimental animals and humans. Acute pancreatitis was observed following the injection of a large dose of Zn. Different metals have different target organs. Using metal pathology, treatments may be developed to save patients suffering from hepatic and renal diseases because Zn is used to a model animal of hepatic or renal disease. ————

zinc; pancreas; metallothionein; lethal organ; metal pathology

Zinc (Zn) is a metal of the IIb family on the periodic table with an atomic number of 30 and an atomic weight of 65.38. In Japan, it is believed that metals of the IIb family are highly toxic and some metals are toxic but not pollutants environmental pollutants. Indeed, mercury (Hg) is the causative agent of Minamata disease and cadmium (Cd) of Itai-itai disease.

Zn is, however, an essential metal, the required amount being 10～20 mg/day/person. It is present in amounts of about 2.5 g in adult humans, and Zn has functions in gene expression, growth and the immune system. The first symptom of Zn deficiency in humans is a taste malfunction. Growth inhibition is observed in severe Zn deficiency. Zn is a coenzyme for many enzymes and essential for insulin to control the blood glucose level.

The toxicity of Zn is lower than that of Cd or Hg and there have been few reports on the effects of excess Zn (Koyama 1994). It should be mentioned that Zn is contaminated with about 1 % Cd.

Zn in tissues

Zn is a common element in animals and plants. The concentration of Zn is 10～30 μg/g in rat tissues except the prostate in which it is extremely high. Zn in cells distributes mainly in the cytoplasm. Zn in tissues is eluted in the

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TABLE 1. Accumulation of zinc in the tissues of rats

Tissue	Normal	Zn 20 mg/kg ×7
Brain	15±1.0	20±2.4
Lung	17±4.2	29±8.0
Heart	17±1.0	18±0.5
Liver	26±0.6	101±5.0
Kidney	23±3.1	50±8.8
Stomach	27±2.5	22±4.3
Small intestine	25±4.0	70±8.0
Pancreas	29±3.1	226±46
Spleen	20±1.5	26±1.3
Testis	27±1.0	33±3.7
Muscle	7±0.6	10±1.4

Rats were injected 7 times s.c. with $ZnSO_4$ at the dose of 20 mgZn/kg every 24 hours. The Zn concentration in the tissues was determined by the use of atomic absorption spectrophotometer. Data indicate mean±S.D. of 3 mice (Onosaka and Cherian 1982).

high-molecular-weight fraction and difficult to detect in the low-molecular-weight fraction following gel filtration. This means that most of basal Zn in tissues is bound to proteins.

Chicks fed a diet supplemented with Zn accumulated Zn in only four tissues. The highest concentrations of Zn were observed in the pancreas, liver, intestinal mucosa and kidney in decreasing order (Oh et al. 1979). No significant accumulation of Zn was observed in the brain, heart, lungs, stomach, spleen, muscle or testes of rats after repeated injections of a salt of this metal (Onosaka and Cherian 1982) (Table 1). In tissues in which the Zn levels were increased, Zn was observed mainly in the metallothionein fraction. Metallothionein is indispensable for Zn accumulation in tissues.

Zn in plasma

The concentration of Zn in the plasma is constant at about 1 μg/ml. It is unclear how the Zn level in the plasma is regulated as no hormone is known to function as a regulating factor of plasma Zn levels. The concentration of Zn in the plasma was increased after injection of a Zn salt and decreased to normal level within 12 hours. Prior injection of actinomycin D maintained the increase in the plasma Zn level until 24 hours (Richards and Cousins 1975). This suggests that synthesis of a particular protein is related to Zn homeostasis, and it was proposed that the protein was metallothionein (MT) which is necessary for hepatic Zn uptake.

The plasma Zn concentration decrease to below 1 μg/ml after feeding them with a Zn-deficient diet. This plasma Zn level is a good indicator of Zn deficiency. A cycle feed intake of 3.5 days was observed in rats fed with a Zn-deficient diet (Tamaki et al. 1995).

Zn and metallothionein

Metallothionein is a low-molecular-weight protein. It binds to metals and is cystein-rich (20 of 61 amino acids residues). Metallothionein has been implicated a wide range of potential homeostatic mechanisms, either in catalysis, storage, the immune system, or detoxication (Kagi and Vallee 1960).

Protein synthesis is inducible by metals (Cd, Cu, Hg, Zn), hormones (glucocorticoids), and organic solvents (ethanol, hexane) among others. In all cases, Zn concentration elevation occurred in tissues in which metallothionein was induced. This indicates that the tissue Zn level is a good indicator of whether metallothionein is induced or not.

The induction of metallothionein by Cd was modified under conditions of Zn deficiency. The metallothionein levels in the liver and kidney were the same in normal and Zn-deficient mice. However, the metallothionein concentration in the pancreas of Zn-deficient mice was lower than that of normal mice (Onosaka and Cherian 1982).

