Metallothioneins and diseases with special reference to cadmium poisoning

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Metallothionein (MT) a low molecular weight metal binding protein, is involved in several human diseases. Brain tissue from Alzheimer´s patients contains lower concentrations of Growth Inhibitory Factor or MT-3. Induction of MT by bismuth increases resistance to renal toxicity of cis-platinum and may be advantageous in cancer therapy. Cadmium exposure induces MT-synthesis in liver, binding Cd and protecting against acute toxicity. Cd-MT is released from liver into plasma, filtered in renal glomerulus and absorbed in tubules. Lysosomal breakdown of MT releases toxic Cd. This mechanism explains renal tubular damage after long-term exposure to Cd. Impaired tubular regulation of calcium and vitamin D metabolism contributes to the development of the adverse effects on the skeleton. Quantitative, specific polymerase chain reaction (PCR) studies showed increased expression of mRNA of MT in testicles after Cd exposure, supporting the notion that MT increases cellular resistance to metals and protects from Cd toxicity. This idea was advanced by one of the present authors thirty years ago.

Introduction

Metallothionein (MT) is a low molecular weight, sulfur rich, metal binding protein, which may be related to several human diseases as a marker of tissue reaction or sensitivity or as an etiologic component in the disease process. There is also data pointing to its possible usefulness as a marker of importance in the therapy of certain diseases [1].

A role for metallothionein is well established in the etiology of cadmium poisoning. A major part of this paper will deal with various aspects – both experimental and clinical – of cadmium poisoning. However, first, brief overviews of other aspects of MT – and diseases will be given.

MT as an etiologic or modifying factor in human diseases. MT in disease treatment

Metallothionein is related to some diseases. It has been shown that the concentration of the recently discovered new form of the protein, MT-3 or GIF (Growth Inhibitory Factor), is decreased in brain tissue from Alzheimer patients. Neurotropic activity of neonatal rat brain neurones was inhibited by GIF (MT3) [2]. The role of MT-3 in brain development and function is presently under investigation. MT-3 deficient mice show no behavioural disturbance or learning difficulties, even in old age [3]. Even if these studies have been performed on mice there is a need for further human research in this area. It should be mentioned that behavioural disturbances or learning difficulties are different from disturbances seen in Alzheimer diseases, which starts later on in life after the subject has had a normal development. The chemical and structural characteristics of MT-3 have recently been investigated (Vasak personal communication). The overall nomenclature for MT as a family name for low molecular weight sulfur rich metal binding proteins according to the consensus reached at the international meeting on MT [4,5] is still applicable. The molecular mechanisms involved in explaining a role of MT in elicitation of neurotoxicity and the possible involvement of various metals needs to be studied further [6].

A role of MT in Copper (Cu) metabolism has been established [7]. In Wilson's disease liver accumulation of copper occurs to a considerable extent as Cu-MT. This is due to a genetically determined deficiency in transport of Cu into bile in this disease with increased liver levels of Cu [8]. MT has also been shown to be present in the placenta of patients suffering from Menke's disease. This disease is a genetically determined disease with deficient intestinal and placental transport of Cu. The role of MT in Indian liver cirrhosis is still a matter of discussion. In addition to high dietary intake of Cu, a genetic factor is likely to be involved in the aetiology of this disease [8]. It is possible that children with deficient MT synthesis develop this disease more frequently upon exposure to excessive copper in the diet than children with normal synthesis of MT. Further evidence is required before this hypothesis can be considered established. MT-3 is considered to be expressed particularly in brain tissue.

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However, it has been reported that MT-3 mRNA is expressed also in some other tissues [9].

During the last years [10] various types of tumours in humans and in animals have been characterised with regard to their expression of MT genes. The susceptibility of MT-null mice to topical application of carcinogenic compounds [11] is a related finding. Observations of variation of tumour sensitivity depending on MT level has lead to the suggestion that cadmium (Cd) could possibly be used as a cancer treatment agent. This suggestion is based on the observation that liver tumours did not develop in mice that were treated with a combination of Cd and NDEA (N-nitrosodiethylamine) whereas animals treated orally only with the tumorigenic agent NDEA had liver tumours [12]. It was shown that metallothionein levels were markedly reduced in livers of tumour bearing animals compared to normal animals without tumours in their livers. The effect of cadmium treatment on liver tumours, thus could well be explained by their higher sensitivity to cadmium due to lack of expression of the metallothionein gene.

It has also been shown that bismuth can induce metallothionein in normal renal tissue and such induction can increase the resistance to renal side effects of cis-Pt. This allows larger doses of cis-Pt to be used in cancer treatment and better therapeutic effect to be achieved [13]. These observations in animals are presently being considered as a basis for clinical use in Japan.

MT induction by zinc given orally is used in the treatment of Wilson's disease. This kind of induction can block the intestinal uptake of copper and decrease the toxic tissue levels of copper usually accumulating in this disease [14].

The potential role of MT in disease treatment is shown in table I.

Table I. Treatment of diseases.

