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STATE-OF-THE-ART REVIEW

Mitochondrial Membrane Permeability Inhibitors in Acute Myocardial Infarction

Still Awaiting Translation

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SUMMARY

Despite therapeutic advances, acute myocardial infarction (AMI) remains a leading cause of morbidity and mortality worldwide. One potential limitation of the current treatment paradigm is the lack of effective therapies to optimize reperfusion after ischemia and prevent reperfusion-mediated injury. Experimental studies indicate that this process accounts for up to 50% of the final infarct size, lending it importance as a potential target for cardioprotection. However, multiple therapeutic approaches have shown potential in pre-clinical and early phase trials but a paucity of clear clinical benefit when expanded to larger studies. Here we explore this history of trials and errors of the studies of cyclosporine A and other mitochondrial membrane permeability inhibitors, agents that appeared to have a promising pre-clinical record yet provided disappointing results in phase III clinical trials. (J Am Coll Cardiol Basic Trans Science 2016;1:524–35)

Despite therapeutic advances, acute myocardial infarction (AMI) remains a leading cause of morbidity and mortality worldwide. Sustained research efforts over the years have achieved numerous milestones (1–3). Despite the success stories, the rate of progression to heart failure and related complications remains unacceptably high (3,4). One potential limitation is the lack of effective therapies to optimize reperfusion after ischemia and prevent reperfusion-mediated injury. This has been termed *reperfusion injury*, or alternatively *ischemia-reperfusion injury* (5). Experimental studies indicate that this process accounts for up to 50% of the final infarct size, lending it importance as a potential target for cardioprotection (6). However, multiple therapeutic approaches have failed to translate from the bench to the bedside, or have shown therapeutic potential in early phase II trials (7–9) but have failed to translate into a clear clinical benefit when tested in larger phase III clinical studies (10–12). Here we explore this history of trials and errors, with a particular focus on the studies of cyclosporine A (CsA) and other mitochondrial membrane permeability inhibitors, a therapeutic approach that appeared to have a promising pre-clinical record yet provided disappointing results in phase III clinical trials.

REPERFUSION INJURY

The phenomenon of reperfusion injury was first born out of a demonstration that post-ischemic restoration of blood flow had several potential deleterious effects, including myocardial stunning (13). The idea of harm from reperfusion was later supported by demonstration of smaller infarct size with slower, low-pressure reperfusion over standard abrupt

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reperfusion at normal pressure, a benefit gained from interventions applied after the ischemic period rather than within it (14).

During ischemia, intracellular Na^{+} and Ca^{++} accumulate as downstream results of acidosis from anaerobic glycolysis, ultimately reaching a Ca^{++}-overloaded state. Upon reperfusion, the rapid normalization of pH causes uncontrolled myocyte contraction, intracellular edema, and formation of reactive oxygen species (ROS) (15). Within the context of reperfusion injury, perhaps the most salient component of this process occurs at the inner mitochondrial membrane (IMM). The IMM remains closed throughout ischemia but undergoes an abrupt transition in permeability during reperfusion (16), which collapses the membrane potential and uncouples oxidative phosphorylation (17). As a result, there is increased inorganic phosphate concentration, increased Ca^{++} flux, and mitochondrial edema, ultimately leading to the cytoplasmic release of cytochrome C, a proapoptotic protein that activates caspase-3 and leads to the death of the cardiac myocyte (5,6,15,18) (**Figure 1**).

The biochemical liaison for the increased leakage of the IMM is the mitochondrial permeability transition pore (mPTP). Much remains to be understood about the mPTP, but a leading hypothesis holds that the mPTP forms from F-type adenosine triphosphate synthase dimers within the lipid bilayer of the IMM (19). The channel opens and is forced to remain open in response to high concentrations of calcium, inorganic phosphate, and ROS, or with reduced IMM potential. All of these conditions are active during myocardial ischemia and reperfusion (20).

Many other signaling cascades and processes within and outside the mitochondria are concomitantly activated during ischemia and reperfusion and are likely to contribute to infarct size. For the scope of this review, we focused only on the mechanisms involving a change in permeability in the mitochondrial membrane for which a drug had been tested in both pre-clinical and clinical studies.

ISCHEMIC PRE-CONDITIONING

This discovery that reperfusion could be a double-edged sword (13) spawned a new wave of experiments. One landmark study described how repeating cycles of alternating ischemia and reperfusion performed prior to prolonged coronary artery occlusion significantly reduced final infarct size in dogs (even when total ischemia time was longer); this created the new field of "pre-conditioning" (21), replicated in numerous laboratories around the world (22–24). A "second window" of cardioprotection was also shown to begin 24 h after the initial window of protection. In 1 study, infarct size was reduced in rabbits subjected to four 5-min cycles of coronary artery occlusion prior to 24-h recovery, followed by a 30-min reocclusion (25) or a 90-min reocclusion (26). The clinical translational value of having identified the second window of cardioprotection, however, is still uncertain.

