

# Heavy metal ions in normal physiology, toxic stress and cytoprotection

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**ABSTRACT:** As a group, heavy metals include both those essential for normal biological functioning (e.g. Cu and Zn), and non-essential metals (e.g. Cd, Hg, and Pb). Both essential and non-essential metals can be present at concentrations that disturb normal biological functions, and which evoke cellular stress responses. The cellular targets for metal toxicity include tissues of the kidney, liver, heart, immune response and nervous system. Intriguingly, manipulations of specific metals, their reservoirs, and the cellular stress response can have therapeutic effects on certain diseases. In this minireview, we will consider both the biological responses to stressful levels of heavy metal cations, and experimental and clinical manipulations of these cations as a means to improve human health parameters.

**KEYWORDS:** heavy metal, zinc, cadmium, tin, copper, cardiomyopathy, chelation, ischemia, inflammation, cytoprotection, heme-oxygenase, heat shock proteins, vascular surgery, immunomodulation, metallothionein, humoral immunity, neuronal injury, oxidative stress

## INTRODUCTION

Heavy metals represent both essential components for the maintenance of normal biological functions, and toxic agents with damaging consequences when present in inappropriate amounts. One way to understand these agents as a group is

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to characterize the influences they have on sensitive biological systems. Metals such as copper and zinc play important roles as cofactors for normal enzyme functioning, and zinc contributes to the structural elements of some transcription factors. When in short supply, there are many biological processes that suffer, and the result can impede the normal roles played by many tissues including those of the kidney, liver, heart, immune and nervous system. Toxic heavy metals share some chemical similarities with the essential metals, and in excess can induce the production of reactive oxygen species as well as interact with sulfhydryls to alter protein structure and function. The management of both essential and toxic heavy metals is in part accomplished by metallothionein. In this article, we will consider the management of these heavy metals in several biological systems, and the effects of these agents on proper biological functioning.

#### **METALLOTHIONEIN-MEDIATED IMMUNOMODULATION: NEW ROLES FOR AN OLD STRESS RESPONSE (M.A.L.)**

Metallothionein serves as one of the points of intersection for many of the issues addressed in this review. Although this small stress response protein is not a member of heat shock protein family, it serves many roles in both normal and stressed cells, acting as a reservoir of essential heavy metals (e.g.,  $\text{Cu}^{+2}$  and  $\text{Zn}^{+2}$ ), as a scavenger for both heavy metal toxicants (e.g.,  $\text{Hg}^{+2}$ ,  $\text{Cd}^{+2}$ ) and free radicals, and as a regulator of transcription factor activity.<sup>1</sup> Metallothionein is induced by a range of different agents, including heavy metals, reactive oxygen species, glucocorticoids, acute phase cytokines and interferon, and by endotoxin. Traditionally considered an intracellular protein, recent work has suggested important roles for metallothionein both in intracellular compartments and as an extracellular agent.<sup>2</sup> The protein is widely expressed in highly homologous forms, suggesting that the roles played by metallothionein are essential to a variety of biological processes, and that the protein has a long evolutionary lineage.<sup>3</sup>

Metals have long been known to evoke changes in the immune response. These changes are dependent both on the specific metal and metal valency, as well as on the genetic makeup and physiological status of the exposed individual.<sup>4</sup> The changes induced in leukocyte behavior can result in immunosuppression and a consequent increase in susceptibility to infectious disease. These changes can also result in an autoreactive immune response that subsequently damages self tissues.<sup>5</sup> Our work has focused on the mechanisms of metal-mediated immunomodulation that are influenced by basal levels of metallothionein, and by those levels of metallothionein that are induced by inflammation, autoimmune disease and toxic metal exposure. We have also explored the potential opportunities this immunomodulation provides both for the management of disease that results from toxic metal exposures, and for the management of diseases that are accompanied by

metallothionein synthesis as a consequence of cellular stresses that are associated with infection, inflammation or other biological stressors.