Cancer	
Cd	Possible use against Cd-sensitive liver tumours.
Bi	MT induction in normal tissue reducing renal side effects of cis-Pt.
Wilson´s	
Zn	MT induction in intestine blocks copper uptake and decreases Cu tissue load.

Role of MT in explaining the elicitation of kidney damage in cadmium poisoning

As mentioned, the best-established role of metallothionein in metal toxicology is in relation to cadmium toxicity. It is known [15] that cadmium can give rise to damage to the kidney in the form of proteinuria and calciuria, to the lung, prostate and testis in terms of cancer and to the skeleton as itai-itai disease and osteoporosis. For all these types of damage, MT is directly or indirectly involved, as will be discussed in the following text.

Chronic cadmium poisoning is a condition which may occur as a result of long term industrial exposure, or from long term intake of food with increased cadmium concentrations [15,16]. Itai-itai disease is an extreme form of chronic cadmium poisoning by the oral route, which will be briefly described in the following text. After the World War II, Dr. Hagino, a general practitioner, discovered a number of patients suffering from a bone disease with multiple fractures and deformities of the spine and the long bones occurring in a village (Fuchu) in Toyama, prefecture in western Japan. The patients suffered severe pain and complained itai-itai (ouch-ouch) and the disease was therefore called itai-itai disease. The patients lost body height and had deformities of the spine, a result of multiple vertebral compression fractures. In the long bones, characteristic pathologic fractures with osteoid formation (Milkman´s pseudofractures) occurred.

Table II. Cd-related diseases.

Chronic cadmium poisoning
Renal tubular dysfunction with proteinuria, calciuria, anaemia, osteoporosis, lung cancer, reproductive disturbance (?), cancer of prostate and testis (?).
Itai-itai disease
Osteomalacia, osteoporosis, renal tubular dysfunction, malabsorption, anaemia.

This latter finding is typical of osteomalacia. By analysis of urine from cases and non-cases in the endemic area and persons from non-endemic areas, it was demonstrated that there was a considerably increased excretion of cadmium in urine, particularly in the cases in the endemic area. Kidney damage with proteinuria occurred in these patients. It was thus obvious that itai-itai disease was a form of renal osteomalacia, kidney damage being of basic importance for the development of bone effects. The main factor explaining this disease to the kidney is the excessive Cd intake from Cd contamination of rice, which occurred as a result of cadmium containing waste water from a smelter being discharged into a river, which was used for irrigation of rice fields. The relatively low calcium content of Japanese food may have been a contributing factor. Also the tradition of dressing so as to screen away from the sunshine gave the women in this area only a low contribution of vitamin D, synthesised by the action of UV-light on the skin. The role of MT in explaining the characteristics of this disease derives from the toxicology of cadmium. After uptake of cadmium from the gut into blood, cadmium is initially taken

up in the liver and then, in the long term, gradually moving over to the kidney. The reason for this redistribution of cadmium has been shown to be due to its binding to MT, which has such a low molecular weight, that it is readily filtered through the glomerular membrane and ends up selectively in the renal tubules [17,18,19]. After degradation of CdMT, cadmium causes cellular damage in the renal tubules with typical histological features such as dilated tubules and a flattened epithelium.

These mechanisms initially demonstrated by our research group and subsequently confirmed by others, includes binding of Cd to albumin, immediately after uptake. Cadmium in this form is predominately taken up in the liver where cadmium is released and induces MT, that binds with cadmium to form CdMT [18,19,20,21,22]. Some time after a single dose, a predominant proportion of liver cadmium is bound to MT. A small part of such CdMT is released into blood plasma and because of its low molecular weight it passes freely through the glomerular membrane and occurs in the tubular fluid of the kidney. Like other low molecular weight proteins CdMT is taken up by pinocytosis into the lysosomes of the kidney. CdMT is degraded in this compartment and "free" cadmium is released into the cytoplasm. And this free cadmium can both cause damage to sensitive sites in the renal tubular cells and at the same time induce *de novo* synthesis of MT, forming CdMT as a toxicologically inactive pool of Cd in renal tubular cells.

The balance between influx of CdMT into the renal lysosomal compartment on the one hand and the rate of synthesis of MT in this compartment on the other hand, regulates the pool of intracellular "free" cadmium, that can interact with sensitive cellular targets. When there is efficient MT synthesis and influx of CdMT into the lysosomes is limited, the free cadmium pool is limited and no membrane damage occurs. When CdMT influx into the lysosomal compartment is high and MT-synthesis is efficient, the pool of free cadmium is large enough to cause damage to the renal tubular cell. There has been some controversy over the role of CdMT as a sequestering agent in the course of cadmium induced renal tubular toxicity and the sensitive site in the cell has not been identified. To improve our knowledge in these respects the following experiment was performed.