Interestingly, limitations of the pre-conditioning strategy became apparent quite rapidly. Cardiac protection was seen when occlusion/reperfusion cycles were performed prior to 40- or 60-min occlusion, but not prior to 90- (27) or 180-min (21) occlusion. These experiments highlight that the efficacy of pre-conditioning is limited to a specific window of duration of ischemia. Hence the difficulty in translation to humans: it is difficult to determine exactly when a patient starts experiencing ischemia, and usually by the time the patient is seen in the hospital, the ischemia has been ongoing for hours.

ISCHEMIC POST-CONDITIONING

An additional step forward in the field was provided by the demonstration of protective effects of interventions applied after the ischemia and the initial reperfusion: "post-conditioning." Brief serial episodes of controlled reperfusion interrupted by repeated brief bursts of ischemia reduced final infarct size: 3 cycles of 30 s of reperfusion alternating with 30 s of occlusion (for a total of 3 min), after 60 min of ischemia due to coronary occlusion provided a significant reduction in infarct size in dogs by 30% to 40% (28). The finding of beneficial effects of conditioning being applied after reperfusion provided an intense stimulus to the field, as it suggested a time window for intervention that extended beyond reperfusion. The same studies, however, also highlighted the critical time dependency of these approaches. Post-conditioning as means of controlled reperfusion intermittently interrupted by brief ischemia is effective in reducing infarct size if initiated within seconds of reperfusion (29,30) while failing to reduce infarct size if post-conditioning is delayed by more than 1 min (30,31). These considerations may explain the challenge of translating into clinical benefits in recent clinical trials (32).

PHARMACOLOGIC ISCHEMIC CONDITIONING

The study of the events occurring in ischemic pre- and post-conditioning has stimulated a large number of investigations into the signaling and mechanisms.

ABBREVIATIONS AND ACRONYMS

AMI = acute myocardial infarction

CsA = cyclosporine A

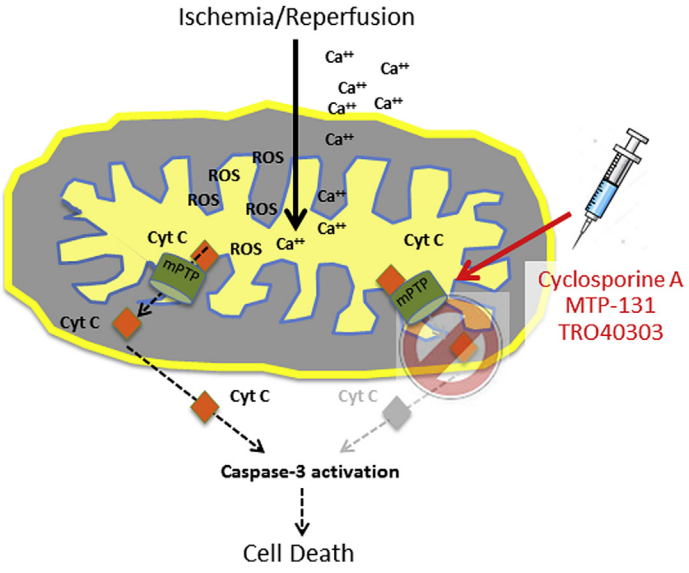
IMM = inner mitochondrial membrane

mPTP = mitochondrial permeability transition pore

PCI = percutaneous coronary intervention

ROS = reactive oxygen species

FIGURE 1 Schematic Representation of the Therapeutic Target of the Mitochondrial Membrane Permeability Inhibitors



Ischemia and reperfusion lead to Ca^{++} overload and reactive oxygen species (ROS) formation leading to opening of the mitochondrial permeability transition pore (mPTP), cytochrome C (Cyt C) release into the cytoplasm, caspase-3 activation, and cell death. Cyclosporine, MTP-131, and TRO40303 inhibit mitochondrial membrane permeability and prevent cell death. mPTP = mitochondrial permeability transition pore.

These studies provided not only a better understanding but also insight into potential therapeutic targets. The hypothesis that a drug given at a specific time during reperfusion could reduce infarct size and improve on the benefit of reperfusion became the search for the "holy grail" in cardiology (6). For the purpose of this review, we will focus on CsA and other drugs targeted at inhibiting the permeability of the mitochondrial membrane during ischemia and reperfusion. CsA and other drugs with similar mechanism had shown promising pre-clinical data, which was then studied in phase II and III clinical trials.

