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Ischemic tolerance in stroke treatment

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Abstract

Although outcome after stroke treatment has significantly improved over the last 30 years, there has been no revolutionary breakthrough. Among different combined approaches, systemic thrombolysis in combination with neuroprotection became a favorite research target. Recent studies suggest that transient ischemic attacks may represent a clinical model of such ischemic tolerance; thus, a new focus on this research has emerged. In this review, we show the parallels between ischemia and neuroprotection and discuss the potential therapeutic options that may be opened by this new molecular knowledge.

Keywords

ischemic tolerance; neuroprotection; stroke; transient ischemic attack; treatment

An acute occlusion of cerebral arteries, if not reversed within a short period of time, usually results in cerebral ischemia, with subsequent cell death occurring within the perfusion territory of the affected vessels [1]. The infarction volume is an important determinant of long-term neurologic outcome and, thus, there has been considerable interest in developing new therapies to reduce tissue damage [2]. At present, the treatment of acute cerebral ischemia is based on two primary therapeutic strategies [3]:

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- Limitation of cerebral ischemia by early reperfusion after cerebral ischemia (revascularization)
- Inhibition of the pathobiochemical cascade leading to secondary ischemic damage (neuroprotection)

In order to reduce neurological morbidity, there must be a thorough understanding of the underlying mechanisms involved in ischemic damage because this will allow us to design new, clinically effective therapeutical options [4]. A necessary prerequisite of every therapeutic strategy is the existence of functionally impaired but viable, and potentially salvageable, brain tissue (the so-called 'zone of penumbra') [4]. The potential time windows for the different primary treatment options may differ from each other; rather short for revascularization approaches and somewhat longer for neuroprotection. However, under some circumstances, reperfusion may itself also have deleterious effects by enhancing neuronal cell death and leading to paradoxical tissue dysfunction or apoptosis (reperfusion injury). Although early reperfusion is accepted as a standard therapeutic option, it remains a controversial topic when linked to different experimental paradigms (e.g., functional outcome, lesion size, markers of inflammation and apoptosis). Improved understanding of the early course of focal ischemia has helped to close the gap between the molecular and biochemical concept of ischemic damage and the potential usefulness of early reperfusion [1,2,5].

In this article, we provide an overview of ischemic tolerance and its potential application in stroke therapy.

Definition

Global or focal sublethal cerebral ischemia for a few minutes confers transient tolerance to a subsequent, more severe and sustained ischemic event. This phenomenon, known as ischemic tolerance, has been demonstrated in animal models of cerebral ischemia and other diseases of the CNS [6–9], and may be observed in humans with different conditions of reduced cerebral blood flow [10–12]. Ischemic tolerance seems to be a fundamental nonspecific cellular response because its signals, transducers and effectors have also been seen in hypoxia-tolerant or hibernating animals [13].

Underlying pathophysiological mechanism

Since ischemic tolerance may confer endogenous neuroprotection, the underlying molecular mechanisms have been studied extensively in animal models and in cell culture [6,7,10,12, 14–16]. From this knowledge, cerebral ischemic tolerance may be subdivided into at least two time profiles: a 'classical' rapid form, in which the trigger induces neuroprotection within minutes and a 'delayed' form, in which the protecting cellular state develops over hours or days and usually involves *de novo* protein synthesis [6]. The underlying pathophysiology of ischemic tolerance is still poorly understood. A stimulus leading to ischemic tolerance is probably followed by functional impairment but not by brain tissue damage, and the induced molecular mechanisms may be categorized into two subgroups [6]. First, a cellular defense against ischemia induced in neurons by post-translational modification of proteins and/or by the expression of new proteins via signaling to the nucleus. This signaling cascade may strengthen the cellular protection or may inhibit apoptosis. Second, a cellular stress response involving synthesis of stress proteins may serve as cellular 'chaperones' by unfolding and helping to eliminate denatured proteins.