We have shown that exogenous metallothionein can suppress elements of the humoral (T-dependent, antibody mediated) response<sup>6</sup> in a manner consistent with the immune enhancements that have been reported in mice that carry targeted disruptions of the *Mt1* and *Mt2* genes.<sup>7</sup> Similarly, a monoclonal anti-metallothionein antibody can enhance the T-dependent humoral response to antigen challenge.<sup>8</sup> Taken together, these results suggest that metallothionein that is released from cells can act to diminish the normal immune response, and that this extracellular compartment of metallothionein is a potential target for therapeutic manipulation. It also suggest that metallothionein that is released from stressed cells can interact with cells that participate in a humoral response, potentially via interactions with the membrane of the target cell population. There is one report that has described a putative metallothionein receptor. El Refaey *et al.*<sup>9</sup> reported that astrocytes express a membrane-associated receptor that potentially regulates astrocyte function. We have observed metallothionein binding on the plasma membranes of leukocytes cultured in the presence of metallothionein<sup>10</sup>, on cells harvested from animals immunized in the presence of adjuvant<sup>8</sup>, and on cells harvested from animals that experience a chronic inflammatory disease.<sup>11</sup>

One of the biological processes activated by this extracellular pool of metallothionein is leukocyte chemotaxis. We have shown that metallothionein binds with surface molecules on many different subpopulations of leukocytes to initiate a chemotactic response.<sup>12</sup> This response can be inhibited by both pertussis toxin and cholera toxin, suggesting that one of the targets of metallothionein binding is a G- coupled protein receptor (GCPR). This suggests that one of the ways that metallothionein may influence immune activities is to initiate cell migration toward tissues that serve as the source of diffusible metallothionein. In instances of metal intoxication, the organs where highest levels of this metallothionein pool originate may become inflamed due to the accumulation of cells responding to the metallothionein gradient, while, simultaneously, other wound sites may experience a less vigorous response due to a decreased immune response directed to those sites. In some instances where chemoattractants originating from the tissue wound overlap with a metal-initiated metallothionein gradient, the resulting inflammatory response may be excessive. A thorough understanding of the role played by metallothionein in these cases of metal intoxication will suggest novel therapeutic avenues, such as intentional induction of endogenous metallothionein, or therapeutic administration of metallothionein to change the course of disease. Metallothionein therapies have already been used experimentally in animals to alter the course of autoimmune disease. For example, injection of metallothionein, or of Zn<sup>+2</sup> at levels known to induce metallothionein can diminish the severity of collagen-induced arthritis in experimental mice.<sup>13</sup>

These observations correspond well with our work showing that combining a targeted disruption of the *Mt1* and *Mt2* genes with *Pttn6<sup>me-v</sup>/Pttn6<sup>me-v</sup>* (“viable motheaten”) results in a dramatic shortening of lifespan in these animals that display a congenital chronic inflammatory disease.<sup>11</sup> This suggests that the propensity to synthesize metallothionein (and the additional propensity to release metallothionein from stressed cells) may represent important susceptibility polymorphisms for certain types of disease in the human population.<sup>14</sup>

We have examined the humoral response in mice that carry an *Mt1* transgene, and in mice with targeted disruption of the *Mt1* and *Mt2* genes. These experiments were done in the presence of  $Zn^{+2}$ ,  $Cd^{+2}$ , or vehicle control at exposure levels that do not alter the response to antigen challenge in wild type mice. Our findings in these studies are intriguing:  $Cd^{+2}$ , but not  $Zn^{+2}$ , suppressed the humoral immune response to antigen challenge below vehicle control levels in the strain carrying the targeted *Mt* gene disruptions. In contrast, both  $Cd^{+2}$  and  $Zn^{+2}$  reduced the humoral response in the *Mt1* transgenic strain (unpublished data). We interpret these results to suggest that, in the absence of functional metallothionein, the animal is susceptible to other mechanisms of Cd-mediated immunotoxicity. In the transgenic strain, both  $Cd^{+2}$  and  $Zn^{+2}$  can induce elevated levels of metallothionein, and in both circumstances humoral immunity is diminished. These results suggest that the metallothionein protein itself is an important component of the immunomodulatory effect, irrespective of the metal species that is complexed with the protein.

In recent experiments, we have explored the role of metallothionein in the progression of an *in vivo* infection with *Listeria monocytogenes*, a model organism for intracellular infection. Our results illustrate that there can be dramatic differences in the ability of mice with different metallothionein gene doses to respond to the infection (unpublished data). This data appears to indicate that metallothionein synthesis influences the course of infection. In light of work from other laboratories that indicates critical influences of metallothionein on various forms of tissue inflammation and ischemia, these results may indicate that preliminary testing of metallothionein biosynthetic capacity in a patient population may represent a valuable prognostic for disease susceptibility and for predictions of the severity of disease.

