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REVIEW ARTICLE

Pharmacologic Preconditioning: Translating the Promise

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Abstract A transient, ischemia-resistant phenotype known as "ischemic tolerance" can be established in brain in a rapid or delayed fashion by a preceding noninjurious "preconditioning" stimulus. Initial preclinical studies of this phenomenon relied primarily on brief periods of ischemia or hypoxia as preconditioning stimuli, but it was later realized that many other stressors, including pharmacologic ones, are also effective. This review highlights the surprisingly wide variety of drugs now known to promote ischemic tolerance, documented and to some extent mechanistically characterized in preclinical animal models of stroke. Although considerably more experimentation is needed to thoroughly validate the ability of any currently identified preconditioning agent to protect ischemic brain, the fact that some of these drugs are already clinically approved for other indications implies that the growing enthusiasm for translational success in the field of pharmacologic preconditioning may be well justified.

Keywords Ischemic tolerance · Stroke · Brain · Neuroprotection · Neurovascular unit · Review

One goal common to all preclinical stroke research is to identify molecular mediators of neurovascular injury or protection and to devise therapies to either block or enhance these mechanisms to improve outcome. Investigations of preconditioning and ischemic tolerance (IT) [1, 2] are no different: The receptors, signal transduction pathways, transcriptional regulatory elements, micro and messenger RNA and protein profiles, and subcellular organelle function that are modified by the preconditioning stimulus are all suitable targets for therapeutics. At first pass, the patient population that suffers from cerebral ischemic injury due to unpredictable focal stroke, cardiac arrest, or subarachnoid hemorrhage represents, by definition, one that is unlikely to derive benefit from preconditioning research. However, the novel endogenous survival pathways identified in preclinical IT studies may ultimately become targets for drugs that protect brain even when acutely administered after the precipitating event. Importantly, a significant number of other patients—those in which we can anticipate a period of cerebral ischemia following transient ischemic attack, aneurysm clipping, subarachnoid hemorrhage, carotid endarterectomy or stenting, asymptomatic carotid stenosis, coronary bypass, and cardiac valve replacement—represent defined at-risk populations ideally suited for translational therapeutic preconditioning. The candidate drugs that might underpin clinical trials for this latter group of patients actually comprise a relatively long—and therefore promising—list, particularly if the current foundation of preclinical studies is expanded with intention. This review will highlight many of these.

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Overview

In the initial years of cerebral IT research, the majority of studies utilized brief ischemia in vivo, or oxygen–glucose deprivation (OGD) in vitro, as the preconditioning stimulus. However, as time passed, the effectiveness of other IT-promoting preconditioning stimuli was slowly realized. The ability of a nonischemic, nonhypoxic stimulus to protect against subsequent cerebral ischemia was initially referred

to as "cross-tolerance," but with the increasing number of preclinical pharmacologic preconditioning studies appearing in the literature, this nomenclature is no longer in frequent use. More important is the implication that the ability of disparate stimuli to trigger both the metabolic changes and the up- or downregulation of expression of the hundreds of genes responsible for establishing IT suggests that many of these stimuli share a common, but limited, set of overlapping molecular signaling pathways that may be amenable to activation by pharmacologic preconditioning mimetics. Some of these preconditioning-inducing agents are particularly attractive from a translational standpoint, given their demonstrated low toxicity and minimal side effects in humans. Even though several nonischemic or nonhypoxic stimuli that are not pharmacologic (hyperthermia, spreading depression, hyperbaric oxygen, exercise, etc.) have shown efficacy as preconditioning triggers in some stroke models, including various "immunological preconditioning" strategies [2], for the purposes of this review, only pharmacology-based preconditioning regimens will garner the spotlight. Not included are prophylactic approaches to neuroprotection that, in essence, represent acute or chronic pretreatments in which the drug is present when ischemia strikes. Rather, the focus here will be on classical or delayed pharmacologic preconditioning wherein the singular or final drug treatment of a series precedes the ischemic event by many hours or days, and the obligatory genomic reprogramming that largely defines the ischemia-tolerant phenotype is promoted.

