

The Role of Zinc in Cerebral Ischemia

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Ischemic stroke is one of the most pervasive life-threatening neurological conditions for which there currently exists limited therapeutic intervention beyond prevention. As calcium-focused neuroprotective strategies have met with limited clinical success, it is imperative that alternative therapeutic targets be considered in the attempt to antagonize ischemic-mediated injury. As such, zinc, which is able to function both as a signaling mediator and neurotoxin, has been implicated in cerebral ischemia. While zinc was first purported to have a role in cerebral ischemia nearly twenty years ago, our understanding of how zinc mediates ischemic injury is still in its relative infancy. Within this review, we examine some of the studies by which zinc has exerted either neuroprotective or neurotoxic effects during global and focal cerebral ischemia.

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Worldwide, stroke is a leading cause of long-term disability and the most common life-threatening neurological disorder (1). While stroke is a heterogeneous condition that encompasses a variety of etiologies, most strokes result from the obstruction of an intra-cranial artery by a thrombus (2). To date, thrombolytic interventions, which aim to lyse clots and restore blood flow to compromised brain regions, represent the only effective treatment for ischemic strokes. Nevertheless, administration of tissue plasminogen activator (tPA), the only approved thrombolytic agent, is constrained by its limited therapeutic window of three hours and by complications derived from hemorrhagic risks, reperfusion injury, and its own intrinsic toxicity (3). Furthermore, the potential for neuroprotective therapies, which aim to antagonize glutamate-induced excitotoxicity or neuronal death mediated by calcium dyshomeostasis, have met with limited clinical success (4). However, effective and alternative therapeutic interventions may be unveiled from a more comprehensive understanding of the biochemical changes

mediating ischemic brain injury. As such, calcium may not be the only divalent metal cation involved in ischemia.

Rather, recent evidence suggests that calcium may serve as an accomplice to zinc, a possibly more potent ionic mediator of ischemic injury (5–8). While zinc and calcium may rely upon common pathways to penetrate and injure cells, recent data also suggests that toxic elevations in intracellular calcium levels, may in part or in its entirety, be induced by zinc (7,8).

Nearly two decades ago, zinc was first implicated in the pathogenesis of ischemia and more than twenty additional *in vivo* studies have since examined the role of zinc during both global and focal experimental paradigms. Some of these studies are summarized in Table 1 and are discussed below. From these studies, zinc has been reported to possess both neurotoxic and neuroprotective capabilities during experimentally-induced ischemia. However, as can be seen in Table 1, few of the findings from these studies are directly comparable, largely owing to considerable diversity in study design, such as variations in models and

duration of ischemia, species and strain, and dosage, route, and regimen of either zinc chelators or zinc supplements.

NEUROTOXICITY OF ZINC IN ISCHEMIA

Tonder and colleagues (9) conducted the first study providing indirect evidence for the toxic translocation of zinc from presynaptic neurons into selective postsynaptic neurons during the experimental paradigm of global ischemia. TSQ (N-[6-methoxy-8-quinoly]-P-toluenesulfonamide) and acid fuchsin staining were used in conjunction to compare changes in zinc staining with the occurrence of degenerating or acidophilic cells between 2 and 24 h post-ischemia. Although degeneration of the cornu ammonis 1 (CA1) subfield was not observed due to the acute survival period of this study, the distribution of TSQ-cell stained bodies of CA4, which were observed as soon as 2 h post-ischemia, corresponded with the distribution of degenerating neurons observed beginning at 18 h post-ischemia. The concomitant decrease in TSQ fluorescence of the mossy fiber terminals and the intracellular accumulation in the CA4 neurons strongly implicated the toxic translocation of zinc.