One group of rats which had preinduced MT synthesis by pre-treatment with cadmium (pre-treated) was compared with a group of non-pre-treated rats with low cellular metallothionein concentration; both groups were given a challenge dose of radiolabelled Cd-MT [23]. A considerably larger proportion of the radiolabelled cadmium in the subcellular membrane fraction (isolated by subcellular ultracentrifugation techniques) was recovered in a high molecular fraction in the non-pre-treated animals than in the pre-treated ones. In the latter group a larger proportion was bound to fractions corresponding to metallothionein and possibly other low molecular weight proteins in the membrane [23]. These findings are in keeping with the hypothesis that cadmium bound to non-metallothionein sites in the cellular membranes is of decisive importance for elicitation of the toxic effects of cadmium on the kidney. It should also be mentioned in this context that the animals, which were pre-treated with Cd, were protected against toxic effects of Cd-MT, whereas non-pre-treated animals later developed nephrotoxic effects. Another observation, which may be of importance when discussing mechanisms of cadmium nephrotoxicity is the early perturbation of calcium metabolism preceding the development of proteinuria after CdMT injection [24]. It is also interesting to note that in studies of uptake and binding of calcium to membranes isolated from the renal cortex of Cd-MT exposed animals, there is a considerably lower binding and uptake in Cd-exposed animals than in controls. This is true both in luminal and particularly in basolateral membranes [24,25]. Thus, it is likely that the basolateral calcium pumps constitute a primary target for Cd. While these observations were made in single-dose-experiments, when the disturbances of renal tubular function were reversible, it is quite possible that similar mechanisms, if occurring in the situation of continuous inflow of Cd-MT into the renal tubular compartment, would give rise to permanent damage. Since cellular calcium metabolism is perturbed, apoptosis may be involved in the process leading to permanent damage. When a model with repeated CdMT administrations (interval two hours) was used, calciuria remained for a much longer time [26]. In a subchronic experiment Tanimoto *et al*. [27] demonstrated apoptotic cells. These last mentioned experiments mimic the situation in human exposures, where influx of CdMT in to the renal tubular compartment can be assumed to be more continuous and renal damage is often permanent.

Increasing levels of intracellular MT, seen in renal tissue upon repeated or long-term administration of Cd protect against nephrotoxicitry of parenterally administered MT. This phenomenon was previously demonstrated in Wistar rats [23,28] and has recently been confirmed by comparison of CdMT nephrotoxicity in wild type and MT-null mice [29]. The role of MT in explaining the protection afforded by pretreatment by Zn against CdMT induced nephrotoxicity in experimental animals [30,31] remains controversial. An important role of induced heat shock proteins in addition to MT [32] may be the explanation why MT-null mice were as susceptible as controls to intravenous CdMT nephrotoxicity [33].

Role of MT in modifying Cd effects in reproductive tissues

Since the first reports in the 1970's, a considerable number of publications have discussed the involvement of metallothionein as a protective agent against tissue damage from cadmium in reproductive tissues. In the first study performed by one of the present authors, [34] it was demonstrated that in mice, pre-treated with small not testicle damaging doses of cadmium, a normally testicle damaging dose did not cause testicular damage. Compared to non-pre-treated animals a larger proportion of testicular cadmium in

pre-treated animals was bound to a low molecular weight protein separated by gel filtration chromatography (Sephadex G-75) and assumed to be MT [34]. Subsequent studies by others have questioned the existence of MT in the testis and the prostate and this has been the subject of considerable controversy. Some authors consider that the lack of expression of MT in these tissues would explain their sensitivity to development of cancer subsequent to cadmium exposure.

In our own studies in rats, apoptosis was indicated by DNA-elecrophoresis 48 and 72 hours after a single injection of 5-10 µmol/kg of $CdCl_2$. Expression of MT-1 occurred to a similar extent in controls and cadmium treated animals according to these RT-PCR-studies [35]. On the other hand expression of p53 gene decreased markedly with increasing doses of cadmium in testicular tissue. In the ventral lobe of the prostate a slight increase in MT-1 expression was noted, while p53 remained largely unchanged. In subsequent studies, extending the survival time to 96 hours [36], similar findings occur. But additional studies [36] of the expression of the c-jun gene showed an expression of this protooncogene at 96 hours in the testis and ventral prostate. There was an uncertain increase in the expression of MT-1 in the prostate and a clear increase in relation to controls in the liver.

Further studies (unpublished) using a quantitative RT-PCR method employing computerised assessment of integrated optical density (IOD) of the expression of the MT-1-gene in comparison to the β-actin gene showed that there was an increased expression of both MT-1 and MT-2 in the testicle subsequent to exposure to 10 µmol/kg of Cd in rats. These data are in accordance with data published by Suzuki *et al.* [37]. The long-standing debate about whether MT is expressed in testicular tissue thus seems to be resolved.

Conclusions

MT is related to several human diseases as a marker of tissue reaction or sensitivity or as an etiologic component in the disease process.

MT as marker of tumour susceptibility has potential use in anti cancer chemotherapy.

In relation to etiology of disease, the role of MT is best established in chronic cadmium poisoning. But a role of MT-3 in Alzheimer´s disease is also possible.

MT induced by Cd in liver, binds Cd and transports it to the kidney where Cd MT is degraded and Cd causes tubular damage, probably involving blockage of basolateral Ca-pumps.

MTs induced in tissues increase their resistance to Cd-toxicity.

Controversy over expression of MT genes in reproductive tissues appears to be resolved by detection of increased mRNA levels in Cd-exposed animals using quantitative PCR-methods.

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