Cyclosporine A, a Potential Therapy of Ischemic Reperfusion Injury. A Common History for Heart and Brain

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Key Words

Ischemic stroke · Reperfusion injury · Neuroprotection · Cyclosporine

tential neuroprotective properties of cyclosporine; however, further larger clinical trials are needed to demonstrate that CsA improves clinical outcome in acute IS patients.

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Abstract

Background: Ischemic stroke (IS) and acute myocardial infarction require emergency reperfusion tissue in order to improve functional outcome. Intra-arterial thrombectomy recently showed very encouraging improvement in IS patients' outcome. However, endovascular methods enhancing reperfusion may expose patients to increase in ischemic reperfusion injury. Experimental evidence indicates that brain ischemic reperfusion injury may be attenuated by ischemic pre- and postconditioning. The opening of mitochondrial permeability transition pore plays a critical role in the onset of reperfusion damage. This mechanism can be inhibited by immunosuppressive drugs like cyclosporine A (CsA). **Summary:** In this review, we present existing experimental and clinical data suggesting that conditioning interventions may prevent brain ischemic reperfusion injury and future challenge for neuroprotection by CsA in acute IS. **Key Messages:** The concept of conditioning has been recently investigated clinically but to a lesser extent in the realm of IS. Recent experimental and phase II clinical research has suggested po-

Introduction

Ischemic stroke (IS) and acute myocardial infarction require emergency reperfusion in order to improve functional outcome. In both conditions, treatments have changed in parallel during the last 2 decades [1]. Intravenous tissue-type plasminogen activator has long been the only therapy with proven clinical benefit in patients with acute IS [2], despite incomplete benefit in patients with large cerebral artery occlusion. Although previous randomized controlled trials [3–5] did not show a benefit over standard treatment, recent studies confirm the effectiveness of this strategy [6–10]. Endovascular methods enhancing reperfusion may expose patients to increased ischemic reperfusion injury thereby hampering the benefit of recanalization by promoting hemorrhagic transformation (HT) and severe vasogenic edema both considered as markers of reperfusion injury [11–14].

Experimental evidence indicates that brain ischemic reperfusion injury may be attenuated by ischemic pre- and postconditioning. Basically, brief episodes of ischemia and reperfusion applied prior to (preconditioning) or immediately after (postconditioning) the sustained artery occlusion have been shown to dramatically reduce stroke volume in animal models [15]. Experimental evidence suggests that mitochondrial permeability transition pore (MPTP) plays a critical role in this process [16]. This phenomenon occurs in the early minutes of reperfusion following a prolonged (i.e., responsible for irreversible tissue damage) ischemia; it is due to the opening in the inner mitochondrial membrane of a mega channel the MPTP that can be prevented by administration of cyclosporine A (CsA) [17, 18]. However, the ability of any pre- and postconditioning intervention to attenuate reperfusion injury depends on the duration of the preceding ischemia and on the application of this intervention within a limited time window (<15 min) following successful recanalization, in line with the therapeutic window validated by ECASS III study [19].

General Background of Ischemic Reperfusion Injury in Brain and Potential Impact of CsA

Following a prolonged interruption of cerebral perfusion, part of the neurons that lie distal to the obstructed vessel may die. Obviously, timely cerebral blood flow restoration will salvage a majority of cells that did not die by the end of the ischemia phase. Unfortunately, reperfusion may have noxious side effects and promote substantial additional brain damage termed ‘reperfusion injury’, that is, part of the cells that were still viable at the end of the ischemic phase, will die as a result of this second insult occurring at the onset of reflow [20, 21]. Hence, the final irreversible damage observed after reperfusion following an ischemic insult results from the addition of a first damage occurring during ischemia and a second damage specifically induced by reperfusion. Noteworthy, ischemic reperfusion injury is not limited to the parenchymal and stromal tissues but also involves the cerebral vasculature [22].

The mechanism of reperfusion-induced cellular damage remains unclear. However, experimental evidence indicates a crucial role of a specific mitochondrial dysfunction named ‘mitochondrial permeability transition’. At the time of reperfusion after a prolonged ischemia, abrupt matrix accumulation of Ca^{2+} and overproduction of reactive oxygen species (ROS) trigger the opening of a mega

channel in the inner mitochondrial membrane, named the ‘permeability transition pore’ (PTP) [16, 23, 24]. Accordingly, during reperfusion, there is overproduction of ROS in mitochondria, which rapidly exhaust endogenous antioxidant scavenging capacity. The resulting flood of ROS directly causes oxidative damage on cellular macromolecules, such as proteins, nucleic acids and lipids, which lead to mitochondrial swelling, cell injury and death [25, 26].

