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ISCHEMIC TOLERANCE OF THE BRAIN
FROM BENCH TO BEDSIDE

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ABSTRACT:
Neurons can be preconditioned by various procedures to resist ischemic events. The preconditioning mechanism induced is characterized by a brief episode of ischemia that renders the brain more resistant against subsequent longer ischemic events. This ischemic tolerance has been shown in experimental animal model of cerebral ischemia and recently in humans. In contrary to the heart, the basic molecular mechanisms of cerebral ischemic tolerance are largely unknown in detail, but is initiated over several hours or days, and can persists for up to a week or more. The pathophysiological course can be divided in (i) induction, (ii) transduction and (iii) tolerance. The cellular mechanism is explained leading to the synthesis of stress proteins. These proteins works as cellular „chaperons" by unfolding misfolded cellular proteins and helping the cell to dispose of unneeded denatured proteins. Implications to clinical and therapeutic implications are drawn.

Key words:
cerebral ischemia – ischemic tolerance – preconditioning – apoptosis – neuronal death

Introduction

Stroke is the third leading cause of death in highly industrial countries. Ischemic stroke counts for 80 % of these cases. At present, the only therapeutic option demonstrated to improve outcome from acute ischemic stroke is thrombolysis of the clot responsible for the ischemic event (Higashida et al., 2003). Accordingly, stroke patients treated by thrombolysis, have better chance of survival with little or no residual disability, but at the same time they may suffer the consequences of a reperfusion damage (Heiss et al. 1999). Much has been learned about the presumed target of acute stroke therapy, the ischemic penumbra, and clinically available imaging modalities such as magnetic resonance imaging and computed tomography hold great promise for at least partially identifying this region of potentially salvageable ischemic tissue (Schellinger et al. 2005). Understanding the biology of ischemia-related cell injury has also evolved rapidly (Schaller et al. 2004) so that emerging therapeutic strategies should be targeted to the initially critically perfused subcompartments (Heiss et al. 1999). In order to minimize the damage caused by the early critical flow disturbances leading to rapid cell damage as the predominant cause of infarction (Heiss et al. 1999), the protocol for the thrombolysis treatment should include for this reason simultaneous administration of protective compounds (Schaller et al. 2004). Such new treatment approaches to improve outcome after focal brain ischemia will likely be derived by looking at naturally occurring adaptive mechanisms such as those related to ischemic preconditioning and hibernation (Schaller et al. 2004; Schaller et al. 2003). Combining knowledge from these three areas provides optimism that additional acute stroke therapies can be developed to maximize beneficial functional outcome in the greatest proportion of acute stroke patients possible (Fisher et al. 2003). Furthermore, finding of long-acting protective compounds could enable preventive administration of such drugs to stroke-prone subjects (Fisher et al. 2003). The development of such potent protective compounds depends on elucidation of protective mechanisms, enabling to focus on specific targets conferring maximal protection (Reshef et al. 2000) with the long-term goal of allowing therapeutic augmentation of the endogenous protective mechanisms in cerebral ischemia and possibly development of new neuroprotective strategies for ischemic stroke treatment (Blanco et al. 2006). However, these broad-spectrum approaches also made clear that preventing neuronal death and thus reducing neurological damage are complex tasks that cannot be successfully resolved by targeting single mechanisms. One of the most promising approaches is to create ischemic tolerance in the brain as represented by the use of pharmacological preconditioning paradigms.

We give therefore on overview about our current understanding of how preconditioning stimuli trigger a neuroprotective state known as cerebral ischemic tolerance in humans and animals in the light of its clinical relevance in the near future.