### **COPPER METABOLIC DISORDER IN MYOCARDIAL PATHOGENESIS (Y.J.K.)**

Copper (Cu) is an essential mineral nutrient that participates in important cellular metabolism and function as a component of a number of cuproenzymes, an

integrated structural element, and a regulatory agent.<sup>15</sup> However, Cu also catalyzes the production of highly reactive oxygen species leading to oxidative damage of lipid, protein, DNA and other molecules.<sup>16</sup> Therefore, either Cu deficiency or excess can lead to diseases or affect the progression of diseases. There is virtually no free Cu in biological system under physiological conditions.<sup>17</sup> Cu intracellular movement is tightly controlled by Cu chaperons<sup>18</sup> and inter-organ movement is mediated by different Cu carrier proteins.<sup>19</sup>

The current US-Canadian Recommended Dietary Allowance (RDA) for Cu is 0.9 mg/day for adult of 19-year-old or older.<sup>20</sup> A debate continues regarding the appropriateness of the RDA for Cu.<sup>21</sup> A human study has examined potential adverse effect of marginal dietary Cu restriction on cardiovascular system.<sup>22</sup> Among 24 subjects consuming a typical American diet (1.03 mg Cu/day), one mildly obese subject sustained a myocardial infarction 4 wks after consuming a starch-based diet and two subjects experienced severe tachycardia 7 or 10 wks after consuming a fructose-based diet. Another subject experienced a type II, second-degree heart block 11 wks after consuming the starch-based diet. It appears that pre-existing cardiac conditions cannot be excluded for the outcome, however, the fact that these abnormalities appeared after the subjects were fed the controlled low Cu diet indicates the triggering effects of low levels of Cu. Moreover, these cardiac defects disappeared after the affected subjects were fed diets supplemented with 3.0 mg Cu/day<sup>22</sup>.

Cu supplementation would prevent or ameliorate cardiomyopathy if this pathogenesis is associated with Cu depression in the heart. We have observed<sup>23</sup> that dietary Cu supplementation (20 mg Cu/kg diet) can reverse an experimentally induced cardiac hypertrophy and improve heart contractile function in mice with established heart hypertrophy and dysfunction induced by ascending aortic constriction (AAC) if they were fed adequate Cu diet (6 mg Cu/kg). Moreover, Cu supplementation-induced recovery occurs in the presence of sustained pressure overload. We further found that AAC caused cardiac Cu depression, which can be corrected by dietary Cu supplementation.

Sco2 is an important Cu chaperon for cytochrome c oxidase (CCO)<sup>24</sup> and mutations in Sco2 result in suppressed CCO activity.<sup>25,26</sup> Patients with mutations in Sco2 developed severe hypertrophic cardiomyopathy.<sup>25-27</sup> A SCO2 patient with severe hypertrophic cardiomyopathy and heart dysfunction was treated with Cu-histidine. This Cu supplement therapy caused a reversal of the hypertrophic cardiomyopathy along with a significant improvement in all parameters of heart function and normalization of ECG signs and blood pressure.<sup>28</sup> A recent study with a small population of chronic heart failure patients has shown that dietary supplementation with micronutrients for 9 months increase left ventricle ejection and decreases left ventricle volume along with an improvement of the quality of life.<sup>29</sup> Among the formulated micronutrients is Cu (1.2 mg/day).

Increased Cu concentrations and a high Cu/zinc (Zn) ratio were found in patients with chronic rheumatic heart disease.<sup>27</sup> Some studies have shown that the plasma activity of semicarbazide-sensitive amine oxidase (SSAO), a Cu-containing protein, was elevated relative to the severity of diabetes mellitus and chronic heart failure.<sup>30</sup> Evidence obtained from epidemiological studies has suggested that serum ceruloplasmin, the main Cu-containing protein in plasma, is an important risk factor for myocardial infarction and cardiovascular disease because it is a potent catalyst of LDL oxidation *in vitro*.<sup>31</sup> We have observed that Cu concentrations in the plasma of streptozotocin (STZ)-induced diabetic mice significantly increased along with a significant depression of Cu concentrations in the liver and a slight increase in the heart.<sup>32-34</sup>

