Published in final edited form as:
Liver Int. 2009 September ; 29(8): 1222–1229. doi:10.1111/j.1478-3231.2009.02007.x.

Changes in hepatic gene expression in response to hepatoprotective levels of zinc

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Abstract

Background—Zinc (Zn) administration at non-toxic doses protects against the hepatotoxicity produced by many agents, but the underlying mechanisms remain elusive.

Aim—To examine the basis of Zn-induced generalised hepato-protective effects.

Methods—Rats and mice were given Zn at known hepato-protective levels (100 μmol $ZnCl_2$/kg/day, s.c., for 4 days) and molecular responses were assessed.

Results—Zn treatment produced changes in 5% of the genes on custom-designed mouse liver array and Rat Toxicology-II array. Changes in gene expression were further confirmed and extended by real-time reverse transcriptase-polymerase chain reaction. Zn treatment dramatically increased the expression of the metallothionein (*Mt*), and modestly increased the expression of acute-phase protein genes (*ceruloplasmin*, *Stat3*, *egr1*, *Cxc chemokines* and heat-shock proteins). For genes encoding for antioxidant enzymes, some were increased (*Nrf2* and *Nqo1*), while others remained unaltered (*Cu, Zn SOD* and glutathione *S*-transferases). Expressions of cytokine and pro-inflammatory genes were not affected, while genes related to cell proliferation (*cyclin D1*) were modestly upregulated. Some metabolic enzyme genes, including cytochrome P450s and UDP-glucuronosyltransferase, were modestly suppressed, perhaps to switch cellular metabolic energy to acute-phase responses. Liver Zn content was increased between 1.6- and 2.1-fold, while hepatic MT protein was increased between 50 and 200-fold. Mice typically showed greater responses than rats.

Conclusion—Such gene expression changes, particularly the dramatic induction of MT and Nrf2 antioxidant pathway, occur in the absence of overt liver injury, and are probably important in the hepatoprotective effects of Zn against toxic insults.

Keywords

gene expression; hepatoprotection; metallothionein; Nrf2; zinc

Zinc (Zn) is an essential trace element with many biological roles as, for example, in hundreds of Zn-containing enzymes and thousands of Zn-finger proteins (1). Zn also acts as a growth cofactor, immunoregulator and antioxidant with anti-inflammatory and anti-

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apoptotic effects (2–4). The liver is an important tissue for regulation of Zn storage and homeostasis, and Zn is clearly necessary for appropriate liver function (4).

Zinc administration at non-toxic levels protects against chemically induced acute and chronic liver injury in experimental animals (Table 1). Zn treatment reduces the hepatotoxicity produced by a wide variety of diverse agents such as carbon tetrachloride (5), bromobenzene (6), thioacetamide (7), ethanol (8), D-galactosamine (9), endotoxin (10), D-galactosamine plus lipopolysaccharides or tumour necrosis factor (TNF)-α (11). Zn pretreatment also decreases liver injury produced by drug overdose, such as acetaminophen (12, 13), salicylate (14) and cisplatin (15). Zn is also well known to protect against hepatotoxicity of metals, such as cadmium (16), mercury (17), arsenic (18), nickel (19) and copper (20). However, the precise mechanisms through which Zn produces this generalised hepatoprotection to such a widely divergent group of hepatotoxicants are not fully defined.

Microarray analysis of gene expression has been recently used to profile genetic response patterns associated with Zn deficiency (21–23). The most consistent change in the livers of Zn-deficient rats is a dramatic (−13 to −100 fold) downregulation of metallothionein (MT), which is encoded by the *Mt1* and *Mt2* genes, along with a variety of aberrant expression of genes encoding lipid metabolism, xenobiotic metabolism, stress response and signal transduction (21–23). MT is an important Zn-regulated small metal-binding protein, often playing an important role in the magnitude and progression of toxic insult in the liver (24). This is probably through maintenance of cellular Zn homeostasis, as well as through its antioxidant properties, as MT is rich in sulphydryl groups (1, 3, 25). However, little is known about the gene expression profiles following Zn supplementation, or after treatment with a hepatoprotective level of Zn. Thus, it is clearly important to profile the gene expression patterns following a hepatoprotective low dose of Zn exposure in experimental animals for a variety of reasons, including both toxicological and potentially nutritional.