After almost two decades of preclinical research, the number of pharmacologic stimuli that induce a state of IT is noteworthy (Table 1). However, the relative depth and breadth of research on any specific pharmacologic paradigm for establishing preconditioning-induced IT remains extremely uneven. For example, some agents that are clinically approved for other indications and that are safe and well tolerated in human patients have received scant attention as preconditioning stimuli, even though a precedent exists for demonstrated protection from ischemic brain injury in at least one laboratory preconditioning study. Conversely, some compounds continue to receive considerable experimental attention in animal studies (albeit sometimes disproportionately from only a handful of laboratories), and we have uncovered a number of their respective induction and expression mechanisms, despite the fact that, even though efficacious in rodents, these agents are unlikely to be approved for clinical use. The volatile anesthetics and the K_{ATP} channel openers (KCOs) are the two classes of drugs that break this pattern, given that a relatively large number of laboratory studies have characterized the effectiveness of these already clinically approved drugs; these and other examples from this latter category will be discussed further below. While not dismissing the value of in vitro models (including organotypic slices and cell culture), the majority of studies cited in this review will be those conducted in animals subjected to transient or permanent focal ischemia, or global ischemia, since the latter models are necessary stepping stones on the road to demonstrating the neuro-, glial-, and vasculoprotective efficacy of a particular preconditioning treatment, which, in turn, lay the groundwork for clinical trials [3].

Tested at the Bench and Clinically Approved

The volatile anesthetics and the KCOs probably rank as the most well-studied and best understood pharmacologic preconditioning agents already in widespread clinical use (Table 1). One family of drugs that have received a significant amount of preclinical attention in adult [4–6] and neonatal [7–9] rodent IT models are the volatile anesthetics; together with their proven safety profiles, these agents are ripe for translational application. To date, isoflurane is the most thoroughly investigated preconditioning anesthetic, but more recently, xenon [10, 11] and sevoflurane [11–14] have garnered attention as well. Further mechanism-based animal studies of both rapid and delayed ischemic preconditioning with sevoflurane, the current inhalational anesthetic of choice for human surgery, are warranted. Mechanistically, studies implicate inducible nitric oxide synthase (iNOS), the MAP kinases, Akt, and K_{ATP} channels, as critical to establishing the IT phenotype following anesthetic preconditioning, and also reveal gender and sex hormone dependencies [15, 16] (Table 1).

Different subtypes of K_{ATP} channels exist in different subcellular locations and in different tissues; at least nine structure-dependent families of KCOs have now been identified, some of which, like diazoxide, are selective for the mitochondrial K_{ATP} channel. Studies of the ability of chromakalim and selective mitochondrial KCOs like the blood–brain barrier-permeable diazoxide to precondition in a classical fashion against global [17–19] and focal [19, 20] ischemia in rodents are many, as are investigations conducted in in vitro models. Moreover, rapid diazoxide preconditioning provided both morphological and functional protection in a canine model of brain injury by hypothermic cardiac arrest [21]. In vitro studies, and some animal investigations, suggest that KCO-induced IT is associated with postischemic reductions in proinflammatory and apoptotic mediators, reactive oxygen species, and blood–brain barrier breakdown, along with increases in Akt, endothelial nitric oxide synthase (eNOS), heat shock proteins, and antioxidant enzymes (Table 1), but the many molecular pathways leading from channel opening to the manifestation of cytoprotective neuronal and vascular phenotypes remain to be clarified. Notably, accumulating

Table 1 Mechanistic basis of pharmacologic preconditioning

Preconditioning stimulus	Anti-inflammatory	Inhibition of apoptosis	Reduction in ROS	Reduction in excitotoxicity	Mitochondrial or plasmalemmal K_{ATP} channel activation	NOS activation	Transducers	Transcription factors	Growth factors	Mitochondrial metabolism/ redox	BBB stabilization	CBF/ angiogenesis	Neurogenesis	Behavioral/ functional outcome
Bench-proven success and clinically approved														
Volatile anesthetics														
Isoflurane		X		X	X	iNOS	Akt, MAPK			X		CBF		X
Xenon		X		X	X			CREB	BDNF	X				X
Sevoflurane	X	X			X		MAPK				X	CBF		
K_{ATP} channel openers														
Diazoxide	X	X	X		X	eNOS	PKC			X	X			
BMS-191095			X		X		Akt, PKC			X				
Bepridil		X	X		X		PKC							
Iptakalim					X					X	X			
Bench poor but clinically approved														
HIF stabilizers														
Deferoxamine								HIF	EPO, ADM					X
Dipyridyl								HIF			X			
Tilorone								HIF						
FG2216 and FG4592								HIF						
EPO	X	X	X				JAK/STAT, Akt, MAPK	HIF, NFkB						
Antibiotics	X	X		X						X				
Opioids					X				CGRP					X
Statins	X		X			eNOS	Akt	CREB						
Phytochemicals and dietary factors														
Resveratrol	X	X	X					PGC-1α, CREB, Nrf-2						
Linolenate		X						NFkB						
Ethanol			X							X				
Ginkoglides							Akt, MAPK	HIF	EPO					
PPAR agonists	X		X								X	CBF		
Estrogen/ estradiol-17β								CREB						