A recent study has shown that Cu chelation using trientine substantially improves cardiomyocyte structure and contractile function and reverses left ventricular collagen deposition in diabetic patients and STZ-induced diabetic rat model.<sup>35</sup> It is unknown what forms of Cu interact with trientine *in vivo* and how trientine affects overall balance among different forms of Cu. However, the distinct effects of trientine in contrary to Cu supplementation described above may reflect the fundamental difference in Cu metabolic disorders between diabetic and pressure overload cardiomyopathy.

It is possible that systemic complications in diabetes lead to Cu accumulation in the blood due to disruption of Cu metabolism in the liver, thus increasing the risk of Cu toxicity to other organs including the heart. However, in pressure overload the heart is the primary organ of Cu metabolic disorder but the liver remains functional. Therefore, Cu functional deficiency would occur in the pressure overload heart. In this context, dietary Cu supplementation should reverse pressure overload-induced hypertrophic cardiomyopathy, but Cu chelation should ameliorate diabetic cardiomyopathy, as observed in previous studies.<sup>23, 35</sup> Although further studies are required to provide scientific understanding of Cu metabolic disorders under different disease conditions, differential manipulation of Cu metabolism under diabetic and pressure overload conditions appears beneficial to patients with cardiomyopathy.

### **BRAIN INJURY AND OXIDATIVE STRESS: THINK ZINC! (S.L.S.)**

Oxidative stress is considered one of the major triggers of neuronal degeneration in the brain. Zn<sup>2+</sup> has been implicated in the regulation of many channels and receptors, but the cation can also act as a trigger for neuronal injury.<sup>36</sup> Zn<sup>2+</sup> is co-released with glutamate at many excitatory synapses and

synaptic  $Zn^{2+}$  can eventually enter neurons through channels associated with glutamatergic post-synaptic receptors such as NMDA and calcium-permeable AMPA/kainate receptors, or through voltage sensitive calcium channels (VSCCs) and  $Zn^{2+}$  transporters. Neurons maintain relatively low levels of intracellular free  $Zn^{2+}$  ( $[Zn^{2+}]_i$ ), through the coordinated activity of systems involving the extrusion, buffering, and sequestration of the cation. A key emerging concept is that many biological systems possess a finely tuned “ $Zn^{2+}$  set-point” and deregulation of this set point can have important consequences. Prenatal  $Zn^{2+}$  deficiency can impair brain development and results in serious cognitive deficits later in life. On the other hand, toxic  $[Zn^{2+}]_i$  rises (resulting from either cation influx or its release from intracellular sites such as metallothioneins (MTs) and mitochondria) mediates toxic effects in a variety of pathological conditions, including cerebral ischemia, brain trauma and epilepsy.<sup>36</sup>

Interestingly,  $Zn^{2+}$  is also a strong inducer of oxidative stress. In neurons,  $Zn^{2+}$  can trigger ROS production through mitochondrial pathways by interfering with the activity of the electron transport chain (ETC).<sup>37,38</sup>  $Zn^{2+}$  can also modulate extra-mitochondrial pathways involved in ROS generation by promoting the increased activity of NADPH oxidase, protein kinase C (PKC) activation, as well as induction of neuronal nitric oxide synthase (nNOS) which together with superoxide can produce harmful peroxynitrite (ONOO<sup>-</sup>). One of the major targets of ROS-dependent  $Zn^{2+}$  release involves the MTs. Recent findings strongly suggest that nitrosative stress can act as a critical trigger for  $[Zn^{2+}]_i$  mobilization as Nitric Oxide (NO) or -ONOO interact preferentially with MT-3 and induce  $Zn^{2+}$  release from MTs both *in vitro* and *in vivo*.<sup>39, 40</sup>

The fact that  $Zn^{2+}$  trigger ROS generation and cellular oxidation enhances  $[Zn^{2+}]_i$  release, set the stage for a dangerous feed-forward cycle as the cation can elicit neuronal demise by activating multiple, intersecting necrotic and/or apoptotic pathways.  $Zn^{2+}$  can promote apoptosis via the induction of the mitochondrial permeability transition pore (mPTP) and release of mitochondrial pro-apoptotic factors<sup>41-43</sup>, but the cation has also been linked to necrotic disruption of neuronal metabolism, and biochemical studies have shown it can inhibit key enzymes in the glycolytic pathway.