This study was designed to examine hepatic gene expression response following treatment with known hepatoprotective levels of Zn in rats and mice. The dose (100 μmol/kg, s.c.) and duration (once daily for 4 days) of Zn treatment used in this study is well documented to achieve chemical- and self-hepatoprotection without itself producing any liver damage, and thus the gene response patterns involved can be evaluated in the absence of any toxic response. In addition, hepatic Zn and several protective proteins, particularly MT, were determined. The results clearly demonstrate that Zn-induced gene expression patterns are similar in hepato-protected rats and mice, and that such Zn-induced gene expression profiles, particularly the dramatic increases in *Mt* expression, are important in the generalised hepato-protective effects of Zn against diverse toxic insults to the liver.

Material and methods

Chemicals

Zinc chloride ($ZnCl_2$) was obtained from Sigma Chemical Co. (St Louis, MO, USA) and dissolved in isotonic saline. The Mouse Custom Atlas Array (600 liver-selective genes) and Rat Toxicology-(II) Atlas Array (456 genes) were obtained from BD Biosciences (Clontech, Palo Alto, CA, USA). [α-^{32}P]dATP was obtained from Perkin-Elmer Life Sciences (Boston, MA, USA). Mono-clonal antibodies against MT were purchased from Dako Cytometry (San Jose, CA, USA). All other chemicals were commercially available and of reagent grade.

Animal treatment and sample collection

Adult male Sprague–Dawley rats (225–250 g) and CD1 mice (25–30 g) were purchased from Charles River Laboratories (Raleigh, NC, USA) and were maintained in Animal Facilities at the National Institute of Environmental Health Sciences with standard rodent

chow and tap water for 1 week before experimentation. Animal care was provided in accordance with the US Public Health Policy on the Care and Use of Animals, and the study protocol was approved by the Institutional Animal Care and Use Committee.

The hepatoprotective dose for $ZnCl_2$ was selected based on the literature (5–15), and a commonly used dose of Zn (100 μmol/kg) was injected s.c. to mice in a volume of 10 ml/kg, or to rats in a volume of 2 ml/kg, once per day in the morning for four consecutive days. Control animals were injected with the same volume of vehicle (saline). Twenty-four hours after the last dose, animals were killed by CO_2 asphyxiation and the livers were removed, weighed, snap-frozen in liquid nitrogen and stored at −80 °C until analysis.

Microarray analysis

Total RNA was isolated from liver samples with TRIzol agent (Invitrogen, Carlsbad, CA, USA), followed by purification with RNeasy columns (Qiagen, Valencia, CA, USA). Approximately 5 μg of total purified RNA was converted to [α-^{32}P]-dATP-labelled cDNA probe using MuLV reverse transcriptase and the Atlas customer array specific cDNA synthesis primer mix, and then purified with a NucleoSpin column (Clontech). The membranes were prehybridized with Expresshyb from Clontech for 2 h at 68 °C, followed by hybridization with the cDNA probe overnight at 68 °C. The membranes were then washed four times in 2 × SSC/1% SDS, 30 min each, and two times in 0.1 × SSC/0.5% SDS for 30 min. The membranes were then sealed with plastic wrap and exposed to a Molecular Dynamics Phosphoimage Screen. The images were analysed densitometrically using ATLASIMAGE software (version 2.01, Clontech, CA, USA). The gene expression intensities were first corrected with the external background and then globally normalised.

Quantitative reverse transcriptase-polymerase chain reaction analysis

The levels of expression of the selected genes were quantified using real-time reverse transcriptase-polymerase chain reaction (RT-PCR) analysis. Briefly, purified RNA was reverse transcribed with MuLV reverse transcriptase and oligodT primers. The forward and reverse primer sequences for selected genes were designed with the ABI PRIMER EXPRESS software (Foster City, CA, USA). The Power SYBR Green Master Mix (Applied Biosystems, Cheshire, UK) was used for real-time PCR analysis. The relative differences in expression between groups were expressed using cycle time (C_t) values as follows: the C_t values of the interested genes were first normalised with β-actin of the same sample, and then the relative differences between control and treatment groups were calculated and expressed as relative increases, setting control as 100%.