PRECLINICAL STUDIES OF CsA

Finding a targeted medication that could be given during a specific time during ischemia or reperfusion, in an effort to reduce infarct size, become the goal of many therapeutic attempts (6). CsA is a cyclic non-ribosomal peptide that was first extracted from the Norwegian soil fungus *Tolypociadium inflatum* in 1969 (33,34). It is used as an immune suppressant in solid-organ transplant patients, serving as a calcineurin inhibitor in T-cell lymphocytes (35), though experimental studies conducted in the late 1980s identified CsA also as an inhibitor of mPTP opening (36). A study on isolated perfused rat hearts revealed that treatment with CsA conferred cardioprotection in myocardial ischemia-reperfusion experiments: CsA 0.2 mM infused during ischemia restored the ATP/adenosine diphosphate ratio and adenosine monophosphate content to pre-ischemic levels and partially improved left ventricular diastolic pressure (37).

The effects of CsA, however, were exquisitely dose dependent; concentrations either lower (0.1 mM) or higher (0.4 to 1.0 mM) had no protective effects (37). The threshold dose effect at 0.2 mM was confirmed in other studies, and reduced efficacy at higher concentrations was shown (37,38). In vivo studies using weight-based dosing showed protective effects for CsA doses of 2.5 mg/kg but not 1.0 mg/kg; effectiveness for doses of 5.0 mg/kg or greater was seen in some but not all experimental studies in rodents (39–41).

Moreover, the effects of CsA appear to be time dependent. When given before ischemia onset (42) or 15 min prior to reperfusion (43), CsA 10 mg/kg provided powerful cardioprotection; however, when given 7 min (44), 5 min (42), 3 min (45), or 2 min (46) prior to reperfusion, this cardioprotection was no longer provided. This time-dependent effect mirrors ischemic post-conditioning results (30,31).

Table 1 summarizes pre-clinical studies with CsA.

PILOT PHASE II CLINICAL TRIALS OF CsA

In 2008, Piot et al. (47) published the results of the first pilot single-blind phase II clinical trial in patients with ST-segment elevation myocardial infarction (STEMI) (47). Fifty-eight patients were randomized to either 2.5 mg/kg intravenous CsA (Sandimmune, Novartis, Basel, Switzerland) or matching placebo given "less than 10 min before direct stenting," and a reduction of infarct size by the area under the curve for creatine kinase was demonstrated (**Figure 2**), although this reduction was not closely mirrored by the cardiac-specific troponin I area under the curve. Total ischemic time was approximately 5 h in each group, but, unfortunately, the exact time between study medication administration and stenting for each group was not reported. Indeed, the single-blind design allows for the possibility of bias (e.g., operators could have been more likely to wait longer between CsA administration and reperfusion to allow for drug steady state). A subgroup of patients (n = 27) underwent cardiac magnetic resonance 5 days after AMI, revealing a smaller infarct size in those who received CsA (**Figure 2**). There were no significant differences in adverse clinical events between the 2 groups (47).

TABLE 1 Preclinical Studies of Mitochondrial Membrane Permeability Inhibitors in AMI