$Zn^{2+}$  dependent activation of both necrotic and apoptotic pathways may conceivably be linked to its intracellular mobilization upon oxidative stress. Findings in neurons indicate that cell oxidants can induce  $[Zn^{2+}]_i$  rises that, in isolated mitochondria, are sufficient to cause a partial loss of mitochondrial membrane potential and trigger a multi-conductance ion channel activity consistent with mPTP opening.<sup>41-44</sup> These ROS-induced  $[Zn^{2+}]_i$  rises are also able to elicit activation of specific  $K^+$  channels leading to  $K^+$  depletion, a key event in neuronal apoptosis.<sup>45, 46</sup> Conversely,  $Zn^{2+}$ -induced generation of mitochondrial ROS might promote yet more  $Zn^{2+}$  release from the protein-bound

pool. Thus, the two pathways appear to work synergistically to induce a self-perpetuating injurious cycle.

It is worth noting that recent findings have prompted a very intriguing debate about the capability of  $Zn^{2+}$  to interplay with  $Ca^{2+}$  to promote excitotoxicity.<sup>47-49</sup> While for at least two decades excitotoxicity has been considered as a purely  $Ca^{2+}$ -dependent process,  $Ca^{2+}$  dependency has been substantiated mostly on experimental paradigms that employed either  $Ca^{2+}$  chelators or intracellular imaging with  $Ca^{2+}$  sensitive fluorescent indicators. However, a potential caveat to this “ $Ca^{2+}$ -centred” view comes from the fact that both  $Ca^{2+}$  sensitive fluorescent probes and chelators also bind  $Zn^{2+}$  with higher affinity. The confounding effect of  $Zn^{2+}$  on  $Ca^{2+}$  imaging has been so far neglected, moving from the assumption that  $[Zn^{2+}]_i$  levels are negligible. On the other hand, as mentioned above, growing evidence has indicated that upon excitotoxic conditions,  $[Zn^{2+}]_i$  increases can reach levels high enough to greatly interfere with fluorescent measurements of  $[Ca^{2+}]_i$  and/or chelation by divalent chelators.<sup>48, 50, 51</sup>

The deleterious synergism between the two cations, though not yet fully explored, offers intriguing new perspectives on our understanding on the ionic determinants of many excitotoxic conditions. Given the emerging role of  $[Zn^{2+}]_i$  release in neuronal death, and the fact that the cation is capable to trigger injury with greater potency compared to  $Ca^{2+}$ ,  $Zn^{2+}$  is likely to emerge as a major mediator of excitotoxicity. For instance, recent data demonstrating that large  $[Ca^{2+}]_i$  rises trigger important intracellular mobilization of  $Zn^{2+}$ , coupled with the likely probability that  $Ca^{2+}$ -induced mitochondrial ROS generation might also promote further  $Zn^{2+}$  release from MTs, suggest a more complex excitotoxic scenario. In such a model, glutamate-driven  $[Ca^{2+}]_i$  rises might actually serve as a “partner in crime” to initiate the injurious mobilization of the main ionic mediator of neuronal death:  $Zn^{2+}$ .

### CYTOPROTECTIVE METAL IONS (G.A.P AND L.E.H.)

All forms of surgical therapy are stressful and injurious for the patient. The majority of surgical and invasive medical procedures are performed in an elective fashion and thus provide the clinician an opportunity to pre-operatively condition the patient to minimize iatrogenic tissue injury. Presently no pre-operative clinical protocols exist which take advantage of intrinsic cellular mechanisms that have been shown to provide protection against iatrogenic ischemia-reperfusion (IR) and acute inflammatory injuries. We hypothesized that