Since ischemic tissue damage is the result of a complex pathophysiological cascade comprising of many molecular events, not only substrate restriction but also noxious events may induce ischemic tolerance. Thus, different triggers, including global or focal transient cerebral ischemia, hyperbaric oxygenation, inflammation, seizure activity, cortical spreading,

depression, metabolic inhibition, oxygen free radicals, hypothermia or hyperthermia, and cerebellar stimulation, may induce ischemic tolerance [7]. Most of these endogenous or exogenous stressors induce both 'rapid' and 'delayed' ischemic tolerance. However, since many diseases of the CNS share common cell death pathways, one stressor may induce tolerance against another (cross tolerance) [7]. Furthermore, triggers inducing ischemic tolerance in the brain do not necessarily have topographical specificity; ubiquitous, stereotypical molecular responses of cells to damage may exist, which means that the same stressor that elicits ischemic tolerance in the brain may elicit tolerance in other organs. Induction and mechanisms of ischemic tolerance in different organs have similar features [7]. In fact, induction of tolerance in one organ may spread via the nervous system or paracrine mechanism to other organs (remote preconditioning) [17]. The further identification and characterization of preconditioning stimuli that may be effective, but relatively harmless, is of utmost importance to apply mechanisms of endogenous ischemic tolerance to therapeutic tools.

Models of ischemic tolerance

To date, models of ischemic tolerance that use clinically approved drugs (e.g., erythropoietin [14], isoflurane [15] or ATP-sensitive potassium ion channel openers [16]) as inducing effectors have been developed. If a multifaceted and coordinated program involving the expression of multiple genes in neurons, glia and endothelial cells guides ischemic tolerance, it seems unlikely that such a complex endogenous response may be mimicked by a single neuroprotective drug [18].

Despite this shortcoming, an approach to identify transcription factors specific for ischemia and ischemic tolerance is to find common DNA-regulatory element(s) in the promoter regions of the genes that are upregulated in distinct cell types during ischemia [19]. These gene loci and transcription factors may become a class of future molecular targets. Drugs capable of activating transcription factors induced by ischemia could augment an endogenous protective response. Identification of a class of transcriptional activators in humans would allow for enhancing endogenous neuroprotection. Stroke and other disorders of the CNS may benefit from this approach. Using this approach will have more translational value and a greater probability of transference from 'bench to bedside'. For example, since *Hsp70* is overexpressed in cells that are more resistant to various stresses, *Hsp70* gene transfer has been successfully used to abrogate ischemic damage [20]. Stress tolerance in neurons is not solely dependent on their own heat-shock proteins (HSPs) but can be supplemented by HSPs from adjacent glial cells. Hence, application of exogenous HSPs at neural injury sites may be an effective strategy to [21]:

- Maintain neuronal viability
- Improve post-ischemic functional outcome
- Increase survival of endogenous neural precursor cells after stroke

The most effective breakthrough in the treatment of cerebral ischemia has been the successful establishment of systemic thrombolysis [22,23]. With better knowledge regarding the molecular mechanisms leading to ischemia-related cell damage and the presumed molecular targets of treatments counteracting these mechanisms, combined strategies based on thrombolysis and endogenous neuroprotection may be developed. Understanding ischemic tolerance is a good approach to unravel the molecular mechanisms involved in neuroprotection, and might improve therapeutic strategies for patients with stroke or other ischemia-related diseases.

Neuroprotection

The clinically demonstrated efficacy of recombinant tissue plasminogen activator for thrombolytic stroke is an important breakthrough in the treatment of acute neurological disorders [22]. The search for effective additional neuroprotectants remains frustrating, particularly with regard to specific pharmaceuticals [2]. However, the mechanisms of cerebral ischemia include glutamate excitoxicity, calcium toxicity, free radicals, nitric oxide, and inflammatory reactions, as well as dysfunctions of endoplasmic reticulum and mitochondria. These injury cascades are interconnected in complex ways; thus, it is difficult to compare their pathogenic importance in ischemia models. For this reason, research in cellular and molecular pathways has spurred studies in potential neuroprotectants, mainly in pharmacological fields, such as anti-excitotoxic treatment, calcium channel antagonism, approaches for inhibition of oxidation, inflammation, and apoptosis [24]. Besides, other protective interventions, including thrombolysis, arteriogenesis, regeneration therapy and ischemia preconditioning or postconditioning, are also under investigation. However, laboratory studies have consistently shown remarkable neuroprotection with such two nonpharmacological strategies: therapeutic hypothermia and ischemic preconditioning. Recent studies have shown that the mechanism of protection underlying both of these treatments is correlated to the downregulation of cellular and tissue metabolism. Thus, understanding the mechanisms underlying such robust protective effects could lead to appropriate translation at the clinical level. Hypothermia is already being used at many centers to improve neurological outcome from cardiac arrest.