Johansen and colleagues (10), as a follow-up to the original study conducted by Tonder and others (9) found that intra-ischemic hypothermia (29°C)

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Table 1. Overview of in vivo Studies Investigating the Role of Zinc during Cerebral Ischemia

Species/Model	Therapeutic Intervention	Assessment Methods/Main Finding(s)	REFERENCE
Rat/Global ischemia; 4-VO ^o (20 min occlusion).	–	TSQ ^o & acid fuchsin staining between 2 and 24 h post-ischemia/At 2 h: TSQ-stained somata in CA4, diminished TSQ-signal in mossy fibers; at 18 h: TSQ-stained cells corresponded to acidophilic cells.	(9)
Rat/Focal ischemia; tMCAO ^c (60 min occlusion).	ZnPP ^d (1–10 µg); topical application.	Brain edema (wet/dry weight method) assessed at 24 h post-ischemia/Modest but significant reduction in brain edema with ZnPP treatment.	(24)
Rat/Global ischemia; 4-VO (20 min occlusion).	Pre-tx ^e & intra-tx ^f hypothermia (29°C) started 1 h prior and maintained for ischemia.	TSQ staining 2 h to 7 days post-ischemia/ischemia at 37°C; TSQ-stained CA3 neuronal bodies present at 2 to 24 h; ischemia at 29°C: TSQ-stained cell bodies absent.	(10)
Rat/Focal ischemia; pMCAO ^g or tMCAO (2 h occlusion).	ZnPP (50 mg/kg) i.p., pre-tx: 30 min (pMCAO & tMCAO); post-tx ^h : 2 or 4 h (tMCAO).	Infarct volume & brain edema assessed 24 h post-ischemia/ZnPP pre-tx in pMCAO: no effect; ZnPP post-tx in tMCAO: no effect; ZnPP pre-tx in tMCAO: dramatic neuroprotective effect reducing infarct volume & brain edema.	(26)
Rat/Global ischemia; 2-VO ⁱ (15 min occlusion).	Ca-EDTA 300 mM (5 µL; i.c.v.), pre-tx: 30 min.	TSQ & acid fuchsin staining 72 h post-ischemia/Zn ^h accumulation leads to degeneration; Ca-EDTA reduced Zn accumulation & degeneration of CA1 neurons.	(11)
Rat/Focal ischemia; tMCAO (2 h occlusion)	ZnCl ₂ ^k (10 mg/kg; i.p.), PP ⁱ (48.5 mg/kg; i.p.), ZnPP (50 mg/kg; i.p.), pre-tx: 30 min.	Infarct volume & brain edema assessed 24 h post-ischemia/ZnCl ₂ reduced infarct volume but had no effect on brain edema; ZnPP and PP: both significantly reduced infarct volume and brain edema.	(27)
Gerbil/Global ischemia; BCCAO ^m (3 min occlusion).	ZnCl ₂ (20 mg/kg; s.c.) pre-tx: 1 h, or 48 and 24 h.	TUNEL ⁿ and H-E ^o at 3 and 4 days, respectively/1 h ZnCl ₂ pre-tx: no effect; 48 and 24 h ZnCl ₂ pre-tx: modest but significant protection of CA1 region.	(28)
Gerbil/Global ischemia; BCCAO (5 min occlusion).	–	Examined ZnT-1 mRNA expression between 12 h to 1 week post ischemia/ZnT-1 mRNA expression was induced in CA1 neurons exhibiting Zn accumulation. Without subsequent ZnT-1 protein expression, these cells started to degenerate at 72 h.	(12)
Rat/Focal ischemia; pMCAO.	–	Neo-Timm stain to show synaptic Zn levels from 7 min to 7 days post-ischemia/Decrease in Zn staining at 7 min and continued throughout 7 days; at 1 h, Zn-positive cell bodies seen.	(31)
Rat/Global ischemia; 2-VO (15 min occlusion).	Ca-EDTA 300 mM (3 µL; i.c.v.); pre-tx: 30 min.	TFL-Zn ^o and TUNEL staining 24, 48, and 72 h post-ischemia/Ca-EDTA markedly reduced Zn accumulation and degeneration of CA1 neurons at all time points.	(14)
Rat/Global ischemia; 2-VO (12 min occlusion).	Sodium Pyruvate (500 mg/kg; i.p.); pre-tx: 30 min; post-tx: 0 ^o , 0.5, 1, 2, 3 h.	TFL-Zn & TUNEL staining 3, 15, and 30 days post-ischemia/Sodium pyruvate almost completely blocked neuronal injury when given within 1 h of ischemia.	(16)
Rat/Focal ischemia; tMCAO (30 min occlusion – mild ischemia) or (60 min occlusion – severe ischemia).	Ca-EDTA 500 mM (5 µL; i.c.v.); pre-tx: 15 min or post-tx: continuous infusion for 1 week (10–100 nmole/h) 1 µL/h; i.c.v.	TSQ, HE, acid fuchsin, CM1-IHC ^r staining 3 h to 14 days post-ischemia/Ca-EDTA pre-tx reduced intracellular Zn accumulation, infarct volume, and degeneration at 3 days but protective effects lost at 14 days or if insult intensity increased (30 min occlusion to 60 min occlusion) and if Ca-EDTA was continuously infused for 7 days.	(17)
Mouse/Global ischemia; BCCAO (20 min occlusion).	Hypothermia (33°C) initiated at occlusion; continued 35 min.	TSQ & H-E staining 72 h post-ischemia/Hypothermia markedly reduced Zn accumulation and degeneration of CA1, CA2, and CA4 neurons.	(18)
Mouse/Focal ischemia photothrombosis.	–	Infarct volumes & ZnSe ^{AM/GS} assessed 30 min to 24 h post-ischemia/Infarct core devoid of Zn by 0.5 h until 24 h; 20% increase in Zn in peri-infarct region up to 6 h; Lesions markedly larger at later (12 and 24 h) than at earlier (0.5–6 h) times.	(33)