tissues could be protected from IR injury by pretreatment of the organism by using brief whole-body hyperthermia (heat shock, HS, 42.5°C) or the systemic administration of metal ions (SnCl<sub>2</sub> or ZnCl<sub>2</sub>) followed by a period of recovery (37°C, 6-8 hr.) prior to major surgical procedures. We have successfully used HS to provide protection in diverse animal models of surgically induced I/R, reviewed elsewhere.<sup>52</sup> The transient state of cytoprotection or protected phenotype is achieved via a complex adaptation in cellular metabolism analogous to that described for the thermotolerance phenomena.<sup>53,54</sup> The dominant metabolic change associated with hyperthermia-induced cytoprotection is the increased expression of the HS-related genes resulting in the rapid synthesis of heat shock proteins (HSP).<sup>55</sup> The HSPs have been recently classified as molecular chaperones and, as such, contribute new insight into cellular resistance to and recovery from IR injury. Additionally, heat shock related effects on acute inflammation, vascular biology and non-HS gene expression have been described.<sup>56,57</sup> We wish to develop clinically relevant protocols for stress conditioning humans prior to invasive surgical and medical procedures. Pharmacologic induction of the cellular stress response has been reported for a number of chemical agents. We have focused our work on the relatively nontoxic stannous and zinc metal ions as potential agents to develop pharmacologic stress conditioning protocols.

It is known that the degree of acute inflammatory response to noxious agents can be radically altered by up or down regulation of heat-shock proteins, specifically heme-oxygenase-1 (HO-1, a.k.a. hsp32) and heat-shock protein 70 (hsp70).<sup>58</sup> Stannous chloride (SnCl<sub>2</sub>), a tin salt, is a relatively nontoxic metal of the transition series of elements. Stannous chloride has been shown to be a potent inducer of HO-1 activity in many animal models<sup>59, 60</sup> and of HO-1 and hsp70 mRNA's in human cells.<sup>61</sup> Acute inflammatory responses, such as acute lung injury associated with the post-operative fat embolism syndrome, are observed in many medically relevant pathologic states.<sup>62</sup>

Ischemia-reperfusion and acute inflammation are fundamental mechanisms by which tissues can become injured during major surgical and invasive medical procedures. Four models of acute ischemia-reperfusion or acute inflammation have been used to test the pharmacologic stress conditioning hypothesis: rodent renal artery occlusion (RAO), rabbit spinal cord ischemia, acute pulmonary inflammation (rabbit fat embolism syndrome by intravenous administration of oleic acid), and acute inflammation within the rodent mesenteric blood vessels.

HS-associated ischemic protection preserves renal microvascular integrity and is dependent upon new HS-gene expression.<sup>63</sup> Sprague-Dawley rats (200-250 g, male) were divided into four pretreatment groups. Animals that

received no pretreatment and no ischemia provided baseline renal vascular resistance data. Sham control animals received normal saline injections (0.9% NaCl) at 16 hours prior to surgery. Tin and zinc pretreated animals received 0.15mg/kg of the metal solution by the subcutaneous route 16 hours prior to RAO. Bilateral RAO was performed in hydrated (3 cc, 0.9% NaCl, IV), anesthetized (5 mg pentobarbital/100 g body weight, IP), animals for 60 min at 37°C. Immediately following RAO, all kidneys were perfused *in-situ* via the renal artery with phosphate buffered saline (PBS, 27°C) at a constant flow rate (0.76 mL/min). Perfusion pressures were measured (mmHg) using an in-line pressure monitor (Tektronix) and renal vascular resistance was calculated (RVR, mmHg/mL/gm). Kidneys pretreated with either tin or zinc demonstrate a significantly reduced RVR following 60 minutes of *in-situ* warm ischemia (Table 1).

**Table 1. Renal Vascular Resistance Following Warm Ischemia**

Exp Group	N	RVR (mmHg/mL/gm) Mean± SD	P vs sham
Baseline/No ischemia	5	43.5 ± 10.1	nd
Sham/ NaCl	8	65 ± 10.3	nd
SnCl <sub>2</sub>	4	44.7 ± 13.8	0.016
ZnCl <sub>2</sub>	5	48.3 ± 7.5	0.010

