Ischemic Tolerance: what does not kill you makes you stronger.

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Stroke affects over 750,000 people per year in the USA. Stroke is particularly prevalent in the south eastern US, with an age-adjusted stroke mortality rate more than 10% above the national average. In Slovenia, stroke is regarded as the number 3 leading cause of death. Current trends suggest that since survival rates from stroke are improving, a focus on reducing disability will become a high priority in the coming years. Stroke can take two major forms, either hemorrhage, due to the rupture of a major or minor blood vessel in brain, or ischemic due to a reduction in blood flow to either the entire brain, or a smaller defined regional territory.

The pathophysiology of brain injury following stroke suggest that the damage is not immediate and evolves, such that over 50 % of the infarct region is potentially salvageable if therapies can be administered. However, there are no currently approved therapies to reduce brain injury following stroke. The only stroke therapy is that of improving reperfusion, such as the "clot buster" tPA or mechanical clot retrieval, which have narrow therapeutic time windows and reach a small percentage of patients. In addition many experimental approaches to reduce brain injury, while promising in pre-clinical trial have failed in the clinic, hence a different approach may be warranted.

Our strategy is for parenchymal protection via the identification of molecular mechanisms which underlie the phenomenon of ischemic tolerance. prior exposure to sub-lethal ischemic stress primes cells to be tolerant to subsequent and normally injurious durations of ischemic stress[1]. Tolerance to a harmful environment can be developmental or acquired. Tolerance can be induced in an organisms a target organ, or a single cell. Tolerance can be induced by multiple stressful environments, including excitotoxicity, temperature changes and metabolic inhibition[2-4]. Clinical retrospective and prospective studies suggest that ischemic preconditioning may occur in human stroke [5-8]. Ischemic preconditioning is being evaluated in the clinic for conditions with raised stroke risk, i.e. ischemia from vasospasm in patients following sub-arachnoid hemorrhage [9]. Yet little is known about the molecular mechanisms whereby the preconditioning stimulus activates a tolerant phenotype. Tolerance has two temporal profiles of protection: delayed tolerance mediated by a cascade of genomic-encoded adaptions to stress[2]. In contrast, rapid tolerance is mediated by a series of new protein synthesis-independent biochemical signaling events, and these will be considered further.

One of our first observations on rapid tolerance, was the inhibition of cell death signaling in rapid ischemic tolerance[2]. This suggested that mediators of apoptosis may be regulated in rapid ischemic tolerance. We screened Bcl-2 family proteins, because these proteins are the major regulators of cell death signaling. We discovered that the procell death protein Bim was down regulated with a temporal profile matching rapid tolerance. Our studies also show that the halflife of Bim is too slow to account for the loss of protein levels, suggesting Bim was being actively degraded in tolerance[2].

Our investigations show that Bim is degraded by the ubiquitin-proteasome system. Proteasome inhibitors block rapid tolerance and the loss of Bim protein levels. Our studies also show p42/p44 MAPK regulated the ubiquitination of Bim following preconditioning ischemia[2]. The significance of this is shown given that even small reductions in Bim are protective. Our studies also show that this same mechanism can account for some of the neuroprotective effects of adenosine. [10]

Our studies then focused on what else would be subject to regulation by the ubiquitin-proteasome system following a preconditioning dose of ischemia. We utilized a shotgun proteomics approach to identify proteins that were ubiquitinated and precipitated using an ubiquitin binding matrix. Informatic analysis of the candidate proteins revealed a signature suggesting a biological event in dendritic spines[11]. First we determined that actin binding proteins in the post synaptic density are degraded by the proteasome following preconditioning ischemia. Second we observed a change in actin remodeling in neurons. The importance of this is shown given the actin stabilizing compound jasplakinolide blocks rapid ischemic tolerance. The impact of these changes to the post synaptic density is quite profound on the morphology of dendritic spines. Following precondition, there is a rapid and transient loss of dendritic spines on neurons, an effect that is blocked by the proteasome inhibitor MG132, and the actin stabilizer jasplakinolide[11].

The consequence for neuronal function is shown given NMDA receptor-mediated excitotoxicity is muted following preconditioning ischemia. Remodeling of the post synaptic density results in release of NMDA receptors from the actin cytoskeleton, and a reduction in NMDA currents, further analysis of these channel properties suggest multiple subunits may play a role in tolerance. [11]

In order to exploit the therapeutic potential of our observations the identity of the E3-ligase mediating these rapid protective events must be found. Yet two previous candidates for a Bim E3-ligase exist, yet neither appear to match the biology of Bim degradation in our system. We utilized a phospho-Bim as a bait to identify potential E3-ligases. Our candidate was identified as TRIM2, a member of a large family of RING-containing proteins with a signature of a Ring, B-box and coil coiled domain. Few studies have investigated TRIM2, but it has been shown to regulate neuronal polarity, and loss of TRIM results in a neurodegenerative phenotype[12,13]. Validation studies in human, mouse and rat cell show TRIM2 interacts with Bim in a MAPK dependent manner. Critically blocking TRIM2 expression using ShRNA stabilizes Bim protein levels and blocks rapid ischemic tolerance.

So what does the future hold for this approach? We continue our studies to identify additional proteins that regulate Bim ubiquitination, including additional binding proteins, and E2-ligases. We are looking at other published substrates of TRIM2, to determine if they too are degraded in rapid tolerance. We are investigating the role of TRIM2 in mediating the morphological phenotype of rapid tolerance that we have defined. Finally, we are investigating other therapeutic agents to determine whether TRIM2 plays a critical role in their protective effects.

In summary, we have discovered the role of multiple cellular signaling cascades in mediating rapid ischemic tolerance. These studies will provide information relevant not only to acute brain injury protection, but also will aid in understating synaptic structure, with relevance to additional neuropathological conditions such as schizophrenia, autism and neurodegeneration. Understanding the response of the synapse to ischemia could also help promote reinnervation and recovery of function following injury. This information could be utilized to develop a high throughput screen for assessing the neuroprotective potential of small molecule drug libraries. Given the dearth of effective therapies for stroke, we feel that our approach could yield novel therapies to reduce brain injury following stroke.

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