Continued

Table 1. Continued

Rat/Focal ischemia; Embolic MCAO.	ZnCl ₂ (80 μmol/kg; i.p.); Bicuculline (48.5 μmol/kg; i.p.) or the above in combination; pre-tx: 30 min.	(19)
Gerbil/ Focal ischemia; right pMCAO.	–	(35)
Gerbil/Global ischemia BCCAO (5 min occlusion) and Rat/Global ischemia 4-VO (10 min occlusion).	Ca-EDTA 300 mM (5 μL; i.c.v.); pre-tx: 30 min or post-tx: 3, 6, 48, 60, or 72 h.	(22)
Rabbit/Global ischemia; inflatable neck tourniquet & systemic hypoperfusion (30 min occlusion).	–	(38)
Rat/Focal ischemia tMCAO (60 min occlusion).	Ca-EDTA 100 mM (5 μL; i.c.v.); pre-tx: 30 min	(30)
Rat/Global ischemia 4-VO (30 min occlusion).	–	(36)
Rat/Focal ischemia tMCAO (60 min occlusion).	–	(37)

^a4-VO, 4-vessel occlusion.

^bTSQ, N-(6-methoxy-8-quinolyl)-P-toluenesulfonamide.

^ctMCAO, transient middle cerebral artery occlusion.

^dZnPP, zinc protoporphyrin.

^epre-tx, pre-treatment.

^fintra-tx, intra-treatment.

^gpMCAO, permanent middle cerebral artery occlusion.

^hpost-tx, post-treatment.

ⁱ2-VO, 2-vessel occlusion.

^jZn, zinc.

^kZnCl₂, zinc chloride.

^lPP, protoporphyrin.

^mBCCAO, bilateral common carotid occlusion.

ⁿTUNEL, Terminal Deoxynucleotidyl Transferase Mediated dUTP Nick End Labeling.

^oH-E, hematoxylin and eosin stain.

^pIFL-Zn, N-(6-methoxy-8-quinolyl)-P-carboxybenzoylsulfonamide.

^q“0” hr refers to the time coinciding with the onset of reperfusion

^rCM1-IHC, antibody against activated caspase-3 immunohistochemistry.

^sZnSe^{AMC}, zinc-selenium autometallography technique.

^tGF-AAS, graphite furnace atomic absorption spectroscopy.

^uAAS, atomic absorption spectroscopy.

prevented ischemic-induced intracellular zinc accumulation and subsequent cellular demise, likely by inhibiting zinc translocation.

More direct evidence for the translocation of zinc was provided by the findings of Koh and others (11), who demonstrated that during a brief period of global ischemia, intracellular zinc accumulation in vulnerable CA1 pyramidal hippocampal neurons preceded degeneration, which could be prevented with the intracerebroventricular administration of the high affinity, membrane-impermeable zinc-chelator, ethylenediaminetetraacetic acid (EDTA) saturated with calcium (Ca-EDTA). This finding convincingly postulated that selective neuronal death of CA1 neurons during global ischemia was mediated by the release of synaptic vesicle zinc from a subset of excitatory terminals and its subsequent translocation into vulnerable post-synaptic neurons. Furthermore, intracellular zinc accumulation preceded neuronal degeneration, which could be prevented with the administration of an extracellular zinc chelator.