Table 1 (continued)

Preconditioning stimulus	Anti-inflammatory	Inhibition of apoptosis	Reduction in ROS	Reduction in excitotoxicity	Mitochondrial or plasmalemmal K_{ATP} channel activation	NOS activation	Transducers	Transcription factors	Growth factors	Mitochondrial metabolism/ redox	BBB stabilization	CBF/ angiogenesis	Neurogenesis	Behavioral/ functional outcome
Fingolimod (FTY720)											X			
Aspirin														
Bench-proven success but not clinically approved														
LPS	X		X			eNOS	TNFα, MAPK	IRF3	Ceramide		X	CBF		
CpG	X						TNFα, IFNβ							
Diphosphoryl lipid A	X		X											
3-NPA		X		X	X		Adenosine, MAPK			X				
Thrombin							MAPK, JNK, TNFα	HIF			X			
Adenosine					X		PKC			X		CBF		
TNFα	X							NFkB	Ceramide					
Bradykinin									bFGF		X			

Shown is evidence to date, obtained primarily from in vivo preclinical studies of both focal and global ischemia and in both adult and neonate models of preconditioning-induced delayed ischemic tolerance, regarding the mechanisms whereby various pharmacologic preconditioning stimuli exert their beneficial effects. Drugs are categorized in parallel with their presentation in the text. An "X" indicates that data are available supporting that particular mechanism; in some instances, the specific molecule(s) or "effect" are listed. The lack of an entry in any given row/column space does not necessarily mean that the indicated mediator is not involved in promoting the ischemia-tolerant state in response to that particular stimulus, but only that no studies yet exist to support such involvement

ADM adrenomedullin, *Akt* protein kinase B, *BBB* blood–brain barrier, *BDNF* brain-derived neurotrophic factor, *bFGF* basic fibroblast growth factor, *CBF* cerebral blood flow, *CGRP* calcitonin gene-related peptide, *CREB* cyclic AMP response element binding protein, *EPO* erythropoietin, *HIF* hypoxia-inducible factor, *IFNβ* interferonβ, *IRF3* interferon regulatory factor-3, *JAK/STAT* janus kinase/signal transducers and activators of transcription, *LPS* lipopolysaccharide, *MAPK* mitogen-activated protein kinases (p38, p42/44), *NOS* nitric oxide synthase, *3-NPA* 3-nitropropionic acid, *Nrf-2* nuclear factor E2-related factor, *NFkB* nuclear factor kappa B, *PPAR* peroxisome proliferator-activated receptor, *PGC-1α* peroxisome proliferator-activated receptor gamma coactivator-1α, *ROS* reactive oxygen species, *TNFα* tumor necrosis factor-α

evidence indicates that activation of mitochondrial KCOs and modulation of mitochondrial function are key means by which many other pharmacologic (and nonpharmacologic) preconditioning stimuli ultimately manifest their protective effects [22].

Approved for Clinic Applications, but Relatively Unexplored at the Bench

A surprisingly long list of FDA-approved drugs (e.g., deferoxamine, erythropoietin (EPO), antibiotics, opioids, statins, phytochemicals, peroxisome proliferator-activated receptor (PPAR) agonists, estrogen, and certain immunosuppressants) have shown preconditioning efficacy in a small number of in vitro and/or in vivo models of ischemic brain injury (Table 1). Expanding efforts to assess these pharmacologic stimuli for the robustness of their protective effects in other models of IT should be prioritized given their high translational potential. Some of the primary features that render these compounds particularly attractive in this regard are detailed below.