Zn level is high in some animal tissues. It is high in the pancreas of adult mice and extremely high in the livers of newborn rats but decreases to normal levels one month after birth (Wong and Klaassen 1979).

Rats pretreated with a small dose of Cd

were protected against the effects of a lethal dose of Cd. It was clearly demonstrated that an increase in metallothionein-bound Zn induced by the pretreatment with Cd significantly reduced Cd toxicity. It was concluded that the mechanism involved the replacement of Zn bound to metallothionein with Cd because this affinity of Cd for metallothionein is much higher than that of Zn (Suzuki and Yoshikawa 1974). The same defense mechanism confers protection against the toxicity of Hg in the kidney or Cu in the liver.

Prior to 1993, it was widely believed that metallothionein was an essential protein for life. However, it could not be shown conclusively that life would not be sustained under conditions of metallothionein depletion. In 1993, the generation of transgenic mice completely lacking in metallothionein-I and -II was achieved (Michalska and Choo 1993). No differences were reported in the numbers of newborn mice, growth rates or life spans of the normal and transgenic mice, clearly demonstrating that metallothionien is not an essential protein for life.

Zn in human tissues

The Zn levels in some tissues are different in experimental animals and humans. In humans, the Zn concentration in the adult liver is about 50 μg/g, higher than that of mice or rats, but decreases significantly as a result of liver diseases such as chronic hepatitis and cirrhosis (Kameda 1985). A strong, positive relationship was found between the Zn and metallothionein concentrations in the human liver (Onosaka et al. 1986). The regression equation was MT (μg/g)$=-94.7+11.9$ Zn (μg/g). The slope of the regression equation suggests that excess Zn in human tissues is bound mainly to metallothionein because the weight ratio of metallothionein to metallothionein-bound Zn is 14.2.

Zn level is also high in the adult kidney, particularly in Japanese. This is caused not by increased Zn uptake to the kidney, but by the induction of metallothionein by Cd in the tissue. The concentration of Zn in the human pancreas is similar to that in the mouse pancreas. Thus, mice may be a good model for studying the roles of Zn and metallothionein in the pancreas in vivo.

Acute toxicity of Zn

Zn is an essential metal but little is known regarding its toxicity compared to that of other metals of the IIb family (Koyama 1994). It was found that mice died following the continuous administration of a small dose or a single injection of Zn at 100 mg/kg of Zn. All mice injected s.c. with Zn at a dose of 500 mg Zn/kg died within three days.

The weight of the mouse pancreas increased significantly 24 hours after the injection of Zn at a dose of 300 mg Zn/kg. An amylase activity in the Zn-injected mice was increased to 15 times that of the control (Fig. 1). Acinar cell degradation in the mouse pancreas was observed histopathologically 24 hours after Zn injection. Acinar cell necrosis, interstitial cell infiltration and edema were also observed in the tissue of some mice (Fig.2).

These findings indicate that Zn targets the pancreas and causes acute pancreatitis in mice. Thus, the Zn-injected mouse may be useful as a model animal of acute pancreatitis (Onosaka et al. 1998).

Target organs of metals

A toxic compound usually accumulates in a specific organ, and toxic effects are observed in the organ in which the compound concentration is high. Effects such as cell damage and dysfunction are observed in the target organ. The concept of a target organ is most important when studying metal toxicity as metal toxicity is not observed in all tissues but in only specific or target organs.

The target organ of Cd is the liver. Most of the Cd accumulated in the mouse or rat liver after a single injection of a Cd salt (Friberg et al.

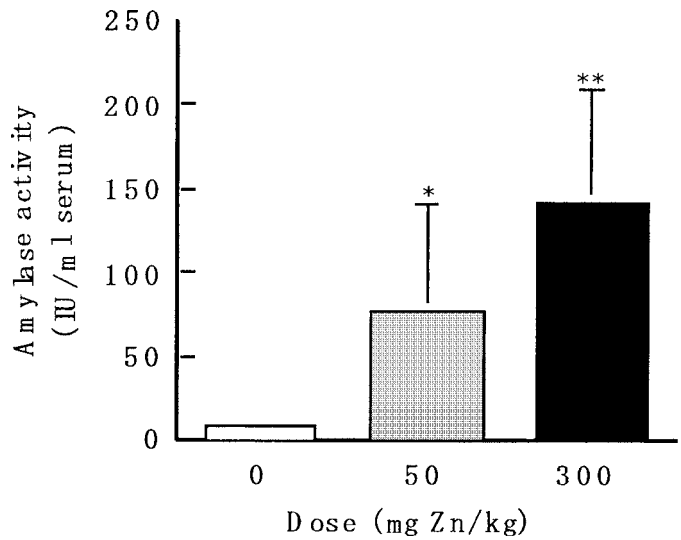

Fig. 1. Amylase activity in mouse serum after zinc injection.
Mice were injected s.c. with $ZnSO_4$ at the dose of 0, 50 or 300 mgZn/10 ml/kg. The amylase activity in serum was determined 24 hour after the injection. Data indicate mean±S.D. of 7 mice (Onosaka et al. 1998).