	Animal	Model	Dose	Timing	Effects on Infarct Size	Other Notes
Cyclosporine A						
Nazareth et al., 1991 (38)	Rat (ex vivo)	Ischemia (60 min) and reperfusion (10 min)	Variable	At start of incubation	N/A	0.2 mM inhibited ATP loss; higher doses reversed this effect
Griffiths and Halestrap, 1993 (37)	Rat (ex vivo)	Ischemia (30 min) and reperfusion (15 min)	0.2 mM	2 min prior to ischemia	N/A	Demonstrated both lower and higher doses of CsA to be less effective
Gomez et al., 2007 (40)	Mouse	Ischemia (25 min) and reperfusion (24 h or 30 days)	10 mg/kg	5 min prior to reperfusion	Reduced by ~50%	LVEF significantly improved and 30-day mortality reduced
Dow and Kloner, 2007 (46)	Rat	Ischemia (30 min) and reperfusion (120 min)	5 mg/kg, 10 mg/kg	~2 min prior to reperfusion	No significant change	Post-conditioning also did not reduce infarct size in this study
Pagel and Krolikowski, 2009 (41)	Rabbit	Ischemia (30 min) and reperfusion (180 min)	5 mg/kg	2 min prior to reperfusion	No significant change when used alone	Benefit when combined with helium and alkalosis pre-conditioning, uncertain significance
Karlsson et al., 2010 (45)	Pig	Ischemia (45 min) and reperfusion (120 min)	10 mg/kg	"for 3 minutes before reperfusion"	No significant change	Data suggest possible deleterious interaction between CsA and isoflurane
Karlsson et al., 2012 (44)	Pig	Ischemia (40 min) and reperfusion (240 min)	2.5 mg/kg	7 min prior to reperfusion	No significant change	Closed-chest model
De Paulis et al., 2013 (42)	Rat	Ischemia (30 min) and reperfusion (120 min)	10 mg/kg	10 min prior to ischemia or 5 min prior to reperfusion	Reduced by >50% if given pre-ischemia; no significant change pre-reperfusion	Highlights potential benefit in combined action on cyclophilin D and complex I (isoflurane)
Huang et al., 2014 (39)	Rat	Ischemia (30 min) and reperfusion (120 min)	1 mg/kg, 2.5 mg/kg, 5 mg/kg	Not reported	2.5 mg/kg and 5 mg/kg reduced infarct size	Difficult to interpret without administration times reported
Zalewski et al., 2015 (43)	Pig	Ischemia (60 min) and reperfusion (180 min)	10 mg/kg	Between 15 min and 10 min prior to reperfusion	Reduced by 14%	CsA also improved myocardial blood flow and LVEF
TRO40303						
Schaller et al., 2010 (54)	Rat	Ischemia (35 min) and reperfusion (24 h)	0.5 mg/kg, 1.25 mg/kg, 2.5 mg/kg	3 min infusion starting 10 min prior to reperfusion	2.5 mg/kg reduced by 38%, lower doses not active	TRO40303 delayed mPTP opening but did not affect Ca^{2+} retention capacity
Le Lamer et al., 2014 (55)	Rat	Ischemia (35 min) and reperfusion (24 h)	0.3 mg/kg, 1.0 mg/kg, 3.0 mg/kg, 10.0 mg/kg	10 min before ischemia, 10 min before reperfusion, or 10 min after reperfusion	1–10 mg/kg pre-reperfusion doses reduced by 40%–50%, 1 mg/kg pre-ischemia reduced by 55%	Separate study established safety and pharmacokinetic data in phase I study in humans
MTP-131						
Cho et al., 2007 (57)	Rat	Ischemia (60 min) and reperfusion (60 min)	3 mg/kg	30 min prior to ischemia, repeated 5 min prior to reperfusion	Reduced by 10%	Arrhythmias were less frequent and less severe in the treatment arm
Kloner et al., 2012 (59)	Sheep	Ischemia (60 min) and reperfusion (180 min)	0.05 mg/kg/h	210 min infusion starting 30 min prior to reperfusion	Infarct size reduced by 15%	Relative infarct size reductions more prominent in larger infarcts, consistently across all models.
	Guinea pig (ex vivo)	Ischemia (20 min) and reperfusion (2 h)	1 nM	10 min prior to ischemia and "at onset" of reperfusion	Infarct size reduced by 38%–42%	
	Rabbit	Ischemia (30 min) and reperfusion (180 min)	0.05–0.10 mg/kg/h	200 min infusion starting 1 min, 10 min, or 20 min prior to reperfusion.	No significant reduction in infarct size.	
Sloan et al., 2012 (58)	Rat (ex vivo)	Ischemia (20 min) and reperfusion (120 min)	1 nM	At onset of reperfusion	Reduced by ~30%	Greater reduction in infarct size in diabetic rats
Brown et al., 2014 (60)	Rabbit	Ischemia (30 min) and reperfusion (3 h)	0.05 mg/kg/h	60 min or 180 min infusion starting 20 min prior to reperfusion	Reduced by 40%–50%	Negative results when infusion started 10 min after reperfusion.
	Guinea pig (ex vivo)	Ischemia (20 min) and reperfusion (2 h)	1 nM	"Beginning at the onset of reperfusion"	Reduced by 40%–50%	

AMI = acute myocardial infarction; ATP = adenosine triphosphate; CsA = cyclosporine A; LVEF = left ventricular ejection fraction; mPTP = mitochondrial permeability transition pore.

In a phase II double-blinded clinical trial of 101 patients with STEMI treated with fibrinolysis, CsA 2.5 mg/kg administered immediately before fibrinolysis did not demonstrate any appreciable benefit over placebo with regard to infarct size (measured with biomarkers), left ventricular ejection fraction, or outcomes (48).

In a clinical trial of 61 patients undergoing elective aortic valve surgery, CsA 2.5 mg/kg given 10 min before aortic cross-clamp removal significantly reduced myocardial injury measured as area under the curve for cardiac troponin I (49). Similarly, in a trial of 78 patients undergoing coronary artery bypass grafting surgery, CsA 2.5 mg/kg given before aorta cross clamping (before ischemia) reduced myocardial injury, especially in patients with longer ischemic times (50).