Drugs that stabilize the transcription factor hypoxia-inducible factor (HIF) isoforms HIF-1α and/or HIF-2α look promising as preconditioning stimuli for anticipated cerebral ischemia. Deferoxamine, an iron chelator used for over 30 years in the treatment of assorted chronic anemias and iron poisoning—and now in clinical trials for intracerebral hemorrhage [23]—is an effective preconditioning compound in models of neonatal [24, 25] and adult [26, 27] stroke. As a result of its ability to inhibit members of the HIF-stabilizing, iron-dependent prolyl hydroxylase family secondary to binding iron, deferoxamine is just one of several preconditioning treatments (e.g., LPS, inflammatory cytokines, thrombin, nitric oxide) that may induce IT in this mechanistic fashion. The transcription of hundreds of survival- and angiogenesis-promoting genes (e.g., vascular endothelial growth factor (VEGF), EPO), as well as the modulation in cellular energy metabolism, that HIF induces [28, 29] is thought to contribute significantly to the ischemia-tolerant state. A number of small molecule inhibitors of prolyl hydroxylase enzymes are under active investigation to leverage this phenotype, including the blood–brain barrier-permeable tilorone [27] and Fibrogen's FG-2216 and FG-4592—now in phase II clinical trials for kidney disease patients with anemia—and may eventually prove useful as preconditioning therapeutics for HIF stabilization. However, studies of the effects of neuron-specific HIF-1α deletion on stroke outcome (in the absence of preconditioning) are controversial [30, 31], suggesting that helpful preischemic, but harmful postischemic, effects of at least neuronal HIF are involved. Therefore, understanding, and controlling, the pharmacokinetics of prolyl hydroxylase inhibition and other HIF regulatory factors will be critical to the success of such approaches.

Evidence from preclinical studies indicates that EPO, like nitric oxide, can serve as both inducer [32, 33] and effector [26, 34–36] of the ischemia-tolerant phenotype. The ability of exogenous EPO to trigger IT might mimic, in part, a paracrine-based signaling system for hypoxic/ischemic preconditioning wherein HIF-driven gene expression changes occurring in astrocytes lead to the synthesis and release of EPO and other downstream target proteins (e.g., adrenomedullin, VEGF), which then mediate the IT response in neurons [33, 37]. The mechanistic basis of EPO's beneficial effects with respect to postischemic treatment protocols in animals is multifold [38–40] (Table 1). Clinically, recombinant human EPO (rhEPO) is FDA-approved for hematopoiesis and has been used by millions of people. However, while a phase II stroke trial for rhEPO showed significant improvements across several outcome measures [41], the outcome of the phase III stroke trial was less encouraging (potentially confounded by cotreatment with recombinant tissue plasminogen activator) [42].

Interestingly, some antibiotics also exhibit preconditioning effects. In particular, administration of the macrolide erythromycin to rats protected pyramidal cell function when hippocampal slices from these animals were rendered severely hypoxic [43] and improved survival of both hippocampal and neocortical neurons following transient global ischemia [44]. Follow-up microarray studies suggest that this protection may be afforded secondary to poststroke transcriptional suppression of proinflammatory genes [45]. The morphologic and functional protection against both transient and permanent focal stroke injury afforded by preconditioning with the third generation cephalosporin antibiotic ceftriaxone was also associated with a reduction in postischemic inflammatory mediators (e.g., TNFα and MMP9), as well as increases in the expression of the astrocyte glutamate transporter protein [46]. Assuming that these improvements in outcome are not the result of reductions in poststroke infection [47, 48], the fact that specific members of the commonly prescribed antibiotic drug family are efficacious as preconditioning stimuli represent provocative findings with high translational potential.

In a similar fashion, opioid preconditioning may be another attractive way to induce rapid or delayed IT in the clinic, particularly if single doses prove effective, and the addiction/withdrawal concerns associated with chronic opioid use can be avoided. However, experimental support for this possibility is limited at present to a handful of studies. In particular, morphine effectively preconditioned Purkinje cells in cerebellar slices against simulated ischemic injury [49], and a delta-opioid receptor antagonist

blocked the ability of both hypoxia to precondition cultured rat cortical neurons against glutamate toxicity [50] and electroacupuncture to precondition rats against transient focal stroke [51]. Elsewhere in the CNS, systemic morphine administration was demonstrated to promote retinal ischemic tolerance, while the nonselective opioid antagonist naloxone blocked ischemic preconditioning-induced retinal IT [52].