Despite the present difficulties, we are quite optimistic regarding future clinical applications of neuroprotective agents, by optimizing experimental approaches and clinical trials.

Transient ischemic attack as a clinical correlate of ischemic tolerance?

A brief episode of ischemia protects against a subsequent and otherwise lethal ischemia. This phenomenon of ischemic tolerance, first described in the myocardium [25,26], has attracted considerable attention as an adaptive mechanism against cardiac ischemia. Although several studies have shown that angina pectoris preceding myocardial infarction represents a clinical correlate of experimental preconditioning protocols [27,28], little is known concerning the situation in the human brain. However, in parallel to an episode of angina pectoris, a transient ischemic attack (TIA) is clinically defined as a functional neurological lesion due to ischemia without structural damage. It has been estimated that, depending on stroke etiology, 7–40% of patients with stroke have had a former episode of TIA [29,30]. The hypothesis, in analogy to the heart, is that if TIA is a clinical correlate of ischemic tolerance [12,31], it might lead to reduced severity of a subsequent stroke.

Using the Lausanne Stroke Registry, Moncayo *et al.* divided patients admitted for first-ever cerebral infarction into two groups on the basis of the presence or absence of prior ipsilateral TIAs [10]. They concluded that patients who had experienced previous TIAs had a more favorable outcome than those who had not. More specifically, TIAs lasting 10–20 min were associated with a favorable outcome after adjusting for other confounding variables, suggesting that short-duration TIAs could lead to ischemic tolerance. The timing between the TIA and stroke was also found to influence stroke outcome. Moncayo *et al.* showed that, if the TIA occurred less than 1 week before the cerebral infarct, there was a higher proportion of favorable outcomes than those with a more remote TIA of greater than 1 week [10]. These findings correspond to the second window of ischemic preconditioning, where protection was demonstrated up to 1 week after a sublethal insult.

In a different study, Castillo *et al.* studied ischemic stroke patients who had atherothrombotic and cardioembolic infarcts [32]. They divided the patients into those who had prior, relevant TIA or not. They determined that the frequency of poor outcome at 3 months in both

atherothrombotic and cardioembolic infarct groups were significantly better among patients who had experienced prior, relevant TIAs and that this correlated with infarct volume, measured on computed tomography on days 4–7.

The size of lesion volume for diffusion-weighted imaging (DWI), mean transit time, cerebral blood flow, cerebral blood volume for perfusion-weighted imaging (PWI) and infarct volume from T2-weighted images at 3–7 days between patients with prodromal TIA and without TIA were compared in a different study by Wegener *et al.* This study showed that, although a similar size and severity of the perfusion deficits occurred in both groups, the initial diffusion lesions tended to be smaller and final infarct volume were significantly smaller among patients with TIA than in those without TIA [33]. This difference was more pronounced when TIA occurred in less than 4 weeks, suggesting that the differences in infarct sizes present in the two subgroups may be due to factors other than time of TIA. Arboix *et al.* observed that the percentage of stroke patients with favorable outcome was significantly greater among those with a history of TIA than those without TIA among the non-lacunar-stroke subgroup [34]. However, in the lacunar-stroke group, the percentage with favorable outcome was similar between the TIA and without TIA groups. Recently, in a retrospective case–control study, Schaller determined that patients with pre-stroke TIA had a more favorable outcome and modified Rankin score compared with patients without TIA [12]. The patients in this study had been treated with local intra-arterial thrombolysis after ischemic stroke due to occlusion of a main cerebral artery. In addition, these patients had no significant differences in baseline characteristics, such as NIH Stroke Scales on admission and recanalization. When a tolerant brain is subjected to ischemia again, the resulting insult (i.e., residual blood flow and disruption of cellular transmembrane gradients) appears to be the same as in the naive brain, but the ensuing lesion is substantially reduced [35]. This suggests that the adaptive changes in the tolerant brain may be primarily directed against delayed processes that contribute to ischemic damage, but adaptive changes that are beneficial during the subsequent test insult cannot be ruled out [35]. It has become clear that multiple effectors contribute to ischemic tolerance, including [36]:

- Activation of fundamental cellular defence mechanisms, such as antioxidant systems, HPSs and cell death/survival determinants
- Responses at tissue level, especially reduced inflammatory responsiveness
- A shift of the neuronal excitatory/inhibitory balance towards inhibition

Accordingly, an improved knowledge of preconditioning and ischemic tolerance may help us to identify neuroprotective strategies.