In follow-up to the findings of Koh and others (11), Tsuda and colleagues (12) examined the induction of ZnT-1 mRNA expression in the CA1 subfield of the hippocampus following global ischemia. It was postulated that in response to the increased levels of intracellular zinc, vulnerable neurons would up-regulate ZnT-1, the plasma membrane-localized zinc transporter that facilitates zinc efflux (13). While ZnT-1 mRNA expression was enhanced as soon as 12 h post-ischemia, without subsequent ZnT-1 protein expression, cellular demise ensued by three days post-ischemia.

Park and colleagues (14) demonstrated that zinc-mediated neuronal death following ischemia may be achieved through the specific induction of p75^{NTR} and its associated death executor, NADE. Following global ischemia, p75^{NTR} and NADE induction was detected in degenerating CA1 pyramidal neurons exhibiting dense zinc accumulation. The co-induction of p75^{NTR} and NADE was found to be dependent

upon zinc levels because the administration of Ca-EDTA completely blocked the induction of p75^{NTR} and NADE and subsequent neurodegeneration of CA1 pyramidal neurons.

Culture studies have demonstrated that zinc neurotoxicity may promote the disruption of different stages of cellular respiration through depletion of ATP and the oxidized form of the coenzyme, nicotinamide adenine dinucleotide (NAD⁺) (15). Animal studies have demonstrated that the administration of pyruvate, the end metabolite of glycolysis, can achieve neuroprotection by normalizing metabolic disturbances and antagonizing zinc neurotoxicity following global ischemia (16). Post-ischemic supplementation with pyruvate was found to provide remarkable, long-lasting neuroprotection when administered within 1 h after the onset of reperfusion (16).

Lee and colleagues (17) found that the intracerebroventricular administration of Ca-EDTA prior to mild focal ischemia achieved early neuroprotection against zinc accumulation and subsequent cellular demise. This effect, however, was lost if either the ischemic insult was more pronounced, if the survival period was extended to two weeks post-ischemia, or if Ca-EDTA treatment was continuously administered.

While Johansen and others (10) demonstrated that deep hypothermia (29°C) reduces interneuronal zinc movement and subsequent death, Tsuchiya and colleagues (18) investigated the impact of mild hypothermia (33°C) on zinc release and associated neuronal death following global ischemia. Mild intra-ischemic hypothermia was found to markedly reduce zinc accumulation and associated degeneration of hippocampal neurons 72 h following ischemia.

Shabanzadeh and colleagues (19) found that intraperitoneal pre-treatment of zinc alone or in conjunction with the GABA_A antagonist, bicuculline had detrimental effects on neurological deficits and the development of the infarct following induction of focal ischemia.

It is now well established that there is an ischemic-mediated regulation of the subunit composition of calcium A/K channels (20). The GluR2 hypothesis postulates that reduced GluR2 expression allows for the toxic calcium, and even zinc entry during ischemia (5,21). Calderone and others (22) demonstrated that zinc plays an integral role in this regulation through its persistent downregulation of GluR2 mRNA (22). Increasing cytosolic zinc levels, for instance, have been shown to induce the expression of a zinc-finger transcription factor REST (restrictive element-1 silencing transcription factor), which is able to suppress neural-specific target genes, including GluR2 (22,23). Calderone and colleagues (22) further demonstrated that during ischemia, zinc triggers neuronal death through temporally distinct mechanisms. It was discovered that intracerebroventricular pre-treatment with Ca-EDTA significantly attenuated the ischemia-induced down-regulation of GluR2 mRNA and protein expression in the CA1 hippocampal subfield. Ca-EDTA pre-treatment further blocked the early stages of apoptosis in CA1 neurons by reducing levels of cytochrome c and caspase 3 activity and DNA fragmentation. Furthermore, Ca-EDTA, when administered between 48 and 60 h post-ischemia, also prevented zinc accumulation and degeneration of CA1 pyramidal neurons, likely by blocking the later stages of apoptosis, but not when administered at 3, 6, or 72 h post-ischemia.