The mechanisms of mitochondrial ROS generation during reperfusion injury may also involve changes in mitochondrial membrane potential. Hyperactive oxidative phosphorylation generates high mitochondrial membrane potentials, a condition known to generate excessive ROS. Such a state would lead to a ‘burst’ of ROS upon reperfusion, thereby causing structural and functional damage to the mitochondria and inducing cell death signaling that eventually culminate in tissue damage [27].

Although superoxide dismutase and NADPH (nicotinamide adenine dinucleotide phosphate) oxidase have been shown to reduce ischemic reperfusion injury in animal models [28, 29], the clinical benefit of antioxidant strategy has also been difficult to show in human trials as shown by SAINT I and II trials [30] and the recent URICO-ICTUS study in which the addition of uric acid to thrombolytic therapy did not increase the proportion of patients who achieved excellent outcome after stroke compared with placebo [31] despite reduced infarct growth in women with acute IS treated with alteplase [32].

CsA, apart from its immunosuppressive activity, inhibits PTP opening and reduces infarct size in animal models of cerebral ischemia [33–44]. The neuroprotective effect appears to depend on several factors, including the route of administration (intravenous (IV), intra-cerebral, ischemic preconditioning (IP) intra peritonea, intracarotid, subcutaneous, intracerebro ventricular), the dosage of CsA, the duration of ischemia, the timing of administration with respect to the onset of ischemia and of reperfusion, the nature of the ischemic model (transient middle cerebral artery occlusion (MCAO) or permanent MCAO, blood–brain barrier (BBB) permeability) and finally the association with other drugs. Preclinical data characteristics and results are summarized in table 1 and the putative mechanism of the effect of cyclosporine on PTP is shown in figure 1.

The molecular structure of the PTP remains unknown. Yet, it is accepted that cyclophilin D (CyPD), a mitochondrial matrix chaperone, is a key component of the PTP [45]. Mitochondrial Ca^{2+} accumulation can activate

Table 1. Experimental data review on CsA postconditioning in murine models

References	Cerebral ischemia			Postconditioning			Results				
	model	occlusion time	start	administration	dose	BBB open	evaluation day after ischemia	side effects	infarct size reduction	neuroscore improvement	conclusion
Uchino et al. [35], 1998	2 carotids occlusion	10 min	Delayed: 30 min	IP	10 mg/kg	Yes traumatic	Day 7	ND	Yes	ND	ptpm inhibition, decrease neural death
Yoshimoto and Stesjö [36], 1999	tMCAO	120 min	Delayed: 1 h	IV	10 mg/kg	Yes traumatic	Day 2	None	40%	ND	PTPm inhibition, improvement with BBB open
			Early: 5 min		50 mg/kg			Seizure death			
			Delayed: 1 h	Ica	5 mg/kg	No			20%		
			Delayed: 3 h		10 mg/kg				10%		
Matsumoto et al. [37], 2002	tMCAO	120 min	Early	Ica	10 mg/kg	Yes mannitol	Day 2	None	10%		20 mg/kg CsA early and at day 1 after reperfusion is neuroprotective
			Early and delayed: 24 h		20 mg/kg	Ica 2 g/kg			20%		
				IP	30 mg/kg	No		None	Yes	ND	
Vachon et al. [38], 2002	pMCAO + carotids occlusion	35 min	Early	Ica	10 mg/kg	No	Day 1	ND	Yes	ND	Ica CsA injection = high concentration in brain
Domańska-Janik et al. [39], 2004	2 carotids occlusion	5 min	Early	Ica	5 and 10 mg/kg	No	Day 7	ND	Yes	ND	PTPm inhibition
			Delayed: 6, 24 h	IP	2 mg/kg				No		
Yu et al. [40], 2004	tMCAO	60 min	Delayed: 3, 24, 48, 72 h	IP	1 mg/kg	No	Day 3	ND	No	Non	CsA with MP less toxic, transient and efficient neuroprotective effect
					10 mg/kg				Yes	Yes	
					10 mg/kg	MP			No	No	
					1 mg/kg*	10 mg/kg				Yes	
Murozono et al. [41], 2004	tMCAO	30 min	Early and delayed: 24 h	IP	10 mg/kg	Yes mutation	Day 2	Yes	No	ND	Neurotoxic effects according intracerebral dose
					1 mg/kg	mdrla			Yes		
Borlongan et al. [18], 2005	tMCAO	60 min	Delayed: 3, 24, 48, 72 h	IP	1 mg/kg	No	Day 3	ND	No	ND	Low dose CsA less toxic, neuroprotective effect with MP
					10 mg/kg				Yes	Yes	
					10 mg/kg				No	ND	
					1 mg/kg*					Yes	
Muramatsu et al. [42], 2007	2 carotids occlusion	5 min	Early	IV	2 ml/kg	Non	Day 4	ND	Yes	ND	CsA better than FR901459
				ICV alzet pump		No		Day 1			