1975). The testis is another target organ of Cd and bleeding in the testes, one of the toxic effects of Cd, is easily observed. In the case of metallothionein-bound Cd, the target organ is the kidney (Tanaka et al. 1975).

Hg targets the kidney. In animals, over 50% of administered Hg was distributed in the kidney (Naganuma 1983). Organic Hg is lipid-soluble and can pass through the blood-brain barrier reaching the brain and resulting in Minamata disease.

It has been shown that the pancreas is the target organ of Zn. After the Zn injection, the total amount of Zn was the highest in the liver, but the Zn level was the highest in the pancreas. Acute pancreatitis was observed as mentioned above.

Fatal organs

Animals have a number of tissues, but most are not essential for survival.

Patients with stomach cancer, for example, can still live following surgical to removal of the entire stomach tissue, suggesting that the stomach is a dispensable tissue.

In contrast to the stomach, five tissues are essential for life. These are the brain, heart, lungs liver and kindey. When a man died, his friends were reconciled to an explanation that he was a heavy drunker. Patients undergoing artificial dialysis have a dysfunction on the kidney. In this paper, these tissues are designated as "fatal organs" because injury or dysfunction of these tissues leads to death.

When severe brain damage occurs, death is inevitable. The lungs and heart are fatal organs, involved in the exchange, thus transport of oxygen and when irreparable damage to these tissues occurs, death is also inevitable. A more being essential tissues explanation for the necessity of oxygen may be required.

Regarding the liver and kidney, no clear answer is given. The role of the liver is in detoxication. Toxic compounds produced in other tissues are transported to the liver and detoxicated as are xenobiotic compounds. What are these toxic compounds? Are they ammonia, amino acids or other related compounds? The purpose of artificial dialysis, without which patients with damaged kidney would not survive, is the excretion of toxic substances. We were not able to determine the

