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Zinc supplementation can reduce accumulation of cadmium in aged metallothionein transgenic mice

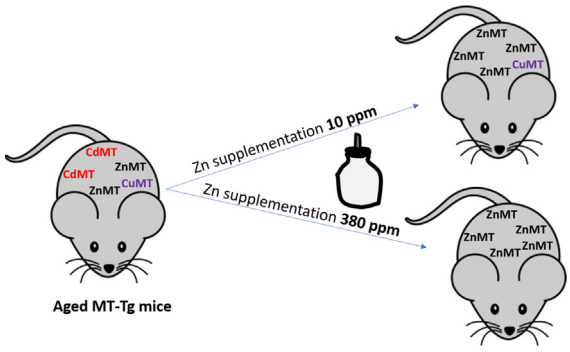
Kamil Pabis [a], Claudia Gundacker [a], Robertina Giacconi [b], Andrea Basso [b], Laura Costarelli [b], Francesco Piacenza [b], Sergio Strizzi [c], Mauro Provinciali [b], Marco Malavolta [b, *]

[a] Center for Pathobiochemistry and Genetics, Institute of Medical Genetics, Medical University of Vienna, 1090, Wien, Vienna, Austria
[b] Advanced Technology Center for Aging Research, Scientific Technological Area, IRCCS-INRCA, Ancona, Italy
[c] Department of Life and Environmental Sciences, Università Politecnica delle Marche, Ancona, Italy

HIGHLIGHTS

- Mice overexpressing MT1 (MT-Tg) show more Cd bound to MT than non-transgenic mice.
- High and low dose Zn supplement reduces (>30%) Cd in organs of aged MT-Tg mice.
- High-dose Zn supplement may cause an imbalance of Zn to Cu ratio.

GRAPHICAL ABSTRACT



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ABSTRACT

Epidemiologic studies suggest that exposure to Cd is related to a multitude of age-related diseases. There is evidence that Cd toxicity emerges from an interference with Zn metabolism as they compete for the same binding sites of ligands. The most responsive proteins to Cd exposure are the metal-binding proteins termed metallothioneins (MTs), which display a much greater affinity for Cd than for Zn. Most studies have considered the effect of Zn on the accumulation of exogenous Cd and tissue damage, whereas observational studies have addressed the association between Zn intake and Cd levels in body fluids. However, it has not been addressed whether supplemental Zn can lower Cd levels in organs of healthy aged animals without affecting Cu stores, a question more pertinent to human aging. We therefore aimed to investigate the effect of Zn supplementation on Cd levels in liver and kidney of aged MT transgenic mice (MT1-tg) overexpressing MT1 at levels more comparable to those observed in humans than non-transgenic mice. We found a >30% reduction of kidney and liver Cd levels in Zn supplemented MT1-tg mice compared to non-supplemented controls, independently of the dose of Zn, without a significant reduction of Cu. Our data support the idea of a causal and inverse relationship between Zn intake and Cd content in organs of aged MT1-tg mice as suggested by observational studies

* Corresponding author. IRCCS-INRCA, Advanced Technology Center for Aging Research, Scientific Technological Area, Ancona, Italy, via Birarelli 8, 60121, Ancona, Italy.
E-mail address: m.malavolta@inrca.it (M. Malavolta).

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in humans. Our work provides the rationale for interventional studies to address the effects of Zn supplementation on Cd burden in elderly people.

1. Introduction

Cadmium (Cd) is a toxic transition metal that has no essential biological role in humans and that has proven to be an important threat to human health (Åkesson et al., 2014; Chen et al., 2018; Jaishankar et al., 2014). Humans are exposed to this metal by inhalation and ingestion. While the daily exposure is generally low, there are no identified means to eliminate excess Cd, so that throughout life it will gradually accumulate in the body, in particular in the liver and kidney which are the main target organs of Cd toxicity (Faroon et al., 2012). Epidemiologic studies suggest that even low level Cd exposure promotes Cd accumulation in tissues and leads to the development of age-related diseases, such as hypertension (Tellez-Plaza et al., 2007), cardiovascular and peripheral arterial diseases (Fagerberg et al., 2013; Tellez-Plaza et al., 2013), chronic kidney disease (Navas-Acien et al., 2009), diabetes (Schwartz et al., 2003), osteoporosis (Gallagher et al., 2008), hearing loss (Choi et al., 2012) and physical decline (Kim et al., 2017). In particular, the prevalence of chronic kidney disease in the general population is estimated at 13% (Navas-Acien et al., 2009) urging the development of preventative therapies.