Western-blot analysis

Tissues were homogenized (1:20, w:v) in PER-Tissue Protein Extraction buffer (Pierce, Rockford, IL, USA) containing freshly added protease inhibitor cocktail (CalBiochem, La Jolla, CA, USA) and 500 μM phenyl-methylsulphonyl fluoride. Cytosols were prepared by centrifugation at 12 000 *g* for 10 min at 4 °C. Protein concentrations were determined using the dye-binding assay (Bio-Rad, Hercules, CA, USA). Total protein (50 μg) was subjected to electrophoresis on NuPAGE® Bis–Tris gels (4–12%) (Invitrogen), followed by electrophoretic transfer to nitrocellulose membranes at 30 V for 1 h. Membranes were blocked in 5% dried milk in TBST (15 mM Tris-HCl, pH 7.4, 150 mM NaCl, and 0.08% Tween 20) for 2 h, followed by incubation with the primary antibody (1:500–1:2000) in Blotto (Pierce) overnight at 4 °C. After washes with TBST, the membranes were incubated in horseradish peroxidase-conjugated secondary antibody (1:4000–1:10 000) for 1 h and washed with TBST three times. Immunoblots were visualised using Super-Signal chemiluminescent substrate (Pierce).

Metallothionein protein determination

Livers were homogenized in 10 mM Tris-HCl buffer (1:5, w:v), followed by centrifugation at 20 000 *g* for 10 min. MT protein concentrations in the cytosol were then determined by the cadmium/haemoglobin assay (26), and were adjusted to μg MT/g wet weight liver.

Liver zinc determination

A known portion of the frozen liver (about 100 mg) was completely digested in nitric acid. Hepatic Zn contents were determined using graphic furnace atomic absorption spectrometry (Perkin-Elmer AAanalst600, Norwalk, CT, USA). Results were expressed as μg Zn/g wet weight liver.

Statistics

For microarray analysis, pooled liver samples were analysed in triplicate while samples from individual animals ($n = 4–5$) were assessed with real time RT-PCR and liver Zn and MT protein content. For comparison of gene expression between two groups, Students' *t*-test was performed. The level of significance was set at $P < 0.05$ in all cases.

Results

Zinc-induced changes in gene expression in rat liver

Treatment of rats with hepatoprotective levels of Zn (see "Material and methods") did not alter animal body weights, or produce any overt toxic manifestations (not shown). Liver weights and gross appearance were normal. Rat Toxicology-II array analysis showed that approximately 5% (28/456) of assessed genes had significantly ($P < 0.05$) altered expression (Table 2). Real-time RT-PCR confirmed and extended this initial microarray screening.

Zinc treatment dramatically increased the expression of *Mt1* and *Mt2* between 8- and 27-fold. Expression of early growth response protein-1 (*Egr1*), heat-shock protein 90 (*Hsp90*), *c*-myc and α1-acid glycoprotein (*Agp*) was increased by two- to three-fold. The expression of other genes for acute-phase response, such as haeme oxygenase-1 (*Hmox1*), CXC chemokine (*Cxcl1*) and ceruloplasmin (*Cp*), were essentially unaltered.

Expression of antioxidant-related genes, such as nuclear factor erythroid 2-related factor 2 (*Nrf2*) and its target gene NAD(P)H quinine dehydrogenase (*Nqo1*), were increased by two- to three-fold after Zn treatment. Rat liver catalase gene expression was also increased. In comparison, other cellular antioxidants, including genes in the glutathione systems and superoxide dismutase, were not appreciably increased.

The expression of interleukin 6 (*Il6*) and inducible nitric oxide synthetase (*Nos2*) was increased approximately twofold following Zn treatment, but the expression of *Il1b*, *Tnfa*, and its receptor *Tnfrsf1b* were basically unaltered.

The expression of cytochrome P450 enzyme genes *Cyp2e1* was modestly suppressed by Zn, while the expression of *Cyp1a1* and *Cyp3a11* was unchanged. The expression of other metabolic enzyme genes, such as UDP-glucuronosyltransferase 1A1 (*Ugt1a1*) and betaine homocysteine methyltransferase (*Bhmt*), was slightly suppressed. The expression of the Zn transporter *ZnT-1* was also increased two-fold by real-time RT-PCR.

The expression of genes encoding for various cell growth and proliferation products were also modestly increased by Zn, such as cyclin D1 (*Ccnd1*), proliferate cell nuclear antigen (*Pcna*) and *p21*, but the expression of insulin-like growth factor-binding protein-1 (*Igfbp1*) was unchanged.