The CYCLE (CYCLosporinE A in Reperfused Acute Myocardial Infarction) trial further tested CsA in a larger randomized phase II open-label study, enrolling 410 patients presenting with STEMI, within 6 h of symptom onset and with angiographic evidence of Thrombolysis In Myocardial Infarction flow grade ≤1 in any epicardial coronary artery (51). Subjects were randomized to receive a bolus of intravenous CsA 2.5 mg/kg (n = 207) "at least 5 min before percutaneous coronary intervention" versus no treatment (n = 203). The exact time incurred from CsA treatment to reperfusion (delay) was not given. Being an open-label study, it is reasonable to assume that patients incurred no delay in the no-treatment arm, better described as a no-treatment or no-delay arm. A PROBE (Prospective Randomized Open Blinded Endpoint) design was employed, in which electrocardiographic tracings and angiogram recordings were centrally assessed by blinded readers. Ultimately treatment with CsA had no effect on the primary endpoint of interest, >70% resolution of ST-segment elevation on electrocardiography 60 min after restoring flow to the culprit vessel (**Figure 2**), or secondary endpoints (biochemical infarct size (**Figure 2**), left ventricular remodeling by echocardiography, or relevant clinical outcomes 6 months after reperfusion. Mean ischemic time in this cohort was approximately 3 h (51). Whether extending ischemia time by 5 min in the CsA treatment arm may have potentially negatively impacted outcomes is unknown.

The inconsistencies in the effects of CsA in the phase II clinical trials are difficult to reconcile. However, a trend may be noted: as seen in the pre-clinical studies, earlier treatment with CsA, such as before ischemia onset (50) or 10 min before reperfusion (47,49) appeared to provide more favorable results than those in which CsA was given 5 min prior to reperfusion (51) or immediately after. **Table 2** summarizes clinical studies with CsA.

PHASE III CLINICAL TRIAL OF CsA IN STEMI

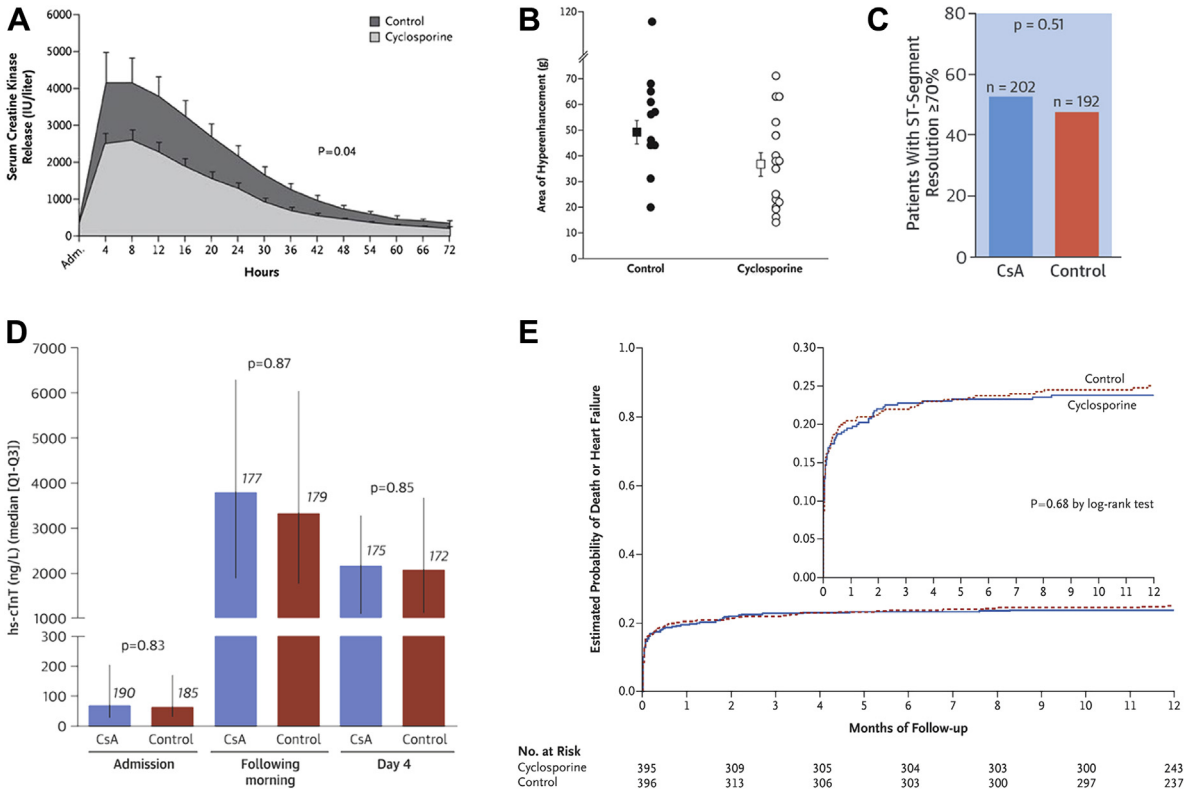
The CIRCUS (Cyclosporine to ImpRove Clinical oUtcome in ST-elevation myocardial infarction patients) trial was a multicenter, double-blinded, randomized phase III clinical trial comparing CsA with placebo in 970 patients presenting with STEMI and angiographically documented occlusion of the left anterior descending artery undergoing percutaneous coronary intervention (PCI) (52). CsA or matched placebo infusion was given "over 2-3 minutes… before the PCI procedure" (52,53). Average ischemic time was approximately 4.5 h in both arms. Again, there was no difference in the composite primary endpoint (all-cause mortality, worsening heart failure during initial hospitalization, rehospitalization for heart failure, or adverse left ventricular remodeling within 1 year of the event) (**Figure 2**) or secondary endpoints (individual components of the composite endpoint, change in left ventricular ejection fraction after 1 year, recurrent AMI, new cerebrovascular accident, or unstable angina). Moreover, CsA failed to reduce ST-segment elevation or reduce infarct size by biochemical criteria (52).