Statins represent another class of widely used drugs that might find utility for preconditioning at risk patients. One finding in the ongoing Stroke Prevention by Aggressive Reduction in Cholesterol Levels (SPARCL) trial was that high-dose atorvastatin reduced the incidence of subsequent stroke in patients without known coronary artery disease, but who had already suffered a recent stroke or transient ischemic attack [53]; whether this is representative of a statin-based preconditioning effect is open to debate, as is the notion that regular statin therapy might chronically precondition the brain and improve stroke outcome in patients taking this drug. The few relevant laboratory studies published to date hint rather strongly that statins might actually be useful for acute preconditioning, given that administration of a single dose of simvastatin is neuroprotective in the postnatal-day-7 rat model of hypoxia–ischemia [54] and that rosuvastatin protects cultured neurons from OGD-induced cell death [55]. Although reductions in the postischemic elaboration of inflammatory cytokines and increases in Akt and CREB activation were observed in the former model [56] (Table 1), more preclinical studies are clearly needed to support this promising avenue of inducing both short- and long-lasting cerebroprotection.

The ability of several agents commonly found in specific foodstuffs to serve as preconditioning triggers is consistent with the notion that, like exercise, phytochemicals and other dietary factors may engender an acute—or with regular consumption perhaps even a chronic—ischemia-tolerant state [57]. Despite the attractiveness of this concept, the study of the CNS effects of phytochemicals with respect to preconditioning and the possible mechanistic basis for such an effect is really in its infancy. The best developed example to date would be resveratrol, a polyphenolic derivative from grape skins, that is, an effective preconditioning compound for focal and global ischemia [58–60]. Whether its mechanism of action involves alterations in histone deacetylation secondary to sirtuin activation, increases in PPAR gamma coactivator 1α (PGC-1α) expression, modulations in mitochondrial redox, protease release, and/or other effects remain unclear. Similarly, in a gerbil forebrain ischemia model, short-term oral administration of grape polyphenol extract is protective [61]. Even the polyunsaturated fatty acid linolenate, a common ingredient of vegetable oil, can precondition the gerbil brain after short-term administration [62]. While obviously more of a causative agent in disease induction and progression than a potential treatment, ethanol is a molecule that can precondition a number of tissues against ischemic and other injuries, including the stroked brain [63]. It should not be unexpected that "Eastern" medicinal herbs and therapeutics—well tolerated by patients for centuries—might also exhibit preconditioning effects suitable for cerebral ischemic protection. To my knowledge, a single study presents evidence for such an effect: Ginkoglides (constituents of the nonflavone fraction of a *Ginkgo biloba* extract) preconditioned against simulated ischemic injury in the C6 rat glioma cell line by a HIF1α-, MAPK-, and Akt-dependent mechanism [64].

Many other clinically approved drugs that promote IT in brain are worthy of mention. In particular, three ligand-activated, nuclear transcription factor isoforms in the PPAR family that regulate gene expression in a number of unique ways exert neuroprotective effects when given after stroke [65]. However, studies also demonstrate that, following a 2-week treatment with the PPARα agonist fenofibrate, reductions in lesion size are realized following focal ischemic injury in the rat, secondary to vascular-based protective effects (reductions in postischemic inflammation and improved vascular reactivity) [66, 67]. Given the rather widespread clinical use of fibrates for hyperlipidemia, the ability of this and other PPAR agonists—perhaps those acting at the gamma (the thiazolidinediones [68]) and delta receptors—to not provide stroke prophylaxis per se but rather to precondition the brain following single or repeated application deserves more research scrutiny. Treating rats with estrogen [69] or the primary naturally occurring estrogen hormone estradiol-17β [70] is also an effective preconditioning strategy and may leverage the natural neuroprotective advantage premenopausal women exhibit regarding their incidence of stroke. The immunosuppressive drug fingolimod (FTY-720), which is in phase II and phase III trials for the treatment of multiple sclerosis, preconditions the mouse brain against focal ischemic injury [71]; its phosphorylation and subsequent activity at different sphingosine-1-phosphate receptors may underlie this effect, although cytosolic phospholipase A2 inhibition and other signaling actions may contribute as well. Thus, studies to determine whether these and/or other immunosuppression-based features of FTY-720 contribute to its preconditioning effect, and whether other FDA-approved immunomodulatory drugs might also promote IT, are worthy pursuits. Finally, one of Western medicine's "miracle" drugs, acetylsalicylic acid (aspirin), shows both rapid and delayed preconditioning-like effects [72, 73], but surprisingly, no follow-up studies were forthcoming from this early provocative work.