Table 1. (continued)

References	Cerebral ischemia		Postconditionning		Results						
	model	occlusion time	start	administration	dose	BBB open	evaluation day after ischemia	side effects	infarct size reduction	neuroscore improvement	conclusion
Yuen et al. [43], 2011	tMCAO	3 h	Delayed: 30 min, 24, 48 h	IP IP SC	20 mg/kg 20 mg/kg + 5,000 UI/kg EPO	No	Day 21	ND	Yes	ND	CsA + EPO neuroprotection better than CsA alone
Leger et al. [17], 2011	pMCAO + carotids occlusion	50 min	Early	IP	20 mg/kg	No	Day 2	ND	No	ND	PTPm inhibition, more efficient in preconditioning
Cho et al. [44], 2013	tMCAO	60 min	Early	Ica	10 mg/kg	No	Day 1	ND	No	No	More efficient in preconditionning
ND = Not documented; tMCAO = transient MCAO; IP = intraperitoneal; Ica = intracarotid; SC = subcutaneous; pMCAO = permanent MCAO; MP = methylprednisolone; ICV = intra-cerebroventricular.											

ND = Not documented; tMCAO = transient MCAO; IP = intraperitoneal; Ica = intracarotid; SC = subcutaneous; pMCAO = permanent MCAO; MP = methylprednisolone; ICV = intra cerebroventricular.

CyPD and subsequently trigger PTP opening. Mice lacking CyPD display a delayed opening of the PTP when Ca^{2+} overload is present and develop smaller cerebral infarcts after prolonged ischemia and reperfusion [46].

Ischemic reperfusion also triggers a sterile inflammatory response [47]. There is emerging evidence that CsA could induce neuroprotective effects via mechanisms different from the inhibition of PTP opening [48]. By preventing calcineurin-mediated dephosphorylation, CsA inhibits the translocation of the nuclear factor of activated T cells (NFAT) family of transcription factors to the nucleus of activated T cells. The NFAT group is involved in the transcriptional activation of the genes encoding interleukin (IL)-2, IL-4 and CD40L; thus, inhibition of the NFAT by CsA results in a specific inhibition of IL production in the T cell [49]. Inhibition of calcineurin by CsA may prevent the dephosphorylation of NOS thereby limiting glutamate neurotoxicity [50]. Endothelial cells contribute to this phenomenon by expressing adhesion molecules that facilitate leukocytes binding and infiltration into reperfused areas. Increasing evidence links the toll-like receptors (TLRs), particularly TLR2 and TLR4, to the deleterious inflammatory effects seen in ischemic reperfusion injury associated with IS [51, 52].

The activation of the phosphorylation of RISK (reperfusion injury salvage kinases) pathway including, that is, phosphatidylinositol 3-kinase/protein kinase B (PI3K-AKT), of the mitogen-activated protein kinase/extracellular signal regulated kinase (MEK1/2-ERK1/2) pathway and/or of signal transducer and activator of transcription 3 may play a role in postconditioning by modulating PTP opening [53–55]. Pharmacological agents targeting these pathways such as CsA may protect the brain from reperfusion injury [56–59]. The neuroprotective effect of CsA may also involve BBB permeability modulation through inhibition of cyclophilin A as suggested by the experimental model of traumatic brain injury [60].

Conditioning Interventions to Prevent Reperfusion Injury

Mechanical Conditioning

In 1986, Murry et al. [61], first demonstrated in the dog heart that brief episodes of ischemia with intervening reperfusion, applied before a prolonged ischemic insult, resulted in a dramatic reduction of infarct size; they named this phenomenon ‘ischemic preconditioning’.

Fig. 1. Schematic representation of cyclosporine inhibiting PTP opening. PTP is in a closed conformation under normoxic conditions. At the time of reperfusion following prolonged ischemia, accumulation of Ca²⁺ in the mitochondrial matrix triggers the translocation of the matrix chaperone CyPD to the inner mito-

chondrial membrane that in turns contributes to set the PTP in its open conformation. Cyclosporine detaches CyPD from the inner membrane and thereby prevents PTP opening (adapted from Nicolli et al. [45]).