Definition of ischemic preconditioning

Preischemic functional state and the level of metabolism are among factors determining the susceptibility of neurons to ischemic events (Ferriero 2005). It has been demonstrated that brief, sublethal cerebral ischemia induces tolerance of neurons to subsequent more prolonged lethal ischemia (Dowden et al. 1999). This phenomenon is called "ischemic preconditioning" and represents probably a fundamental cell/organ response to certain types or levels of injury (Ettaiche et al. 2001). Establishing this protective phenotype in response to stress stimuli depends on a coordinated response at the genomic, molecular, cellular and tissue levels (Schaller et al. 2002, Gidday 2006). Cerebral ischemic tolerance can be established by global and focal ischemia, both of which are capable of protecting the other form of ischemia (Chen J et al. 1997). In addition, cerebral ischemic tolerant states have been induced by chemical, pharmacological, electrical and/or anoxic/ischemic means (cited in Schaller et al. 2002). Further experiments have shown that metabolic and physical stresses can also induce cross-tolerance to cerebral ischemia, but the protection by cross-tolerance is relatively modest (Kirino, 2002). In most of these states of ischemic tolerance,

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the neuronal protection requires several days to develop. The *early phase* lasts for several hours after the preconditioning stimulus and can be related to adenosine receptors and ATP-dependent potassium channels (Schaller et al. 2003). A *delayed phase*, after 1-7 days, is explained by genetic remodeling (Schaller et al. 2003). In exceptional cases, effective tolerance may be achieved only after 12 to 15 days.

The underlying mechanism of ischemic tolerance still is not fully understood. It seems that two distinct phases are involved (Kirino, 2002, Schaller et al 2002). Firstly, a cellular defense function against ischemia may be developed by the mechanisms inherent to neurons such as posttranslational modification of proteins or expression of new proteins via a signal transduction system to the nucleus (Reshef et al. 2000). Secondly, a stress response and synthesis of stress proteins (heat shock proteins) may be activated. These mechanisms are mediated by chaperones by unfolding misfolded cellular proteins and helping the cell to dispose of unneeded denatured proteins (Trendelenburg et al. 2005).

Experimental aspects

Ischemic brain injury evolves over time, often taking days or even weeks to fully develop (Heiss 2003). It is a dynamic process that involves immediate oxidative stress and excitotoxicity followed by inflammation and preprogrammed cell death (Heiss et al. 1999; Ferriero, 2005). Following focal ischemia, a complex and dynamic interaction of vascular cells, glial cells, and neurons determines the extent of the ensuing lesion (Heiss et al. 1999; Trendelenburg et al. 2005). Traditionally, the focus has been on the investigation of mechanisms of damage, while recently it has become clear that endogenous mechanisms of (neuro)protection are equally important for the final outcome after ischemic stroke (Trendelenburg et al. 2005). Such neuroprotective strategies include salvaging neurons through the use of targeted pharmacotherapies, protecting neurons through preconditioning, and repairing neurons by enhancing neurogenesis.

In experimental model organisms, complete or true neuroprotection can be achieved only through preconditioning, a process during which an animal develops tolerance to an otherwise lethal stressor (Kirino 2002). Although of no clinical use, preconditioning models have provided valuable insight into how repair systems work in the brain. Cumulative evidence indicates that the same genes that are up-regulated during preconditioning, those mediating true protection, are also up-regulated during injury and repair (Stenzel-Poore et al. 2004). Numerous mediators and mechanisms have been proposed to explain induced ischemic tolerance (Prass et al. 2003). Specifically, hypoxic preconditioning and hypoxic-ischemic stroke have been shown to induce hypoxia inducible factor-1 (HIF-1) and its target survival genes, vascular endothelial growth factor (VEGF), and erythropoietin (Epo) in animals (Ferriero, 2005). Of particular interest is the up-regulation of Epo, a growth factor that may have therapeutic potential in the treatment of ischemic stroke (Schaller et al. 2006).