Zinc-induced changes in gene expression in mouse liver

Mice treated with a hepatoprotective dosage regiment of Zn (see "Materials and methods") maintained their body weights and liver weights, and showed no overt toxic manifestations (not shown). Custom-designed liver-selective Atlas array showed that the expressions of approximately 5% (30/588) of genes assessed were significantly ($P < 0.05$) altered (Table 3). Real-time RT-PCR confirmed and extended this initial microarray screening.

Zinc treatment dramatically increased *Mt1* and *Mt2* transcripts between 500- and 1000-fold. Transcriptional expression of *Hmox1*, *Egr1* and the transcription factor *Stat3* was increased approximately two-fold by Zn. Other genes for acute-phase response, such as *Cxcl1*, *Cp*, and heat-shock protein 70 (*Hspalb*), and *c-jun* were also significantly increased by Zn.

The expressions of the antioxidant-related genes such as *Nrf2*, *Nqo1* and *Gclm* were increased following Zn treatment. In comparison, expressions of other cellular antioxidant-related genes, including glutathione *S*-transferases and superoxide dismutase, were basically unaltered.

The expression of genes encoding cytokines (*Il1b*, *Il6* and *Il10*) and inflammatory mediators [*Tnf*, *Tnfrsf1b*, *Nos2* and prostaglandin synthase (*Ptgs2*)] were unaltered.

The expression of some metabolic enzyme genes were slightly decreased by Zn, including cytochrome P450 enzyme genes *Cyp2j5*, *Cyp3a11*, *Cyp4a10* and *Cyp4a14*. The expression of *Cyp2e1* and *Cyp2f2* was slightly decreased after addition of Zn in the array, but not by real-time RT-PCR. The expression of other metabolic enzyme genes, such as *Ugt1a2* and *Bhmt*, were also slightly suppressed by Zn.

The expression of genes encoding cell growth and proliferation was also slightly increased with Zn exposure, such as *Ccnd1* and *Pcna*. Zn treatment also slightly increased the expression of insulin-like factor-binding protein-1 (*Igfbp1*).

Zinc-induced changes in hepatic protein and zinc levels

The expression at the translational level for potentially protective proteins was assessed by Western-blot analysis (Fig. 1). Zn treatment dramatically increased the amount of MT protein (~7 kDa) in both the rat and the mouse, while protein levels of glutathione *S*-transferase-α, glutathione *S*-transferase-μ, heat-shock protein 70, heat-shock protein 90 and β-actin were not significantly altered.

Hepatic MT protein levels were also assessed by the cadmium/haemoglobin assay (26), which measures the protein by its metal-binding capacity. MT levels were again dramatically increased by the hepatoprotective Zn treatments (Fig. 2, left). In the rat, MT was increased ~45-fold (4.4 vs 199 μg/g), and in the mouse, it increased ~200-fold (1.6 vs 318 μg/g). Hepatic Zn concentrations were also markedly increased by the Zn treatment in both rats and mice (Fig. 2, right panel) (Table 3).

Discussion

Hepatoprotective levels of Zn treatment in rats or mice induced a pattern of hepatic gene expression changes, the most consistent change of which was increased MT expression at both the transcript and the protein levels. Modest induction of some genes encoding for acute-phase protein genes, antioxidant enzymes and cell proliferation-related genes were also evident. Some, but not all, metabolic enzyme genes were suppressed, perhaps in an attempt to switch cellular metabolic energy to acute-phase responses. The gene expression changes were similar between mice and rats, but mice were generally more responsive to the

Zn treatments. The treatment levels of Zn used in this study were non-toxic, and no Zn-induced overt liver injury was evident. In general, most of the gene expression changes are subtle, or modest. These gene expression changes, particularly the dramatic increase in MT production, are probably important in the hepatoprotective effects of Zn against hepatotoxicant insult.

Metallothionein is a low-molecular-weight, metal-binding protein that plays important roles in the detoxication of heavy metals, in the homeostasis of essential metals and in the scavenging of free radicals (25). Induction of MT is an important adaptive mechanism affecting the magnitude and progression of hepatotoxic lesions (24). Induction of MT by Zn has been proposed to be a primary mechanism of protection against the hepato-toxicity produced by carbon tetrachloride (5) and cadmium (16). MT-null mice are unable to synthesize MT protein in response to hepatotoxicant insults and are clearly more sensitive to hepatotoxicity of cadmium (25), carbon tetrachloride (27), acetaminophen (28), cisplatin (29) and thioacetamide (30). Thus, it can be concluded that the dramatic induction of MT is critical for Zn-induced generalised hepatoprotection.