Finally, there are a number of nonischemic/nonhypoxic, nonpharmacologic interventions reported to date that

trigger robust ischemic tolerance in brain, including exercise [74–77], acupuncture [51, 78, 79], hyperbaric oxygen, transcranial magnetic stimulation [80], and caloric restriction [81], to name a few. With the exception of exercise and hyperbaric oxygen, studies of the ability of antecedent treatment with these agents to protect brain are relatively rare. Ultimately though, the signaling pathways and molecules that lead to the requisite metabolomic and genomic changes induced by these interventions, when identified, may become viable targets of a pharmacologic preconditioning strategy.

Tested at the Bench, but Unsuitable for Clinic Applications

As alluded earlier, there are some pharmacologic agents (e.g., lipopolysaccharide (LPS), NMDA, and the metabolic inhibitor 3-nitropropionic acid (3-NPA)) that have received significant experimental focus. However, these agents are unlikely to be approved for clinical use because of toxicity concerns related to the difficulties of dose titration and the magnitude of noncerebral, off-target side effects. Other drugs in this category include exogenously administered thrombin [82, 83], adenosine [17, 84], TNFα [85], bradykinin [86], and oxidative stress-inducing agents [87] (Table 1). However, as elaborated below, analogs of these compounds may eventually be developed that provide the same therapeutic, IT-promoting benefit with considerably fewer safety, tolerability, and dosing concerns, so careful reviews of the collected mechanistic data underpinning these studies are merited.

The ability of low doses of LPS, a common endotoxin derived from the cell membrane of specific Gram-negative bacteria, to precondition ischemic brain is a case-in-point. Following a very early study showing LPS-mediated protection against permanent focal stroke in rats [88], preconditioning with low doses of this endotoxin in rat [89, 90] and mouse [91] transient focal stroke models and neonatal hypoxia–ischemia models [92] is now well established. Notably, LPS is one of the few preconditioning stimuli to be efficacious in a large animal model of ischemic brain injury (hypothermic circulatory arrest in pigs) [93]. Mechanistically, the ischemia-tolerant state is one exhibiting a transient anti-inflammatory phenotype that, in turn, appears to result from LPS stimulating a proinflammatory activation of the innate immune system. Toll-like receptor activation likely plays a role in mediating this somewhat paradoxical response to LPS and perhaps other preconditioning stimuli as well [94].

3-NPA depresses oxidative phosphorylation secondary to the irreversible inhibition of succinate dehydrogenase. Initially, neuroprotection from OGD was documented by the rapid preconditioning of brain slices with this metabolic inhibitor [95], but even when administered systemically, 3-NPA can promote a state of delayed cerebral ischemic tolerance that protects against both transient [96, 97] and permanent [98, 99] focal stroke in a number of rodent species; its effects in models of global ischemia are more controversial [100–102]. While this particular compound will not be considered for clinical trials, its ability to transiently reduce cellular metabolism, as observed with the prolyl hydroxylase inhibitors and to some extent by preconditioning with aspirin [72] and antibiotics [43], suggests that a finely controlled, tissue- and/or cell-specific suppression of mitochondrial metabolism [22] could serve as a general therapeutic approach to inducing ischemic tolerance, akin to that found in hibernating and anoxia-tolerant vertebrates. If generally true, then how to achieve and temporally regulate such an effect in an organ-specific manner present one of many significant translational research challenges in this field.

Additional Considerations

With respect to treating patients by oral or intravenous routes, the efficacy of some systemically administered, pharmacologic preconditioning strategies suggests many provocative implications for therapeutic consideration. For example, “whole animal” preconditioning with hypoxia or hyperbaric oxygen, or with systemic (intravenous or intraperitoneal) administration of agents such as LPS, 3-NPA, various cytokines, resveratrol, etc., must require the sequential participation of all cells of the neurovascular unit to account for the pan-cerebral IT they promote. In other words, protection by centrally delivered preconditioning drugs that do not readily cross the blood–brain barrier (which excludes morphine, ethanol, and a few others) likely entails the following intercellular signaling sequence: Cerebrovascular endothelial cells sense and respond in an integrated fashion to a circulating molecular signal (or alterations in oxygenation) and transduce the stress/survival signal to surrounding astrocytes and neurons by yet another series of intercellular molecular signals. The extent to which disparate systemic preconditioning stimuli activate distinct or overlapping signaling pathways in and between these different cells, and the degree to which the resulting gene expression patterns are shared among them, is unclear. As one example, there is evidence that TNFα serves as a downstream mediator in response to hypoxia [103], ischemia [104], hyperoxia, LPS [91], exercise [76], and other preconditioning stimuli. From a treatment standpoint, these findings suggest that IT may be achieved in humans by a drug that does not actually have to cross the blood–brain barrier, but rather, one that is capable of