HIF-1 is a key physiological sensor of the oxygen level in most mammalian cells. Hypoxia induces HIF-1 p (Semenza 2000) – a transcription factor heterdimeric complex composed of inducible HIF-1alpha and constitutive HIF-1beta proteins - and then regulates the cellular transcriptional response to reduced oxygen availability to restore homeostasis of oxygen as a substrate for aerobic metabolism (Prass et al. 2003). In various animal models, such activation of HIF-1 has been implicated as a key event in (ischemic) tolerance induction in vitro (Ruscher et al. 2002) and in vivo (Prass et al. 2003, Bergeron et al. 2000) binding to the hypoxic response elements in a number of HIF target genes. Among others, HIF-1 induces Epo (Semenza 2000). Only recently it was found that Epo is also expressed in the central nervous system and that it exerts potent neuroprotective effects (Ehrenreich et al. 2002). Epo transcription as well as Epo translation has been found in preconditioned brains (Prass et al. 2002, Prass et al. 2003)). In addition, there is solid evidence that exogenously applied Epo is neuroprotective in vitro as well as in vivo (cited in Schaller et al. 2006). Epo may be necessary but not sufficient for neuroprotection (Schaller et al. 2006), so that it appears more likely that there are also other protective signaling cascades involved in hypoxia-induced tolerance in the brain that are independent of Epo (Prass et al. 2003; cited in Schaller et al. 2006). Potential candidates include nitric oxide, adenosine, vascular endothelial growth factor, Bcl-2, heat shock protein 70, and tumor necrosis factor-α.

The current objective of ischemic preconditioning research is to identify the underlying endogenous protective cellular receptors and signaling cascades, with the long-term goal of allowing therapeutic augmentation of the endogenous protective mechanisms in cerebral ischemia and possibly development of new neuroprotective strategies for ischemic stroke treatment. At this time, however, the postinjury enhancement of neurogenesis appears to offer the best hope for long-lasting functional recovery following brain injury (Trendelenburg et al. 2005).

Overlapping with hibernation

Stroke, ischemic preconditioning, and ischemic preconditioning plus stroke all induce gene changes that overlap little among conditions (Stenzel-Poore et al. 2004, Schaller et al. 2003). Stroke induce robust up-regulation of gene expression whereas preconditioning followed by stroke results in a marked downregulation. Genes up-regulated by stroke suggest activation of stress/inflammatory pathways and increased metabolism and ion channel function (Stenzel-Poore et al. 2004). Preconditioning tends to decrease genes involved in these pathways. Follow-up experiments show that preconditioning decreased voltage-gated potassium currents in vitro and increased bleeding time (Stenzel-Poore et al. 2004). Preconditioning reprograms the response to ischemic injury via transcriptional changes that may suppress metabolic pathways and immune responses, reduce ion channel activity, and decrease blood coagulation (Stenzel-Poore et al. 2004). These changes resemble evolutionarily conserved responses to decreased blood flow and oxygen availability that occur during hibernation (Stenzel-Poore et al. 2004).

Clinical aspects

There are only some few clinical studies about the phenomenon of ischemic tolerance in human brain. The extend of this phenomenon in the brain can not be judged at present. For this reason, we give here two examples of the clinical importance of this wide experimental phenomenon.

(a) Prestroke transient ischemic attack

The first description of this ischemic tolerance-phenomenon was in the heart, which was reported by Murry in 1986 (Murry et al. 1986). Subsequent studies demonstrated ischemic preconditioning in lung, kidney and liver tissue, whereas more recent studies have concentrated on the brain.

Transient ischemic attacks (TIA) have long been identified as a risk factor for subsequent ischemic stroke. How-

ever, recent clinical evidence suggest that prestroke TIA may improve neurological outcome after stroke (Weih et al. 1999, Schaller 2005), by serving as a ischemic preconditioning stimulus and triggering neuroprotective mechansism (Moncoaya et al. 2000, Chen et al. 1997). It seem that patients with prodromal TIAs display different patterns of diffusion and perfusion deficit in a subsequent stroke (Wegener et al. 2004).

Besides the biochemical explanation in animals, other possibilities such as up-regulated collateral circulation of facilitated thrombolysis, (as observed in the heart (Andreotti et al. 1996)), also must be taken into account in humans (Schaller 2005). An interesting feature in this context, may be that acetylsalicylic acid (ASA) is neuroprotective and independent of its actions on platelets in animal studies (Riepe et al. 1997). It was hypothesized that the protective effect of ASA against hypoxia – at least in part – may be due to a similar increase of endogenous hypoxic tolerance (Riepe et al. 1997). The above presented clinical data underline this hypothesis.