However, MT induction alone is insufficient to completely explain Zn-induced generalised hepatoprotection against all toxic agents. For example, Zn protection against alcohol-induced liver injury is independent of MT (31). Other mechanisms in addition to MT induction must also be involved in Zn-induced hepatoprotection, at least with some hepatotoxicants. The Keap1-Nrf2-ARE signalling pathways have emerged as important regulators of the mammalian defense system for detoxication of toxic agents such as carbon tetrachloride, acetaminophen, bromobenzene and furosemide (32), and are often mediated through the upregulation of *Nqo1* and other cellular antioxidant enzymes (32–34), Thus, the induction of *Nrf2* and *Nqo1*, although modest, may contribute to Zn-induced generalised hepatoprotection.

Other Zn-induced gene expression changes, although small, could also contribute to Zn-induced adaptive responses. For example, the transcription factor Egr1 and heat-shock proteins could mediate hepatic response to inflammatory stimuli (35), and *Hsp70* null mice are more susceptible to carbon tetrachloride hepatotoxicity (36) and acetaminophen-induced liver injury (37). Zn-induced increases in the expression of *Egr1* and *Hsp*, although modest, could be an important part of Zn-induced adaptive responses. Zn-treatment-induced increases in liver proliferation-related genes may also contribute to adaptation, as the proliferating livers are more resistant to liver injury (38), and timely liver regeneration can prevent the progression of injury by upregulation of calpastatin (39). Liver cell regeneration may also involve stem cell proliferation, and the regenerating cells require a large amount of Zn and Zn-binding MT protein during the priming step, soon after toxic insults (40), indicating the important roles of Zn and Zn-induced MT in the liver.

It should also be kept in mind with Zn that the range between a safe level and toxic levels is relatively narrow. An overdose of Zn can be toxic to the liver or elsewhere (41). Zn has a dual function in host defense, and can act as either a pro-oxidant or a pro-antioxidant, depending on cellular Zn metabolism and homeostasis (42). In this regard, the redox biology of MT protein plays an important role in modulating the cytoprotective or cytotoxic capacity of Zn (42). In the livers of Zn-deficient animals, marked downregulation of MT could weaken the host defense against various toxic stimuli and compromise the normal function of the body (21–23). Although proper MT expression protects against hepatotoxicants (i.e. carbon tetrachloride), overexpression of MT or excess dietary Zn supplementation provide no further protection (27). Similar to the dual functions of Zn, MT is also a double-edged sword and elevated extracellular MT could have adverse effects (25, 43). Thus, to maintain a

constant state of cellular Zn homeosatsis is essential for host defense and normal cellular function, at least in part through MT regulation and adaptive response machineries (44, 45).

In summary, the current study demonstrated that non-toxic, hepatoprotective levels of Zn in experimental animals evoked a consistent pattern of gene expressions, including dramatic upregulation of MT, modest activation of Nrf2- and acute-response-related genes, and modest suppression of metabolic enzymes. These gene expression changes could play an integrated role in Zn-induced protection against various hepatotoxicants.

Acknowledgments

The authors thank Drs Erik Tokar, Wei Qu and Larry Keefer for their critical review of this manuscript. This research was supported by the Intramural Research Program of the NIH, National Cancer Institute, Center for Cancer Research, by the National Institute of Environmental Health Sciences and by the NIH grant AA014623 for Dr Zhou. The content of this publication does not necessarily reflect the views or policies of the Department of Health and Human Services.