Not all studies have shown associations between TIAs and more favorable outcomes after stroke. In a cohort study in northern California (USA), no correlation was observed between the occurrence of TIA and reduced disability due to subsequent stroke [37]. The reasons for these discrepancies remain undefined, but potential causes, such as latency between TIA and stroke and duration of TIA, did not explain the results. However, we must point out that a number of factors in clinical settings, such as the duration and timing of TIAs relative to the occurrence of stroke, cannot be controlled as is the case in animal studies. Since stroke and TIA mostly affect the aging population, it is important to determine whether experimental TIA remains effective in electing protection in aged patients with underlying vascular disease and whether the protection is long lasting [12]. In addition, it remains unclear whether there exists an influence of age on ischemic tolerance [38]. Experimental studies demonstrated loss of early-phase preconditioning, which is not surprising, given age-related changes in the vasculature [12,38,39].

Age may alter the potential ischemic preconditioning relationship between TIA and stroke outcome. Several mammalian experimental studies showed an age-related reduction of the

endogenous mechanism of protection in the heart [38,40–42]. Middle-aged adults with pre-infarction angina, the clinical equivalent of ischemic preconditioning, have better outcomes than those without angina following acute myocardial infarction [40]. Recently, Della Morte *et al*. studied the potential effect of TIAs in improving outcome from ischemic stroke among patients over the age of 65 years [42]. In a retrospective study, they evaluated 203 patients (age ≥ 65 years), who had been diagnosed with acute ischemic stroke, and categorized them according to the presence (n = 42 [21%]) or absence (n = 161 [79%]) of TIAs within 72 h of stroke onset. Patients were monitored until their discharge from the hospital (length of hospital stay: 14.5 ± 4.8 days). No significant differences in the NIH Stoke Scales and modified Rankin scale scores were observed between those patients with TIAs and those without TIAs present before stroke onset at admission or discharge. These results suggested that TIAs among those over the age of 65 years may not be associated with much cerebral ischemic preconditioning.

However, from these studies, we can conclude that, overall, stroke outcomes may be better among patients with recent TIAs. The protective effect of TIA is observed mainly among patients with nonlacunar cerebral infarction and when TIAs occur within 1 week, rather than a number of months, before cerebral infarction and last for more than 10 min [10]. Whether these clinical results mean that TIAs lead to ischemic preconditioning and increased ischemic tolerance is not entirely known, but remains an intriguing hypothesis in view of the similarities to observations in animal work on this phenomenon. Until now, discussions of whether prior TIAs had no effect of subsequent lacunar infarctions has been purely speculative, as no literature concerning it existed. The different pathophysiological mechanism between lacunar and nonlacunar stroke may explain this difference.

Genomics of ischemic preconditioning

Stroke, ischemic preconditioning and ischemic preconditioning plus stroke all induce gene changes that overlap, depending on the condition [12,43]. Stroke induces a robust upregulation of gene expression, whereas preconditioning followed by stroke results in a marked downregulation [43]. Genes upregulated by stroke suggest activation of stress and/or inflammatory pathways and increased metabolism and ion channel function. Preconditioning tended to decrease genes involved in those pathways. Follow-up experiments showed that preconditioning decreased the activity of voltage-dependent potassium channels *in vitro* and increased bleeding time. Preconditioning reprograms the response to ischemic injury via transcriptional changes that may inhibit metabolic pathways and immune responses, reduce ion channel activity and decrease blood coagulation. These changes resemble evolutionarily conserved responses to decreased blood flow and oxygen availability that occur during hibernation [8].

One way to identify transcription factors specific for ischemia and ischemic tolerance is to find common DNA-regulatory element(s) in the promoter regions of the genes that are upregulated in distinct cell types by ischemic tolerance [19]. Identification of these sequences and transcription factors that bind to them could become a class of future molecular targets and novel drugs. Small molecules capable of activating transcription factors induced by ischemia could augment an endogenous protective response. One such molecule is resveratrol, a polyphenol found in some plants (e.g., grapes), which is known to be neuroprotective and to emulate ischemic tolerance [44]. Resveratrol activates, among others [44]:

- Silent mating type information regulation 2 homolog (SIRT) 1
- A NAD^+-dependent class III histone deacetylase, which deacetylates histones, modulating the transcription factor NF-κB
- p53 (tumor suppressor protein)

- p73 (p53-related tumor suppressor)
- Ku70 (DNA-repair protein Ku)
- Forkhead box class O (FOXO) transcription factors

Possible future therapeutic management & reperfusion

The previously described concept – based on physiological and pathophysiological data – argues against the possibility of a single-drug therapy for ischemia-reperfusion injury that has characterized the research approaches of basic science laboratories, the pharmaceutical industry and clinical trials so far. However, extensive research has provided promising results that may be integrated into a comprehensive therapy concept of ischemia reperfusion injury. This concept may be complemented with new drug classes that act at the level of gene regulation, as described earlier.

Antioxidants and nitric oxide synthase inhibitors may reduce the rate of membrane damage, but there is no reason to believe that they induce survival, signal transduction, inhibit the activity of calpain or caspase, or induce rapid recovery of translational competence. Similarly, caspase inhibitors may block the progression of apoptotic mechanisms, but, again, there is no reason to expect that they will inhibit either lipid peroxidation or calpain activity or induce rapid recovery of translational competence. Peptide growth factors may induce the early recovery of translational competence, induce survival-signal transduction and may affect the transcription of physiologic inhibitors of calpain and radical damage; however, since signal transduction, transcription and translation take time, it is probably not practical to let calpain remain active and lipid peroxidation progress unimpeded while awaiting full expression of the molecular response to growth factor signaling. Indeed, radical stress can itself induce a 90% reduction in insulin-induced phosphorylation of ser-473 on Akt [45].

These considerations illustrate the strength of a concept based on an integrative, multitherapeutic approach. Interesting targets at molecular level may include:

- Peptide growth factors for the early induction of survival-signal transduction and recovery of translational competence
- Inhibition of calpain
- Inhibition of radical damage
- Caspase inhibition

In addition, first clinical steps with pretreatment with electroacupuncture demonstrated increases in the production of endocannabinoid 2-arachidonylglycerol and N-arachidonoylethanolamine anandamide, which elicit protective effects against transient cerebral ischemia through cannabinoid CB_1 receptors, suggesting a novel mechanism for rapid tolerance to focal cerebral ischemia [46].

Rigorous studies to establish such an approach will require the evaluation of neurologic, histopathologic and/or neuroradiological outcomes, surrogate markers, and molecular end points associated with each intervention component to establish the necessary role of each drug involved. Clinical models (e.g., the TIA model) for neuroprotection will be needed to examine such therapeutic options.

Expert commentary

Ischemia-induced decreases in the mitochondrial capacity for respiratory activity probably contribute to the ongoing impairment of energy metabolism during reperfusion and possibly

also exacerbate the pathology ensuing after ischemia. From our review of the literature, the concept of single-drug intervention in stroke treatment provides dubious potential. The best approach to translate research from ischemic preconditioning to the clinic would be to develop a pharmacological approach to induce the state of ischemic tolerance in patients who have experienced a TIA, so that these patients have better outcomes after a potential subsequent stroke. Therefore, we propose that identification of a class of transcriptional activators specific for ischemia and ischemic tolerance in humans could provide a window to endogenous neuroprotection and, possibly, also towards further therapeutic developments in the treatment of acute stroke and other disorders of the CNS.

Five-year view

Increasing our knowledge regarding the cellular and molecular mechanisms leading to ischemia-related cell damage, combined with strategies based on thrombolysis, might lead to supplementary therapies in the next few years that may arise from the field of endogenous neuroprotection. For that reason, better animal models need to be developed to emulate TIAs, with the goal of improving our understanding of ischemic preconditioning, ischemic tolerance and neuroprotection following TIAs. This approach appears promising and may lead to the development of new additional treatments and/or drugs. Finally, it is entirely plausible that different strategies will need to be implemented in the older population, where both TIAs and ischemic preconditioning may have less potential to induce neuroprotection.

Key issues

- The time to treatment of acute stroke remains critical regarding the onset of symptoms and revascularization.
- From recent clinical studies, there seems to be a tendency to widen the window of treatment.
- This wider treatment window of acute stroke underlines the discussion for combined treatment of thrombolysis and neuroprotection to minimize the revascularization damage.
- With transient ischemic attacks, we now have a clinical model for neuroprotection.

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