The traditional concept of TIA adds to this belittlement, although the short-term risk for any adverse event after TIA, either cerebrovascular or cardiovascular, is greater than previously assumed (Rothwell 2003), and the long-term risk, too, is substantial (Lovett et al. 2003). Because of this and the inability to differentiate TIA from stroke in the hyperacute stage, where thrombolysis is now the state-of-the-art emergency treatment, the term "brain attack" for both, either in combination with cerebral imaging information (Saver, 2004) or not, seems a more adequate approach to improve therapy, mandatory workup, and prediction of prognosis (Daffertshofer et al. 2004). These new findings advocate a new definition or replacement of the term.

(b) Reperfusion

The restoration of cerebral blood flow to ischemic tissues causes additional damage, which is termed reperfusion injury and significantly influences the outcome of patients after ischemic stroke (Heiss et al. 1999). It is now well accepted that central to ischemic/reperfusion-induced injury is what occurs to mitochondria hours to days following the ischemic insult (Perez-Pinzon et al. 2005). For many years, it has been established that reactive oxygen species (ROS) and reactive nitrogen species (RNS) promote lipid, protein, and DNA oxidation that affects normal cell physiology and eventually leads to neuronal demise (Perez-Pinzon et al. 2005). In addition to oxidation of neuronal molecules by ROS and RNS, a novel pathway for molecular modifications has risen from the concept that ROS can activate specific signal transduction pathways that, depending on the insult degree, can lead to either normal plasticity or pathology (Perez-Pinzon et al. 2005). Two examples of these pathways could explain why lethal ischemic insults lead to the translocation of protein kinase Cdelta, which plays a role in apoptosis after cerebral ischemia, or why sublethal ischemic insults, such as in ischemic preconditioning, lead to the translocation of epsilonPKC, which plays a pivotal role in neuroprotection (Reshef et al. 2000).

A better understanding of the mechanisms by which ROS and/or RNS modulate key protein kinases that are involved in signaling pathways that lead to cell death and survival after cerebral ischemia will help devise novel therapeutic strategies (Schaller et al. 2003).

Prospect to the future

The development of alternative new drugs and clinically applicable complex neuroprotective strategies is warranted (Schaller et al. 2003). One of the most promising approaches is to create ischemic tolerance in the brain by using pharmacological preconditioning paradigms. If a neuroprotective drug based on ischemic preconditioning could be considered for prophylactic use in high-risk populations, its prophylactic neuroprotection would include (i) short-term neuroprotection before and after high-stroke risk procedures, (ii) long-term neuroprotection for primary and secondary intervention in populations at high risk for stroke, and (iii) concomitant neuroprotection with agents that have multiple treatment effects (Schaller et al. 2003). Patients undergoing neurosurgical procedures such as aneurysma clipping, endarterectomy, or endovascular therapy, which have a substantial potential risk of cerebral ischemic events during a defined period, might be considered for short-term, periprocedure prophylactic neuroprotection. Several populations at high long-term risk for initial ischemic stroke have been identified and include those with combinations of vascular risk factors, transient ischemic attacks, atrial fibrillation, and asymptomatic carotid stenosis (Fisher et al 1995). Such people, as well as those at risk for stroke recurrence after minor strokes, are readily identifiable and perhaps appropriate for long-term prophylactic neuroprotection.

Conclusion

A brief episode of ischemia renders the brain resistant against subsequent, longer ischemic episodes. This ischemic tolerance has been shown in numerous experimental models of cerebral ischemia. Although the mechanism of ischemic tolerance remains uncertain, its discovery provides the focus for a further understanding of the mechanisms of endogenous neuroprotection and the potential of novel therapeutic strategies for neuroprotection. Clinically, the most effective breakthrough in the treatment of cerebral ischemia has been the successful establishment of systemic thrombolysis. With the increasing knowledge about the dynamics of pathophysiological and molecular mechanisms leading to ischemia-related cell damage and about the presumed molecular targets of treatments counteracting these mechanisms, combinatorial treatment strategies based on thrombolysis and endogenous neuroprotection have to be developed. Ischemic tolerance seems to be a very strong candidate in serving a better understanding of the molecular mechanisms involved in neuroprotection as well as in improving therapeutic strategies for patients with stroke or other ischemia-related diseases.