The results of the CIRCUS study are in line with those of the CYCLE phase II trial (51) but inconsistent with those of the first phase II STEMI study (47). The CIRCUS trial used a different formulation of CsA than the original study, but the CYCLE trial used the same formulation of the first pilot study, thus making it unlikely that the formulation affected outcomes. A significant difference in trial designs is evident in the timing of CsA treatment, with "less than 10 minutes prior to direct stenting" in the first phase II study to "in the minutes preceding PCI" in the CIRCUS trial, and approximately 5 min in the CYCLE study, possibly affecting the effectiveness of CsA (52,53). **Figure 3** shows the effects of timing on CsA efficacy in pre-clinical and clinical trials.

TRO40303: AN INHIBITOR OF THE MITOCHONDRIAL PERMEABILITY TRANSITION PORE

A recently explored compound is TRO40303, which acts on the mPTP by binding translocator protein 18 kDa, an outer mitochondrial membrane protein that interacts with proteins implicated in mPTP formation (54). In a pre-clinical study in rats of myocardial ischemia (35 min), TRO40303 2.5 mg/kg given 10 min

FIGURE 2 Results of Clinical Trials With CsA in Acute Myocardial Infarction



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Plasma total CK (IU/L)	Cyclosporine (n=474)	Control (n=495)
Baseline	531 [103; 445]	626 [114; 541]
H4	4183 [1992; 5744]	3920 [1780; 5691]
H12	3191 [1855; 4182]	3220 [1840; 4224]
H24	1765 [1004; 2276]	1837 [1019; 2368]
Peak	3992 [1910; 5447]	3917 [1878; 5608]

ST segment elevation (mm)	Cyclosporine (n=474)	Control (n=495)
Baseline	5.4 ± 2.7	5.4 ± 2.8
60-90 min post-PCI	2.7 ± 1.9	2.7 ± 1.9
Discharge	1.9 ± 1.2	1.8 ± 1.1
One year	0.8 ± 0.9	0.8 ± 0.8

(A, B) Results of a small phase II clinical trial of cyclosporine A (CsA) in ST-segment elevation myocardial infarction (47): CsA led to a small but statistically significant ($p = 0.04$) reduction in infarct size measured as plasma creatine kinase (CK) levels over time and as delayed gadolinium enhancement at cardiac magnetic resonance in a subgroup of patients. **(C, D)** Lack of benefit of CsA in the open-label phase II CYCLE (CYCLosporinE A in Reperfused Acute Myocardial Infarction) trial (51). **(E, F)** Lack of clinical benefit of CsA in the double-blind CIRCUS (Cyclosporine to ImpRove Clinical oUtcome in ST-elevation myocardial infarction patients) clinical trial (52) in terms of clinical outcomes and surrogate endpoints such as plasma CK levels and resolution of ST-segment elevation. hs-cTnT = high-sensitivity cardiac troponin T; IU = international units; PCI = percutaneous coronary intervention; Q = quartile.