In 2003, Zhao et al. [15] showed that comparable protection could be achieved by altering the conditions of reperfusion. Mimicking the initial experiment by Murry et al. [61], they applied the brief cycles of ischemic reperfusion immediately after (but not before) the sustained ischemic insult; this intervention resulted in a significant infarct size reduction, and they named this phenomenon ‘ischemic postconditioning’. This finding was confirmed by several other groups [62]. In a randomized, controlled, multicenter study, our group first reported that ischemic postconditioning can limit infarct size in ST-elevation myocardial infarction patients [63]. Briefly, the application of 4 episodes of 1 min inflation (obstruction of the coronary artery) and 1 min deflation (reperfusion) of the angioplasty balloon was associated with a 36% myocardial infarct size reduction [64]. Several phase II trials confirmed these findings [65]. However, other studies failed to find a beneficial effect of ischemic postconditioning during myocardial ischemia [66, 67].

In the brain, retrospective and prospective studies have shown that previous transient ischemic attack (TIA) attenuated subsequent stroke with smaller infarct size

and less clinical deficits compared with patients without a preceding transient event [68–71].

In the last 2 decades, our knowledge concerning the underlying molecular basis of postconditioning has substantially improved, and there is hope to potentially mimic the endogenous neuroprotective state in patients with a high risk of cerebral ischemia. This mechanism may promote plastic neuronal changes allowing better tolerance to ischemic insult in animal models [72]. By using a MCAO model of TIA in rats, Malhotra et al. [73] showed that erythropoietin and its receptor were upregulated by IP in the ipsilateral hemisphere by 24 h after IP.

The putative mechanisms of preconditioning may also include inhibiting ROS activities, overexpression of heat shock proteins, immediate-early gene expression, synthesis of new proteins, inflammation inhibition, increase of anti-apoptotic proteins and activation of Akt pathways, a signal transduction mechanism that promotes survival and growth in response to extracellular signals [74–77]. However, the clinical application of preconditioning is very limited due to the fact that the occurrence of most strokes is unpredictable.

Remote Conditioning

Different modalities of ‘conditioning’ interventions have proved to be capable of preventing reperfusion injury. One of the most intriguing is ‘remote conditioning’. Przyklenk et al. [78] initially observed that brief episodes of ischemic reperfusion applied to the circumflex coronary artery were able to protect the left anterior descending artery territory from ischemic reperfusion injury. This suggests that the protection could be ‘transferred’ remote from the initial site of injury. Numerous experimental and phase II clinical trials have confirmed this observation [79]. One popular experimental set up in patients consists of applying 4 episodes of 5 min brachial cuff inflation (i.e., brief ischemia) interspersed by 5 min cuff deflation (brief reperfusion) prior to a prolonged heart, kidney, liver or brain ischemia; as a result, these organs develop significantly smaller infarcts [80]. The mechanism of this intriguing phenomenon remains poorly understood, but appears to require either a neural or a humoral pathway [81, 82].

In acute IS, remote preconditioning has recently been applied during ambulance transportation to the hospital [83]. An open-label blinded outcome proof-of-concept study of pre-hospital, paramedic-administered rPerC at a 1:1 ratio in consecutive patients with suspected acute stroke. After neurological examination and MRI, patients with verified stroke who received alteplase treatment were included, and they received MRI at 24 h and 1 month and clinical re-examination after 3 months. The primary end point was penumbral salvage, defined as the volume of the perfusion-diffusion mismatch not progressing to infarction after 1 month approach showed no global statistically significant effect on penumbral salvage, infarct size or infarct progression as measured by MRI. However, when adjusted for baseline severity of hypoperfusion, a voxel-by-voxel analysis demonstrated increased tissue survival after 1 month suggesting that interventions targeting lethal reperfusion injury may be neuroprotective.

Clinical Assessment of CsA in Reperfusion Injury Ischemic Stroke

In a pilot phase II clinical trial, our group recently examined 127 IS patients to find whether IV administration of cyclosporine in combination with thrombolysis might reduce cerebral infarct size [84]. Patients, presenting with an anterior circulation stroke and eligible for thrombolytic therapy, were enrolled in this multicenter, single-blinded, controlled trial. Fifteen minutes after randomization, patients received either an IV bolus injection of

2.0 mg/kg CsA or placebo. This low dose was chosen after analysis of experimental IS data in murine models. CsA is effective between 0.6 and 2.4 mg, the neuroprotective effect disappears at higher concentrations (>6 mg), which is associated with neurotoxicity [18].