While the above studies demonstrate that elevated intracellular zinc levels during ischemia serve as a critical mediator of neuronal death, zinc inhibition achieved through either early or late chelation paradigms may be effective in combating zinc neurotoxicity.

NEUROPROTECTION BY ZINC IN ISCHEMIA

In contrast to chelation-based therapeutic intervention, various studies have demonstrated neuroprotective benefits following the administration of various zinc compounds. Yamasaki and col-

leagues (24), for example, examined the effectiveness of zinc protoporphyrin (ZnPP) in mediating post-ischemic brain edema by selectively blocking the cytokine, interleukin-1 (IL-1). In this study, following transient focal ischemia, ZnPP was topically applied to the lateral ventricle at the onset of reperfusion. Brain edema was assessed 24 h later using the wet and dry method (25) and the topical application of ZnPP was found to reduce ischemic brain edema significantly by blocking IL-1 activity.

As a follow-up investigation into the reported anti-inflammatory effects of ZnPP demonstrated by Yamasaki and others (24), Kadoya and colleagues (26) demonstrated that ZnPP was limited in its level of neuroprotection by the temporal parameters of its administration and whether or not reperfusion followed the onset of ischemia. Specifically, intraperitoneal ZnPP pretreatment was found to significantly reduce infarct volume and post-ischemic brain edema in the transient model of ischemia (tMCAO). However, the neuroprotective effects of ZnPP were lost if either the severity of the ischemic insult increased (permanent MCAO) or if treatment was delayed two or four hours following ischemic onset.

In a follow-up study to Kadoya and others (26), Zhao and colleagues (27) set out to determine whether the zinc or protoporphyrin complement of ZnPP possessed neuroprotective capabilities. Equimolar doses of zinc chloride (ZnCl₂), PP, and ZnPP were all found to reduce the lesion size, but only ZnPP and PP were found to ameliorate ischemic brain edema. As such, this study suggests that zinc ions, in comparison to protoporphyrin, provide neuroprotection by mechanisms other than reducing brain edema.

Matsushita and colleagues (28) later demonstrated that zinc supplementation could provide neuroprotection to the CA1 hippocampal subfield during global ischemia in the gerbil. Because the gerbil possesses isolated cerebral hemispheres and an incomplete circle of Willis, the bilateral occlusion of the common carotid

arteries (BCCAO) results in pronounced global ischemia (29). It was found that while superacute (1 h) subcutaneous pretreatment with ZnCl₂ had no effect, subacute (48 and 24 h) pretreatment afforded significant neuroprotection to vulnerable CA1 cells against delayed neuronal death.

Recently, Kitamura and colleagues (30) have found that reduction in zinc levels following intracerebroventricular injection of Ca-EDTA prior to focal ischemia accelerated the early development of the infarct, suggesting the need for a minimum complement of zinc to maintain cellular viability, even during cerebral ischemia.

CHANGES IN ZINC LEVELS DURING ISCHEMIA

Alternatively, other studies have attempted to monitor dynamic changes in zinc levels during cerebral ischemia in the absence of therapeutic interventions. Sorensen and colleagues (31) set out to examine if altered levels of synaptic vesicle zinc during the course of focal ischemia could be detected histochemically (32). Relying on the neo-Timm stain, it was demonstrated that as soon as 7 min following ischemic onset, zinc positive-terminal staining was visibly decreased within the ischemic region and was relatively absent for all remaining times examined, up to seven days post-ischemia. The rapid reduction and eventual absence of zinc staining was attributed to the likely release of zinc from synaptic vesicles. The emergence of zinc-stained neurons at one hour post-ischemia may reflect zinc accumulation following trans-synaptic movement of zinc from surrounding zinc-enriched terminals.