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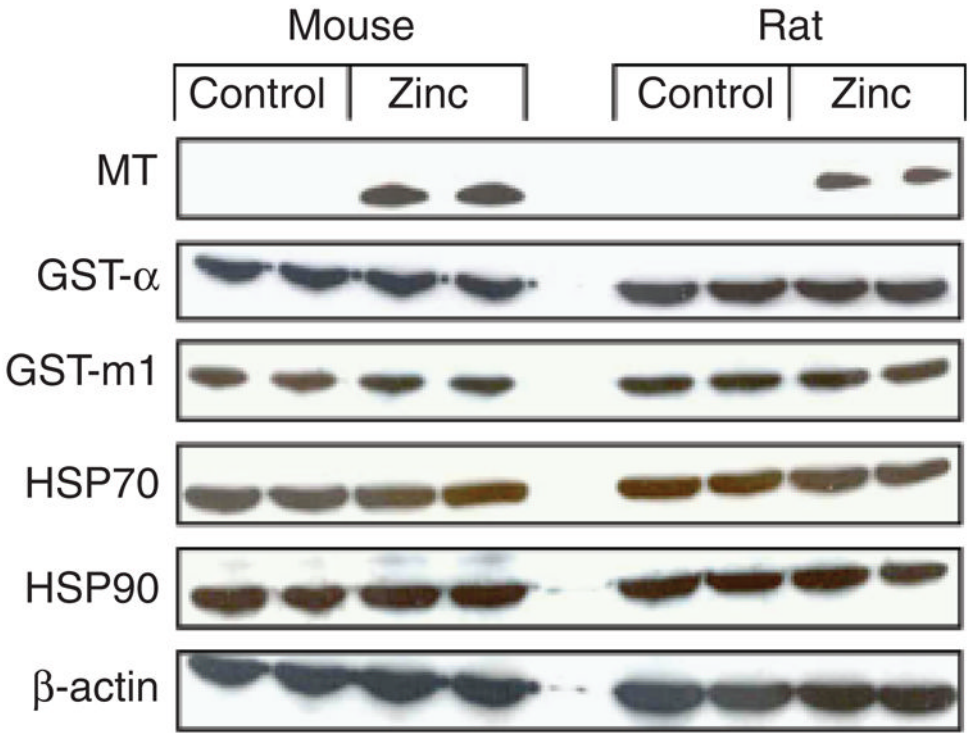

Fig. 1.

Representative Western-blot analysis of liver proteins. Rats and mice were treated with zinc chloride at a non-toxic, hepatoprotective level (100 μmol/kg, s.c. daily for 4 days), and hepatic protein was extracted for Western-blot analysis as detailed in "Materials and methods". The approximate size of bands are metallothionein (~7 kDa), GST-α (~31 kDa), GST-μ (~30 kDa), HSP70 (~68 kDa), HSP90 (~86 kDa) and β-actin (~42 kDa).

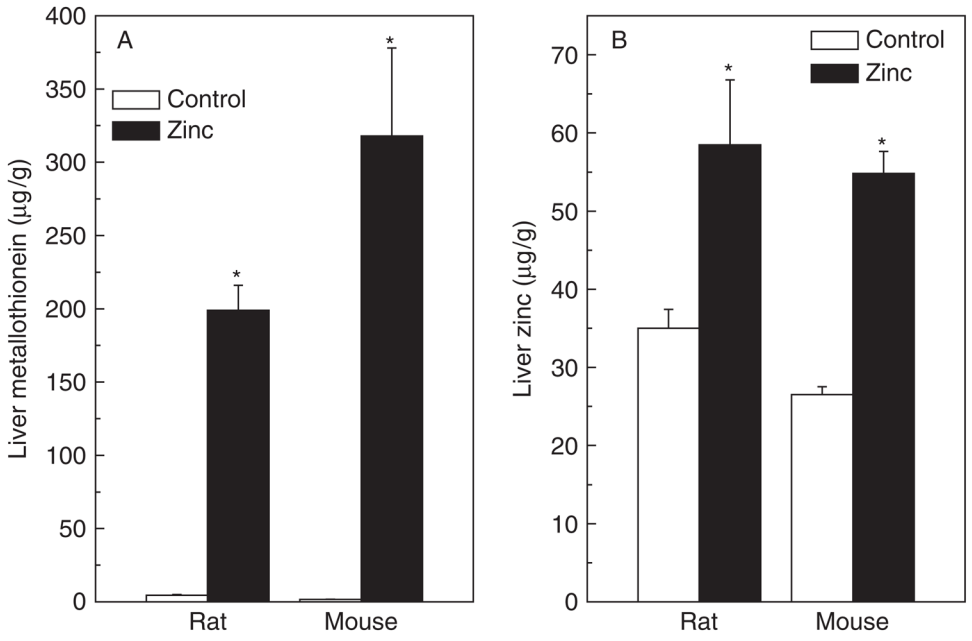

Fig. 2.