There is evidence that Cd toxicity is related to its interference with Zn metabolism. Cd has lower stability than Zn in complexes with oxygen and nitrogen ligands but is much more thiophilic than Zn. Cd concentrations therefore must be higher than Zn concentrations to displace Zn from coordination sites with oxygen and nitrogen but need to be only one thousandth of those of Zn to displace it from sulfur sites (Jacobson and Turner, 1980). Low serum Zn is associated with increased risk of Cd-induced renal damage (Lin et al., 2014) and increased cancer mortality in adults exposed to Cd (Lin et al., 2013). The most responsive proteins to Cd exposure are the metal-binding proteins named metallothioneins (MTs). MTs comprise a family of ubiquitous (i.e., MT1 and MT2) and tissue specific (i.e., MT3 and MT4) isoforms that participate in the cellular response to numerous forms of stress (Maret and Krezel, 2007). The much higher affinity of MTs for Cd than Zn contributes to the long half-life (~26 y) of total body cadmium. Even though MTs are responsible for the accumulation of Cd in the body, these proteins play a positive role in longevity, as suggested by the long-lived and short-lived phenotype of MT overexpressing (Malavolta et al., 2016, 2012; Yang et al., 2006) and MT-KO mice (Kadota et al., 2015), respectively.

The interplay among MTs, Cd and Zn may be of particular interest in the elderly, due to the high prevalence age-related Zn deficiency (Giacconi et al., 2017; Wong et al., 2013), the possibility that MTs are increased in response to inflammatory changes (Mocchegiani et al., 2004) and the increased levels of urinary Cd (Duc Phuc et al., 2016; Moriguchi et al., 2005; Phuc et al., 2017; Sun et al., 2016) and tissue Cd (Friberg et al., 1986; Wills et al., 2008) with aging.

The establishment of a causal relationship between Zn intake and organ levels of Cd in presence of high levels of MTs would pave the way to reduce the levels of Cd accumulated in aged organs by Zn supplementation.

Surprisingly, most experimental studies focused on the interaction between Zn and exogenous Cd administration and have relied on mice and rats, which have unusually low MT levels compared to large mammals like humans (Henry et al., 1994). For example, it has been long known that Zn deficiency exacerbates accumulation of Cd (Reeves et al., 2005; Waalkes, 1986) and its carcinogenic effects (Waalkes et al., 1991). More recently authors shifted their attention to supplemental Zn showing that Zn protects against liver injury induced by Cd administration (Rogalska et al., 2011), reduces Cd accumulation in vitro (Zhang et al., 2014) and attenuates Cd-induced lipid peroxidation in plasma (Jemai et al., 2007).

Although, Cd competes with Zn for binding sites on MTs (Goyer, 1995), it is unknown if a reduction of Cd in organs of aged animals is achievable by Zn supplementation in the presence of a high basal expression of MTs. Moreover, there is the possibility that such supplementation would further induce deficiency of Cu (Willis et al., 2005) thus aggravating tissue Cd loads. To address these questions, we determined Cd levels in the liver and kidney of Zn-supplemented MT transgenic mice (MT1-tg) and measured the levels of Zn and Cu in organ protein extracts from supplemented mice.

2. Methods

2.1. Mice and Zn supplementation

Mouse tissues were recovered from a biobank of mouse tissues established at INRCA. Samples were derived from C57BL/6J-Tg(Mt1) 174Bri/J mice (MT1-tg) obtained from The Jackson Laboratories (Bar Harbor, ME) and previously characterized (Iszard et al., 1995). MT1-tg mice are visually indistinguishable from non-transgenic littermate controls (C57BL/6J strain) but they display 6–20-fold higher basal levels of MT protein in liver and kidney as well as in other organs. Part of these mice were given Zn supplemented drinking water with 10 ppm (n = 6 mice) or 380 ppm (n = 6 mice) of Zn for 1 month in the form of $ZnSO_4$. The 10 ppm dose one previously reported to exert partial protection against the formation of preneoplastic lesions in mice fed high levels of dietary iron (Park et al., 2012), while the 380 ppm dose was shown to be among the highest concentration of Zn that can be administered in mice without evident toxicological effects (Malavolta et al., 2012). The supplementation started at around 600 days of age (equivalent to 60–65 years in humans). Another group of MT1-tg mice was bred in identical condition without Zn supplementation (n = 5) and served as control. A few samples of liver explants from non-supplemented adult (3–6 months) C57BL/6J and MT1-Tg mice were additionally used to compare Cd distribution and levels in protein extracts. All tissues from mice stored at INRCA biobank received a standard diet (Mucedola 25/18 CR basic, 31 ppm Zn) which included only trace amounts of Cd. However, due to their high MT expression, these mice accumulate more Cd than C57BL/6J mice making them more similar to humans. Daily Zn consumption from diet amounted to a mean 4.5 mg/kg bodyweight in aged mice plus 2–2.5 mg/kg bodyweight from the lowest dose of Zn supplementation (10 ppm). While the amount of Zn consumed with the diet is higher than Zn intake from supplemented water at 10 ppm, the Zn in the form of $ZnSO_4$ should be more easily absorbed than the Zn included in the pellet. Moreover, the 10 ppm dose is grossly comparable to a supplementation with 10–15 mg/day in humans based on a conversion