The primary end point was infarct volume on MRI at 30 days. Secondary end points included infarct volume according to the site (proximal/distal) of arterial occlusion and recanalization after thrombolysis. When considering the whole study population, the reduction of infarct volume in the CsA compared with the control group was not significant. However, in the subgroup of patients with proximal occlusion and effective recanalization, infarct volume was significantly reduced in the cyclosporine compared with the control group.

Myocardial Infarction

We recently published the results of the CIRCUS trial that investigated the ability of CsA to improve clinical outcomes of ST elevation myocardial infarction in patients [85]. A total of 395 patients in the cyclosporine group and 396 in the placebo group received the assigned study drug and had data that could be evaluated for the primary outcome at 1 year. The rate of the primary outcome was 59.0% in the cyclosporine group and 58.1% in the control group (OR 1.04; 95% CI 0.78–1.39; $p = 0.77$). Cyclosporine did not reduce the incidence of the separate clinical components of the primary outcome or other events, including recurrent infarction, unstable angina and stroke. No significant difference in the safety profile was observed between the 2 treatment groups.

In patients with anterior STEMI who had been referred for primary PCI, IV cyclosporine did not result in better clinical outcomes than those with placebo and did not prevent adverse left ventricular remodeling at 1 year.

CsA, Future Studies in IS

The absence of effect of CsA in the heart, in this specific clinical setting, does not preclude the potential efficacy of CsA in IS patients. Based on our encouraging recent phase II study results, we are therefore currently designing a phase III trial combining thrombectomy and CsA within the 6 h time window in patients with proximal occlusion with a dose similar to that of the phase II trial.

Imaging and Reperfusion Injury

Although it is undoubtedly a major progress, the recent advance in reperfusion therapy by intra-arterial thrombectomy, brings into light the issue of reperfusion

injury, with more patients being now exposed to this specific type of injury. We therefore need to measure reperfusion injury not only as part of the prognosis evaluation but also examine the impact of neuroprotective agents. HT and vasogenic edema are classical markers of reperfusion damage. Nevertheless, imaging methods revealing early and specific markers of reperfusion damage are currently insufficient. Hence, this area may deserve future research.

Post-Ischemic Hyperperfusion and Perfusion-Weighted Imaging

Several animal studies suggest that the restoration of cerebral circulation consistently results in a hyperemic phase. Hyperemia may contribute to the development of reperfusion injury including via brain edema or hemorrhage. Furthermore, following post-ischemic hyperemia, a phase of secondary hypoperfusion can occur, which results in harmful effects on the reperfused tissue. Kidwell et al. [86] used perfusion-weighted (PW)-MRI to characterize hyperemia in 12 patients following intra-arterial thrombolysis. Hyperemia was visualized in 5 of 12 patients. On day 7, 79% of voxels with hyperemia demonstrated infarction, whereas only 36% not showing hyperemia demonstrated infarction. Despite the voxel-by-voxel association of increased perfusion with infarction, there were no significant differences in the degree of clinical improvement in patients with regions of hyperemia versus those without. Recently, Yu et al. [87] using arterial spin labeling (ASL) showed that late ASL increased perfusion might predict high-grade HT. Larger future MRI studies are necessary to assess the extent to which hyperemia may result in unfavorable clinical outcome in human stroke.

Breakdown of the BBB and Contrast-Enhanced MRI

Pathological responses to ischemia in the microvasculature play a central role in the evolution of infarction; a critical event after ischemia is BBB breakdown [88].

The BBB impairment consists of complex ultrastructural disruptions, including damaged capillary endothelial cells, degeneration of astrocytes and pericytes, as well as perivascular edema [89]. Inhibition of microglial activation may protect the brain after IS by improving BBB viability and integrity [90]. Given these perspectives, it has become essential to assess BBB breakdown by optimal imaging methods.

Using a novel MRI marker that can detect BBB disruption, Latour et al. [91] studied a total of 144 acute stroke patients to test the association between reperfu-

sion, HT and clinical outcome. BBB disruption was more common in patients who reperfused than in patients who did not reperfuse. In the reperfused group, patients with BBB disruption were more likely to have a poor clinical outcome than those without disruption. The post-contrast enhancement of the cerebrospinal fluid in FLAIR images (termed hyperintense acute reperfusion marker, HARM) is also an established marker of early BBB disruption [92].

Conclusion

Although the concept of conditioning has been breached clinically in the cardiovascular setting, it has been investigated clinically to a lesser extent in the realm of neurological disease. Recent experimental and phase II clinical research has suggested potential neuroprotective properties of cyclosporine in acute IS. Although accumulating experimental and clinical research evidence are encouraging, further larger clinical trials are needed to demonstrate that CsA improves clinical outcome in acute IS patients.

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