Similarly, our laboratory has examined the temporo-spatial changes in synaptic vesicle zinc levels following focal ischemia using the zinc-selenium autometallography technique (33). Utilizing the technique of photothrombosis (34), we found that the core of the infarct was devoid of zinc staining up until 24 h post-ischemia, the last time point examined, while the peri-infarct region showed a significant increase (at least 20%) in

staining intensity up to six hours post-ischemia. Interestingly, infarct volumes were found to be significantly larger, at least double in size, at the latter time points (12 and 24 h) compared with infarct volumes assessed at the earlier time points (30 min, 1–6 h). We also verified these findings in a transient model of focal ischemia to confirm the vascular access of sodium selenite. The reason for the elevated levels of synaptic zinc staining within the periphery of the infarct is currently under investigation but may represent a compensatory mechanism to buffer the release of synaptic vesicle zinc and, moreover, may even delineate the putative penumbral region.

In addition to utilizing histochemical stains or fluorescent dyes to measure changes in zinc levels during ischemia, detection using micro-dialysis has also been employed. Using dual probe micro-dialysis coupled with graphite furnace atomic absorption spectroscopy (MD-GFAAS), Yang and colleagues (35) detected a significant decrease in zinc levels from baseline in the ipsilateral cortical hemisphere and slight changes in the contralateral hemisphere during focal ischemia. Although this study suggested that extracellular zinc levels drop during ischemia, more recent micro-dialysis studies suggest that while a temporal derangement in extracellular zinc levels during ischemia occurs, levels are increasing rather than decreasing.

Kitamura and colleagues (36) examined the temporal release profile of extracellular zinc using micro-dialysis and also examined subsequent intracellular zinc accumulation in the CA1 subfield of the hippocampus during global ischemia. Within 15 min post-ischemia, extracellular zinc levels reached a peak (~600 nM) that was double the basal level. Subsequently, extracellular zinc levels decreased and returned to baseline 15 min following reperfusion. A similar release profile also was found for glutamate, although glutamate levels reached peak level within 30 min and were more than 20× the basal level. Additionally, using TSQ fluorescence, evidence for intracellu-

lar zinc-accumulation was found within the CA1 pyramidal neurons but not before 24 h post-ischemia, suggesting that the release of zinc from synaptic vesicle stores is neither excessive nor immediately accumulated in vulnerable post-synaptic neurons. Similarly, Kitamura and colleagues (37) also found that extracellular zinc levels in the focal ischemic cortex increased within 15 min, peaked to twice the basal level (~300 nM) within 30 min post-ischemia, and returned to basal level within 15 min after reperfusion.

Recently, Frederickson and colleagues (38) also have used micro-dialysis to examine extracellular zinc levels during global ischemia and reperfusion. Following ischemic onset, extracellular zinc levels increased and were synchronous with glutamate release. However, the reperfusion-induced zinc release was more pronounced (> 100 nM, in some cases), in both intensity and duration than the initial ischemic-induced zinc release. Moreover, the delayed reperfusion-induced zinc release was unaccompanied by glutamate release, possibly reflecting a release of zinc from intracellular stores, such as metallothioneins (39–41) or mitochondria (42–44).

Although the predominance of available literature from *in vivo* ischemic studies has focused on the involvement of synaptic vesicle zinc, it is likely that intracellular zinc accumulation during ischemia is achieved through a possible synergism of zinc release from both synaptic and intracellular stores of zinc, as most recently demonstrated during global ischemia (38) and reported in other experimental paradigms (45–51).

CONCLUSION

Despite recognizing the involvement of zinc in cerebral ischemia nearly twenty years ago, we are only beginning to unravel the physiological functions of zinc during ischemia. Considering the multimodal impact that zinc has on cellular physiology, undoubtedly intricate, overlapping, and even synergistic mechanisms are liable to account for its toxic or protective capabilities (5,6,52–54; Figure 1).