References

1. Bergeron M, Gidday JM, Yu AY, et al (2000). Role of hypoxia-inducible factor-1 in hypoxia-induced ischemic tolerance in neonatal rat brain. Ann Neurol 48: 285–296.
2. Blanco M, Lizasoain I, Sobrino T, et al..(2006) Ischemic preconditioning: a novel target for neuroprotective therapy. Cerebrovasc Dis. 21 Suppl 2:38-47.
3. Chen J, Simon R. .Ischemic tolerance in the brain. Neurology. 1997;48:306-11
4. Higashida RT, Furlan AJ, Roberts H, et al. .(2003) Trial design and reporting standards for intra-arterial cerebral thrombolysis for acute ischemic stroke. Stroke. 34:e109-37.
5. Daffertshofer M, Mielke O, Pullwitt A, et al. (2004) Transient ischemic attacks are more than "ministrokes". Stroke. 35:2453-8
6. Dowden J, Corbett D. (1999) Ischemic preconditioning in 18- to 20-month-old gerbils: long-term survival with functional outcome measures. Stroke. 30:1240-6.

7. Ehrenreich H, Hasselblatt M, Dembowski C, et al. (2002) Erythropoietin therapy for acute stroke is both safe and beneficial. Mol Med 8:495-505.
8. Ettaiche M, Heurteaux C, Blondeau N, et al. (2001) ATP-sensitive potassium channels (K(ATP)) in retina: a key role for delayed ischemic tolerance. Brain Res. 890:118-29.
9. Ferriero DM. (2005) Protecting neurons. Epilepsia. 46 Suppl 7:45-51
10. Fisher M, Ratan R (2003) New perspectives on developing acute stroke therapy. Ann Neurol 53:10-20.
11. Fisher M, Takano K. (1995) The penumbra, therapeutic time window and acute ischaemic stroke. Baillieres Clin Neurol. 4:279-95
12. Gidday JM. (2006) Cerebral preconditioning and ischaemic tolerance. Nat Rev Neurosci. 7(6):437-48
13. Heiss WD, Thiel A, Grond M, et al. (1999) Which targets are relevant for therapy of acute ischemic stroke? Stroke 30:1486-9.
14. Heiss WD (2003) Best measure of ischemic penumbra Positron emission tomography. Stroke 34:2534-5.
15. Kirino T. (2002) Ischemic tolerance. J Cereb Blood Flow Metab. 22:1283-96
16. Lovett JK, Dennis MS, Sandercock PA, et al. (2003) Very early risk of stroke after a first transient ischemic attack. Stroke. 34: e138–e140.
17. Moncayo J, de Freitas GR, Bogousslavsky J, et al. (2000) Do transient ischemic attacks have a neuroprotective effect? Neurology. 54:2089-94.
18. Murry CE, Jennings RB, Reimer KA (1986) Preconditioning with ischemia: A delay of lethal cell injury in ischemic myocardium, Circulation, 74: 1124-1136.
19. Perez-Pinzon MA, Dave KR, et al. (2005) Role of reactive oxygen species and protein kinase C in ischemic tolerance in the brain. Antioxid Redox Signal. 7:1150-7.
20. Prass K, Scharff A, Ruscher K, et al. (2003) Hypoxia-induced stroke tolerance in the mouse is mediated by erythropoietin. Stroke. 34:1981-6
21. Prass K, Ruscher K, Karsch M, et al. (2002) Desferrioxamine induces delayed tolerance against cerebral ischemia in vivo and in vitro. J Cereb Blood Flow Metab. 22: 520–525.
22. Reshef A, Sperling O, Zoref-Shani E. (2000) Role of K (ATP) channels in the induction of ischemic tolerance by the 'adenosine mechanism' in neuronal cultures. Adv Exp Med Biol. 486:217-21
23. Riepe MW, Kasischke K, Raupach A. (1997) Acetylsalicylic acid increases tolerance against hypoxic and chemical hypoxia. Stroke. 28:2006-11