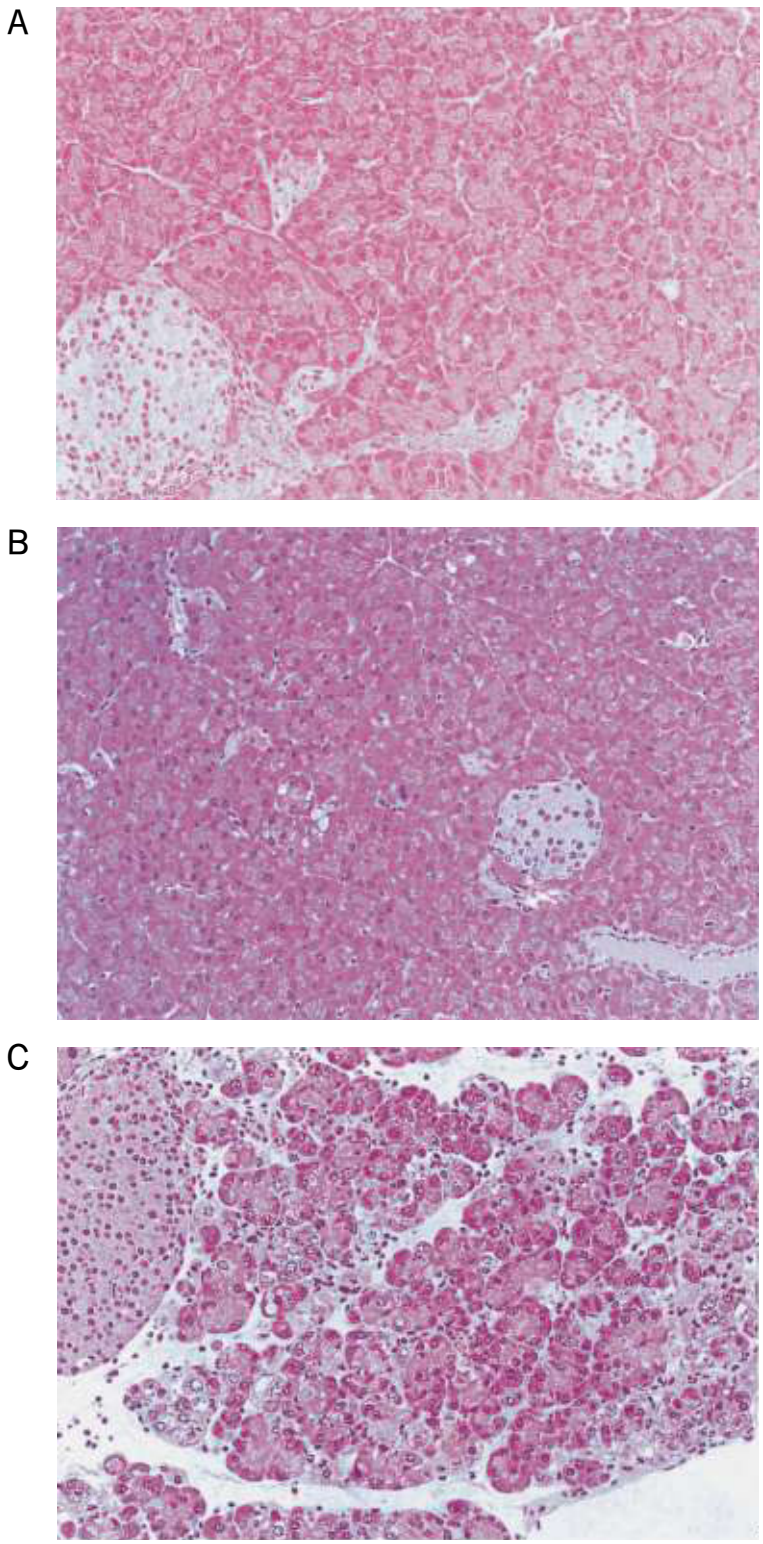

Fig. 2. Pancreatitis by zinc injection in mice.
Mice were injected s.c. with $ZnSO_4$ at the dose of 0(A), 50(B) or 300 mgZn/10 ml/kg(C). The pancreas was removed 24 hour after the injection, and treated with H-E stain (×170). (A) no remarkable changes were found. (B) an acinar cell degradation was observed. (C) acinar cell degradation, acinar cell necrosis, interstitial cell infiltration and edema were observed. (A) Animal No. 3, (B) Animal No. 1, (C) Animal No. 5.

toxic compounds that accumulate in the liver because no suitable animal models were available for these experiments.

It has been reported that the death of rats i.v. administered with large doses of Cd may have been due to liver failure (Dudley et al. 1982). Mice survived an injection of Cd at the lethal dose following an injection of olive oil, phytine, heptane, thiopronine or shosaikoto. The common feature of these compounds is that they induce metallothionein (over 100 μg/g) only in the liver (Onosaka and Tanaka 1991). These findings indicate that the target organ of Cd is the liver, and Cd is useful when studying the death of animals due to hepatic damage.

The fatal organ of Hg is the kidney because of the high distribution of administered Hg in this organ. In the case of Cu, its fatal organ is the liver, based on the accumulation of injected Cu in this organ.

Patients with pancreatic cancer who undergo total extirpation of the organ can remain alive if supplies of insulin and digestie enzymes are sufficient. However, patients still die due to acute pancreatitis every year. It was observed that mice died following injection of a large dose of Zn, and acute pancreatitis was observed in the mice. Thus, whether the pancreas is a fatal organ needs to be clarified.

Metal pathology

The greatest difficulty for toxicologists is to obtain suitable animal models. There are many toxic metal compounds, but few are mortal in a short period. Moreover, the determination of target tissues of toxic lethal metal compounds is difficult in many cases.

While metals are essential for animals, heavy metals such as Cd and Hg are extremely toxic. The common toxicologically features of heavy metals are difficult to determine because different metals have different forget organs. Thus, it is necessary to study why patients die as a result of damage to fatal tissues and this may lead to a new field of metal pathology in which experimental animal models are used. Metal pathology is designated as the branch of science dealing with the study of metals toxic to experimental animals.

In metal pathology

1) Acute toxicity of metals can be studied when animals die in a short period of time following injection of a large dose of a toxic metal compound.

2) Different metals have different target organs. Cd and Hg target the liver and kidney, respectively, to cause the death of animals (this should be conclusively shown in the future). It is expected that Cd can be used to clarify the functions of the liver and Hg the functions of the kidney.

3) An experimental animal dies following administration of a toxic compound. The levels of compounds change leading to the death of an experimental animal. In this case, it is extremely difficult to detect the fatal compound produced. Heavy metals are so toxic that in most cases their injection experimental animals causes death. The animals, however, can survive a lethal dose of such metals following pre-induction of metallothionein. The level of the toxic compound in the tissues must change following administration of the toxic metal, and be the same between normal and metallothionein pre-induced animals.

4) Organic compounds are metabolized in vivo, but metals cannot be decomposed chemically. It is easy to determine metal concentrations in tissues using AAS, ICP or ICP-MS.

It is expected that by means of metal pathology toxic compounds that target the liver and kidney will be identified as will be the target organ of Zn. A new method should be developed to extend the lifespan of patients suffering from diseases related to heavy metal-poisoning by inhibiting the toxicity of such lethal compounds.

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