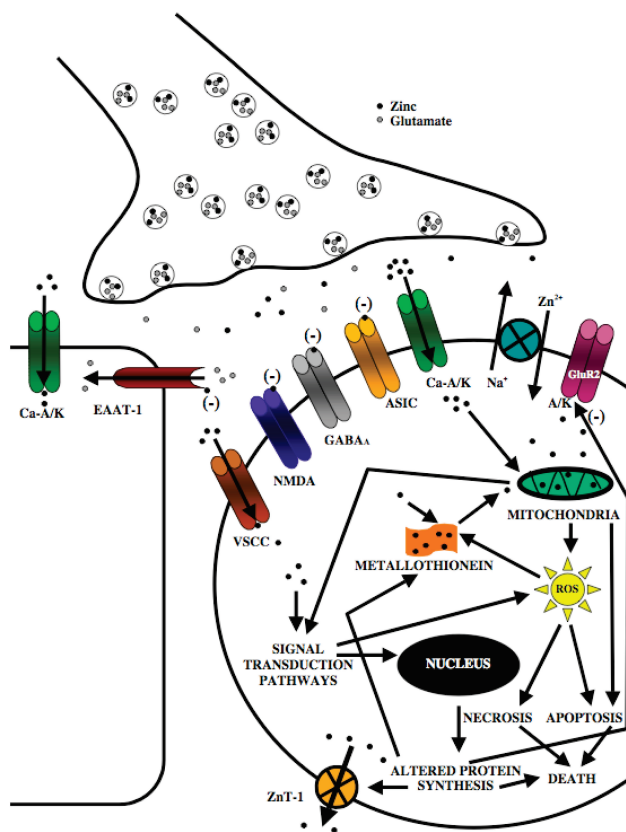


Figure 1. Schematic Overview of the Putative Toxic and Protective Mechanisms Elicited by Zinc during Cerebral Ischemia

During ischemia, heightened release of zinc from a subset of glutamatergic terminals likely promotes the translocation and accumulation of zinc in vulnerable post-synaptic neurons. Following release, synaptic zinc is thought to achieve cellular access predominately through subpopulations of calcium-permeable AMPA and/or kainate channels (Ca-A/K) (56–58). Zinc entry may also be facilitated by the zinc-sodium exchanger and less predominately through voltage sensitive calcium channels (VSCC) or NMDA-type glutamate receptors (56,57,59,60). Intense cytosolic zinc overloads, likely mediated by Ca-A/K receptor channels, can promote pronounced mitochondrial dysfunction and reactive oxygen species (ROS) generation to trigger necrosis, whereas milder cytosolic zinc loads may augment apoptotic pathways (43,57,61–63). The cellular oxidative stress and acidosis achieved during ischemia may additionally promote the liberation of zinc from zinc-ligands, such as metallothioneins (39,49,64,65). In the attempt to confer resistance to the rising cytosolic zinc levels, the zinc transporter, ZnT-1 and metallothionein III can be upregulated during ischemia to promote zinc efflux and cytosolic buffering, respectively (12,66,67). Zinc also plays an integral role in the subunit expression of Ca-A/K channels during ischemia by altering transcriptional regulation that leads to GluR2 subunit downregulation, the presence of which renders A/K receptor channels calcium-impermeable (20–23). Zinc also can activate signal transduction pathways, such as protein kinase C, which can promote ROS generation (68,69). Zinc also can augment glutamate-induced neuronal injury by directly inhibiting GABA_A channels and inhibiting glutamate re-uptake by blocking excitatory amino acid transporters (EAAT-1) expressed on glial cells (70–72). During ischemia, activation of Ca-A/K receptor channels, acidosis, and elevated zinc levels can also work synergistically to promote glial injury (73). Conversely, during ischemia, zinc also may achieve protective effects by substantially inhibiting calcium influx by blocking NMDA-type glutamate receptor channels or acid-sensing ion channels (ASICs) (74–80). Zinc also can exert anti-apoptotic efforts through the inhibition of various caspases, pro-apoptotic genes, and endonucleases (81–83).

While the mechanisms by which zinc mediates or prevents ischemic-induced injury are complex, conceivably, as is the case for calcium, cells are also expected to possess a specific zinc set-point, by which too little or too much zinc can promote cellular demise (5). As such, studies examining either zinc chelation or supplementation should heed the delicate balance by which zinc achieves its toxic or protective capabilities. Moreover, in view of the clinical importance of zinc in mediating ischemic injury, future investigation is warranted to develop more effective, potentially zinc-based therapies. To this effect, a novel lipophilic BAPTA diester, DP-b99 already has shown remarkable clinical promise in treating patients with ischemia. The success of DP-b99 in the laboratory and in the clinic, thus far, has, in part, been attributed to the delicate manner by which it buffers and redistributes zinc ions (55).

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