TABLE 2 Clinical Trials of Mitochondrial Membrane Permeability Inhibitors in AMI

	Clinical Indication	Inclusion Criteria (Selected)	Dose	Timing	Effects on Infarct Size	Other Notes
Cyclosporine A						
Piot et al., 2008 (47) Single-blind RCT	STEMI N = 58	1) Anterior STEMI 2) TIMI flow grade <1 3) Slated for PCI (primary or rescue)	2.5 mg/kg	"less than 10 minutes" prior to reperfusion	40% reduction in AUC for CK 20% reduction in infarct size by cardiac MRI	A 1-year follow-up in a subcohort found more favorable cardiac remodeling at cardiac MRI
Ghaffari et al., 2013 (48) Double-blind RCT	STEMI N = 101	1) Anterior STEMI 2) Candidate for thrombolytic therapy	2.5 mg/kg	"immediately" prior to reperfusion	No difference in CK-MB or troponin I	No effects on clinical outcomes
Chiari et al., 2014 (49) Single-blind RCT	Scheduled for aortic valve surgery N = 61	1) Age >18 yrs 2) Scheduled for aortic valve surgery	2.5 mg/kg	"less than 10 minutes" prior to aortic unclamping	35% reduction AUC for cardiac troponin I	Beneficial effect remained significant after adjustment for cross-clamping duration
Hausenloy et al., 2014 (50) Double-blind RCT	Referred for elective CABG surgery N = 78	1) Adult 2) Referred for elective CABG surgery	2.5 mg/kg	Prior to cross-clamping of the aorta	38% reduction in AUC for cardiac troponin T	Beneficial effect was optimized in patients with longer ischemic times
CYCLE51 (2016) (51) Open label RCT with PROBE design	STEMI N = 410	1) First STEMI 2) TIMI flow grade <2 3) Slated for PCI 4) Within 4–6 h of onset of chest pain	2.5 mg/kg	"at least 5 min" prior to reperfusion	No difference in ST-segment normalization or cardiac troponin T at day 4	No effects on cardiac remodeling No effects on clinical outcomes
CIRCUS52 (2015) (52) Double-blind RCT	STEMI N = 791	1) Anterior STEMI 2) TIMI flow grade <1 in LAD 3) Slated for PCI	2.5 mg/kg	"prior to PCI"	No difference in peak CK	No effects on clinical outcomes
TRO40303						
MITOCARE56 (2015) (56) Double-blind RCT	STEMI N = 163	1) First STEMI 2) TIMI flow grade <1 3) Slated for PCI	6 mg/kg	"15 min (and preferably 5 min)" prior to reperfusion	No difference in AUC for CK or cardiac troponin I over 3 days	No effects on cardiac remodeling No effects on clinical outcomes
MTP-131						
EMBRACE-STEMI61 (2016) (61) Double-blind RCT	STEMI N = 297	1) First anterior STEMI 2) TIMI flow grade <1 3) Slated for PCI	0.1 mg/kg/h	≥15 min but <60 min and for 1 h following reperfusion	No difference in AUC for CK or cardiac troponin I over 3 days	No effects on cardiac remodeling No effects on clinical outcomes

AUC = area under the curve; CABG = coronary artery bypass grafting; CK = creatine kinase; CK-MB = creatine kinase-myocardial band; LAD = left anterior descending artery; MRI = magnetic resonance imaging; PCI = percutaneous coronary intervention; PROBE = prospective open blinded endpoint; RCT = randomized controlled trial; STEMI = ST-segment elevation myocardial infarction; TIMI = Thrombolysis In Myocardial Infarction.

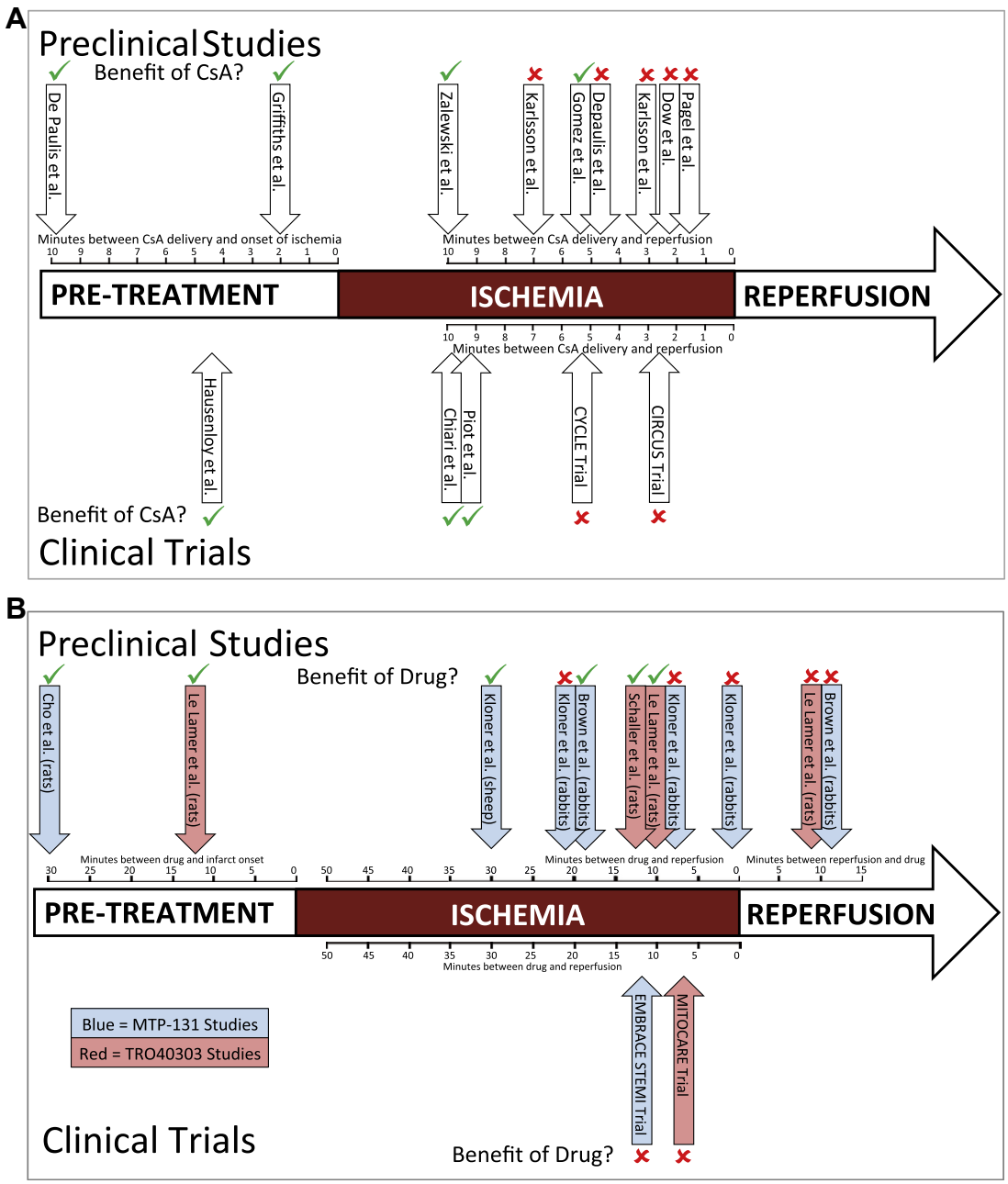
prior to reperfusion significantly reduced infarct size (54). Further work with the rat model demonstrated increased infarct size sparing with higher doses of the agent, which was beneficial if given before ischemia onset or 10 min prior to reperfusion but not if given after reperfusion; the same investigators executed a phase I trial in healthy subjects to obtain pharmacokinetic and favorable safety data as part of the overall translational process (55).