activating the appropriate cerebral endothelial receptors, and even though isolated neurons or other resident brain cells can be preconditioned in culture with a variety of stimuli, it may be possible that a noncerebral tissue (e.g., the liver), or its specialized vascular endothelium, is the indirect "mediator" of systemically delivered preconditioning treatments by virtue of its "response" to such treatments (e.g., releasing cytokines into the blood). Thus, these tissues, and not the brain per se, may be suitable therapeutic targets for stroke preconditioning. The ability of remote preconditioning (brief mesenteric or limb skeletal muscle ischemia) to protect ischemic brain [105, 106] underscores such a possibility.

Next Steps

To date, the endpoints used in many preclinical IT studies, even for the more "popular" pharmacologic preconditioning agents, tend to be morphologic; more functional outcome measures, and more long-term follow-up studies at clinically relevant time points, are needed in both focal and global ischemia models. Ultimately, drumming up solid preclinical efficacy for any lead preconditioning drug will require detailed pharmacokinetic studies to identify the time dependency of its effect and the dose that is neither impotent nor toxic. Ideally, documentation that such a treatment is efficacious in large animal models [21, 93], in females [73, 107], in aged animals where IT may be blunted [108], and those with comorbidities and other known stroke risk factors is also needed.

Of course, no pharmacologic treatment is without unintended side effects and related concerns. Analogs of one or more of the aforementioned pharmacologic agents that exhibit less than ideal clinical profiles might ultimately promote cerebral IT just as effectively but with a wider safety and tolerability profile. Current examples of this include dipyridyl, a lipid soluble iron chelator effective in preconditioning in a photothrombosis model [109] that may prove less problematic in certain preconditioning paradigms than deferoxamine. The nonmethylated cytosine-guanine bacterial oligonucleotide CpG, which acts as a toll-like receptor 9 ligand [110, 111], and the nonharmful endotoxin analog diphosphoryl lipid A [112] seem to protect the mouse brain at magnitudes similar to those achieved with LPS. One or more of the non-hematopoietic, cerebroprotective EPO analogs that appear to activate unique EPO receptor populations in brain that are linked to neuroprotective signaling pathways [40] may also exhibit robust qualities as a preconditioning agent.

The hypotensive, hyperglycemic, and other side effects of mitochondrial-selective KCO drugs like diazoxide might be avoided by using the analogs BMS-191095 [113] and bepridil [114], the latter a clinically approved antianginal medication. Iptakalim, a relatively new KCO that crosses the blood–brain barrier to act selectively at SUR2 type of K_{ATP} channels, without adversely affecting the pancreatic SUR1 type of channels, shows preconditioning-like neurovascular protective effects in a rat model of high altitude hypoxic brain injury, even when administered by gavage [115]. Ultimately, phase I and II clinical trials will be necessary for our most promising preconditioning drugs to define dosing, tolerability, and efficacy in humans, but these translational steps may fail like many others if such preclinical studies are not designed carefully and do not adhere to the updated STAIR criteria [116].

Given the number and diversity of pharmacologic treatments that currently promote IT in animals, some of which are already approved for clinical use, and considering that the very concept of finding utility in what was viewed by many not so long ago as a field of endeavor without clinical ramifications, the potential for translational success looks bright. In all likelihood, the preconditioning treatment regimen of choice will have to be modified significantly depending on the nature of the anticipated ischemic event (as is true in our animal models), be it stroke, subarachnoid hemorrhage, or any number of planned or emergency neurosurgical or cardiac surgeries, or neuroradiological interventions. Preconditioning "cocktails" may ultimately be utilized to induce mechanism- and cell-specific IT across all cells of the neurovascular unit. Also, we may find pharmacologic preconditioning employed in conjunction with post-stroke treatments—particularly with the thrombolytics. As the field of pharmacogenomics evolves [117], it will be exciting to define and implement individualized preconditioning treatments based on personal genetic profiles.

Despite the many issues regarding chronic drug administration of any kind, some can envision a future in which a form of preconditioning-like prophylaxis is pharmacologically established in a vitamin-mimicking fashion for patients with defined combinations of the more "standard" risk factors (e.g., age, race, genetic history, smoking, hypertension, diabetes) that we already know are associated with adverse cerebrovascular events.

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