Hepatic metallothionein (A) and Zn contents (B). Rats and mice were treated with zinc chloride at a non-toxic, hepatoprotective level (100 μmol/kg, s.c. daily for 4 days). Hepatic MT protein was determined by the cadmium/haemoglobin assay and hepatic zinc content was determined by atomic absorption spectrophotometry (see “Materials and methods”). Data are mean ±SEM (n = 4 for rats and n = 5 for mice). *Significantly different from controls ($P < 0.05$).

Table 1

Hepatoprotective levels of zinc protects against liver injury from various chemicals and metals

Hepatotoxicant	Metabolic activation	Proposed mechanism of toxicity	Zn protection	Reference
Chemicals				
Carbon tetrachloride	$CCl_3^{\bullet}$ by P450	Oxidative stress	++++	(5)
Bromobenzene	BB 3,4-epoxide by P450	Covalent binding	++	(6)
Thioacetamide	TA sulphoxide by P450	Covalent binding	+	(7)
Ethanol	Acetaldehyde by ADH	Inflammation	++	(8)
D-galactosamine	Unknown	Deplete uridine nucleotide	++	(9)
Endotoxin	None	ROS (reactive oxygen species)	+++	(10)
GlaN1LPS/TNF-α	Unknown	Inflammation, ROS	+++	(11)
Drugs				
Acetaminophen	NAPQI by P450	Covalent binding, ROS	++	(12), (13)
Salicylate	Unknown	ROS, inflammation	++	(14)
Cisplatin	None	Inflammation, ROS	++	(15)
Metals				
Cadmium	None	Binding to sulphydryl groups	++++	(16)
Mercury	None	Inflammation	++	(17)
Arsenic	Unknown	Inflammation	++	(18)
Nickel	Unknown	ROS, inflammation	+	(19)
Copper	None	Wilson's disease	+++	(20)

+ Partial protection;

++ protection;

+++ good protection;

++++ excellent protection.

Table 2

Gene expression changes following a hepatoprotective dose of zinc in rats

Symbol	GeneBank#	Encoded proteins	Ratio Zn/control array	Ratio Zn/control RT-PCR
Acute phase protein genes				
Mt1a	NM_138826	Metallothionein-1a	**8.0**	**8.0**
Mt2	M11794	Meathiothinein-2	N/A	**27.0**
Egr1	NM_012551	Early growth response 1	**3.4**	**2.4**
Hspaa1	NM_175761	Heat-shock protein 90	**2.4**	**2.6**
Myc	Y00396	c-myc oncogene	**1.8**	**2.3**
Agp	J00696	α1-acid glycoprotein	1.4	**2.9**
Hmox1	J02722	Haeme oxygenase-1	0.8	1.1
Cxcl5	U90448	Chemokine (C-X-C motif) ligand 5	N/A	**2.0**
Cp	L33869	Ceruloplasmin	N/A	1.3
Antioxidants				
Nrf2	AF037350	Nuclear factor Nrf2	N/A	**3.0**
Nqo1	NM_017000	NAD(P)H dehydrogenase, quinone 1	**3.2**	**2.3**
Cat	M11670	Rat Liver catalase	**1.5**	**2.5**
Gstp2	X02904	Glutathione S-transferase P	1.1	1.7
Gpx3	NM_022525	Glutathione peroxidase 3	N/A	1.8
Gclc	NM_012815	Glutamate-cysteine ligase	0.6	1.5
Sod1	Y00404	Superoxide dismutase 1	1.3	1.9
Cytokines and inflammatory mediators				
Il6	NM_012589	Interleukin-6	**2.9**	**2.0**
Nos2	L12562	Inducible nitric oxide synthetase	**2.9**	**1.9**
Il1b	NM_031512	Interleukin-1β	N/A	1.2
Tnfa	X66539	Tumor necrosis factor (TNF)-α	0.6	1.5
Tnfrsf1a	M63122	TNF receptor 1	1.4	1.4
Metabolic enzymes				
Cyp2e1	NM_031543	Cytochrome P450 2E1	**0.8**	0.8
Cyp1a1	NM_012540	Cytochrome P450 1A1	0.8	1.5
Cyp3a2	U09742	Cytochrome P450 3A2	1.1	1.0
Ugt1a1	NM_012683	UDP glycosyltransferase 1A1	**0.6**	0.8
Bhmt	AF033381	Betaine homocysteine Me-transferase	N/A	**0.7**
ZnT1	U17133	Zinc transporter protein 1	N/A	**2.1**
Cell proliferation and others				
Ccnd1	NM_171992	Cyclin D1	**2.5**	**2.0**
Pcna	Y00047	Proliferate cell nuclear antigen	1.4	1.7
p21	L41275	P21, cip1, waf1	2.2	1.5
Igfbp1	NM_013144	IGF-binding protein 1	0.9	1.2