HS-associated ischemic protection can also preserve neurologic function in the rabbit model of acute aortic occlusion (AAO).<sup>64</sup> Three groups of New Zealand White rabbits were subjected to 20 minutes of infra-renal aortic occlusion to induce acute spinal cord ischemia, and divided into 3 pretreatment groups (Table 2). Sham control animals received normal saline injections (0.9%NaCl, intravenous) 16 hours prior to AAO. Unstressed control animals received no pretreatment or handling prior to AAO. The tin pretreatment animals received one injection of SnCl<sub>2</sub> (0.15 mg/kg, subcutaneous) followed by 16 hours of recovery. Following 20 minutes of AAO, all animals were followed with daily neurological exams for two days and then euthanized. Intraoperative hemodynamic parameters were not significantly different between groups. Paralysis developed in 7/8 in the unstressed control group but was not seen in the SnCl<sub>2</sub> group, (0/4, p< 0.001). The sham group demonstrated an intermediate level of protection as one animal developed paralysis, 5 animals developed paresis (weakness) and one animal had normal function.

**Table 2. Neurologic Function Following Acute Spinal Cord Ischemia**

Exp Group (n)	% Normal	% Paretic	% Paralysis
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Sham/NaCl (7)	14	72	14
Control (8)	0	12	88
SnCl <sub>2</sub> (4)	100	0	0 *

\*p < 0.001 SnCl<sub>2</sub> vs Control

Similarly, HS could influence the progression of acute pulmonary inflammation induced by fat embolism syndrome in the rabbit (Table 3). New Zealand White rabbits (2-3 Kg) received a single dose of oleic acid (OA, 0.025cc/kg, IV) 16 hours after randomization to two pretreatment groups. Group 1 (n=13, Sham/NaCl) controls received an injection of normal saline (0.9%NaCl, subcutaneous) and Group 2 (n=11, SnCl<sub>2</sub>) an injection of SnCl<sub>2</sub> (0.15mg/kg, subcutaneous). Pulmonary oxygen transfer and bronchoalveolar lavage for cell infiltrate were performed 24 hours after OA administration. Oxygen transfer was significantly better in animals following pretreatment with SnCl<sub>2</sub> compared to sham treated animals. The improvement in pulmonary function is associated with a significant reduction in WBC infiltration into the alveolar space.

**Table 3. Lung Function and Inflammation Following Oleic Acid injection**

Exp Group	N	Oxygenation (mm Hg)	WBC count (10 <sup>6</sup> cells/ml)
Sham/ NaCl	13	47.7 ±11.5	1.13 ± 0.32
SnCl <sub>2</sub>	11	65.2 ± 20.8	0.81 ± 0.9
P value		0.013	0.04

We have also evaluated the ability of tin to moderate acute inflammation within rodent mesenteric blood vessels that is induced by an injected inflammatory agent (Table 4).<sup>65</sup> Adult male Wistar rats received normal saline or tin pretreatments 16 hours prior to intra-arterial suffusion of the pro-inflammatory agent formyl-methionyl-leucyl-phenylalanine (FMLP, 10<sup>-7</sup>M). Leukocyte adherence to mesenteric vascular endothelium was determined by intra-vital microscopy techniques and reported as cell number per 100 µm of vessel length. Tin pretreatment significantly reduced the leukocyte adhesion to rodent mesenteric blood vessels induced by FMLP treatment.

**Table 4: Leukocyte-Endothelial Adhesion Following FMLP**

Exp. Group	N	Baseline	FMLP Treatment
Sham/NaCl	42	3.1 ± 0.4	6.4 ± 0.6
SnCl <sub>2</sub>	28	2.5 ± 0.3	2.5 ± 0.2
P value		ns	<0.05

\*Units are cell number per 100 µm of vessel length.

The systemic pretreatment of rodents with stannous chloride or zinc

chloride can provide tissue-level protection against acute ischemia and reperfusion injury. The cytoprotected state is associated with a reduction in leukocyte adherence to FMLP-primed endothelium and enhanced expression of hsp70 within vascular tissue. The low toxicity profile of these metal salts makes these agents ideal pharmacologic candidates for future clinical trials targeting preoperative stress conditioning in the human.

In a recent study of human hsp72 and hsp70B' induction in colon cell lines by ZnCl<sub>2</sub>, several cell-specific effects were found, indicating a potentially useful selectivity in human cellular responses to zinc ions. Hsp72 was inducible in HT-29 human colon carcinoma cells but not SW-480 colon cancer cells or CRL-1807 human colonocyte line. Hsp70B' was induced in HT-29 and CRL-1807 cells but not SW-480 cells<sup>66</sup>.

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