24. Rothwell PM. (2003) Incidence, risk factors and prognosis of stroke and TIA: the need for high-quality, large-scale epidemiological studies and meta-analyses. Cerebrovasc Dis. 16 (suppl 3): 2–10
25. Ruscher K, Freyer D, Karsch M, et al. (2002) Erythropoietin is a paracrine mediator of ischemic tolerance in the brain: evidence from an in vitro model. J Neurosci. 22:10291–10301.
26. Saver JL. (2004) Number needed to treat estimates incorporating effects over the entire range of clinical outcomes: novel derivation method and application to thrombolytic therapy for acute stroke. Arch Neurol. 61:1066-70.
27. Schaller BJ, Buchfelder M. (2006) Neuroprotection in primary brain tumors: sense or nonsense? Expert Rev Neurother. 6:723-30.
28. Schaller B (2005).Ischemic preconditioning as induction of ischemic tolerance after transient ischemic attacks in human brain: its clinical relevance. Neurosci Lett. 377:206-11
29. Schaller B, Graf R. (2004) Cerebral ischemia and reperfusion: the pathophysiologic concept as a basis for clinical therapy. J Cereb Blood Flow Metab. 24:351-71.
30. Schaller B, Graf R, Jacobs AH. (2003) Ischaemic tolerance: a window to endogenous neuroprotection? Lancet. 362:1007
31. Schaller B, Graf R. (2002) Cerebral ischemic preconditioning. An experimental phenomenon or a clinical important entity of stroke prevention? J Neurol. 249:1503-11
32. Schellinger PD, Hacke W (2005) Stroke: Advances in therapy. Lancet Neurol 4:2
33. Saver JL, Kidwell C. (2004) Neuroimaging in TIAs. Neurology. 62: S22–S25
34. Semenza GL (2000) HIF-1: mediator of physiological and pathophysiological responses to hypoxia. J Appl Physiol. 88: 1474–1480
35. Stenzel-Poore MP, Stevens SL, Simon RP. (2004) Genomics of preconditioning. Stroke 35(11 Suppl 1):2683-6
36. Trendelenburg G, Dirnagl U.(2005) Neuroprotective role of astrocytes in cerebral ischemia: focus on ischemic preconditioning. Glia. 50:307-20
37. Weih M, Kallenberg K, Bergk A, et al. (1999) Attenuated stroke severity after prodromal TIA: a role for ischemic tolerance in the brain? Stroke 30:1851-4

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PHLEBTHROMBOSIS: CORRELATION BETWEEN RISK FACTORS, SYMPTOMS AND COLOR DOPPLER ULTRASOUND

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Aim of the study:To evaluate risk factors,symptoms and ultrasonografic data in the patients with deep vein thrombosis {DVT}.

Material and Methods:

The study involved the period of time between January 2003 till September 2006.We studied 153 patients admitted in our emergency department or referred by other specialists for DVT.

Time by onset of symptoms varied from some hours to 30 days.The age of the patients ranged 18-75 years old.The diagnosis was verified by color doppler ultrasound and/or CT , or Phlebography.

Results:

Risk factors were revealed in 117 cases.These risk factors were sedentary life, dislipidemia, surgery, traume, neoplasia, pregnancy. The diagnosis was verified with ultrasound in 56.2 % of cases refered by other specialists and 97.6 % of cases firstly seen by vascular surgeons.

Conclusions:

1-The symptoms that generally are seen for DVT,not always verify this diagnosis if we perform more specific investigations.

2-The best prevention of DVT is achieved if we know and control risk factors.

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