Rats were treated with Zn (100 μmol/kg, s.c. daily for 4 days). Data are mean ±SEM (n = 3 for microarrays and n = 4 for real-time RT-PCR analysis of individual rat). Bold indicates significantly different from controls $P < 0.05$.

N/A, not available on the array; RT-PCR, reverse transcriptase-polymerase chain reaction.

Table 3

Gene expression changes following a hepatoprotective dose of zinc in mice

Symbol	GeneBank#	Encoded proteins	Ratio Zn/control array	Ratio Zn/Control RT-PCR
Acute phase protein genes				
Mt1	BC027262	Metallothionein-1	N/A	**514**
Mt2	NM_008630	Meathiothinein-2	N/A	**1020**
Hmox1	M33203	Haeme oxygenase-1	**1.8**	**2.2**
Stat3	U06922	Signal transduction/activator 3	**2.3**	**2.9**
Egr1	M20157	Early growth response 1	**2.6**	**2.3**
Cxcl1	NM_008176	Chemokine (C-X-C motif) ligand 1	1.3	**3.2**
Cp	U49430	Ceruloplasmin	1.3	**2.9**
Hspa1b	M35021	Heat-shock protein 70	1.2	1.9
c-jun	J04115	Jun oncogene	1.3	1.9
Antioxidants				
Nrf2	BC026943	Nuclear reactor factor Nrf2	**2.1**	**1.4**
Nqo1	BC004579	NAD(P)H dehydrogenase, quinone 1	**1.5**	**2.1**
Gclm	NM_002061	Glutamate-cysteine ligase, modifier unit	N/A	**3.0**
Gstp1	D30687	Glutathione S-transferase-π	0.7	0.8
Gpx3	U13705	Glutathione peroxidase 3	0.9	1.8
Sod1	NM011434	Superoxide dismutase 1	1.0	1.7
Cytokines and inflammatory mediators				
Il1b	NM_008361	Interleukin-1β	0.8	1.4
Il6	J03783	Interleukin-6	0.7	1.0
Il10	M37897	Interleukin 10	1.2	1.1
Tnf	NM_013693	Tumour necrosis factor (TNF)-α	0.9	1.2
Tnfrsf1b	M59378	TNF receptor 1	1.3	1.1
Nos2	M87039	Inducible nitric oxide synthetase	1.4	1.2
Ptgs2	M64291	Prostaglandin synthase, Cox2	1.2	1.1
Metabolic enzymes				
Cyp2j5	NM_010007	Cytochrome P450 2J5	**0.5**	0.8
Cyp3a11	X60452	Cytochrome P450 3A11	**0.4**	**0.5**
Cyp4a10	AB018421	Cytochrome P450 4A10	**0.6**	**0.5**
Cyp4a14	Y11638	Cytochrome P4504A11	**0.6**	0.8
Cyp2e1	L11650	Cytochrome P450 2E1	0.8	1.4
Cyp2f2	M77497	Cytochrome P450 2F2	**0.8**	1.2
Ugt1a2	D87866	UDP-glucuronosyltransferase 1A2	**0.7**	**0.8**
Bhmt	AF033381	Betaine homocysteine Me-transferase	**0.5**	**0.8**
Growth and cell proliferation				
Ccnd1	M64403	Cyclin D1	**2.4**	**2.3**
Pcna	X53068	Proliferate cell nuclear antigen	**2.3**	**1.5**
Igfbp1	X81579	Insulin-like factor binding protein 1	**2.2**	**2.5**

Mice were treated with Zn (100 μmol/kg, s.c. daily for 4 days). Data are mean ±SEM (n = 3 for microarrays and n = 5 for real-time reverse transcriptase-polymerase chain reaction (RT-PCR) analysis of individual mouse). Bold indicates significantly different from controls $P < 0.05$.

N/A: not available on the array.