The MITOCARE trial was a proof-of-concept double-blinded study, which investigated TRO40303 6 mg/kg administered after coronary angiography but before balloon inflation during PCI in patients with acute STEMI (56). The exact timing from treatment to reperfusion is not reported, but, given the double-blinded nature of the study, is unlikely to have been significantly delayed, considering the fact that operators would have striven for the earliest possible reperfusion for each subject in case they were randomized to placebo. TRO40303 provided no cardioprotection as measured by biomarker release over 3 days, cardiac magnetic resonance-assessed myocardial salvage index, infarct size, or left ventricular ejection fraction (56). Again, it is reasonable to think this seemingly disconnect between preclinical and clinical trials might have been due to the lack of pretreatment in the latter case, with or without other factors (e.g., differences in equivalent doses between species).

MTP-131: AN INHIBITOR OF THE INTERNAL MITOCHONDRIAL MEMBRANE PERMEABILITY

Another attempt at mitochondrial blockade came recently in the form of MTP-131, 1 of a class of many newly developed small peptides found to target the

FIGURE 3 Timeline in Preclinical and Clinical Trials



Schematic representation of time-dependent effects of **(A)** cyclosporine A (CsA) and **(B)** MTP-131 and TRO40303 in pre-clinical and clinical trials.

internal mitochondrial membrane and reduce ROS production in response to multiple chemical stressors. When infused prior to the onset of ischemia and repeated 5 min prior to reperfusion in an in vivo rat model, infarct size showed a 10% reduction (57); isolated rat heart studies later suggested an infarct-sparing effect when infused at the time of reperfusion after being subjected to 20 min of global ischemia (58). Using this agent, infarct size was also reduced in the sheep model when infused after

TABLE 3 Characteristics of the Ideal Drug for Clinical Translation in Myocardial Reperfusion Injury

	Ideal Drug	Cyclosporine A
Preclinical studies		
MoA	Single known target	Inhibition of mPTP and of calcineurin
Dose-response relationship	Linear response	Inconsistent response, possibly *U*-shaped
Toxicity	Limited or none	Toxic at high doses
Therapeutic index	Large	Narrow
Therapeutic window	Effective when given before, at or after reperfusion	Efficacy appears limited when given <10 min prior to reperfusion
Efficacy across different experimental settings	Exploring longer duration of ischemia and of reperfusion	Limited data
Effects on infarct sparing	Use of at least 2 different independent methodologies	Consistent reduction seen with multiple methods
Effects on cardiac remodeling	Measure of cardiac dimensions and systolic/diastolic function	Preservation of cardiac systolic function
Sufficient length of follow-up	Sufficient to see to full effects on infarct healing and remodeling	Limited data
Validation in 2 or more animal species	Validated in rodents and large animals	Validated in rodents and large animals
Class effect	Validation of the MoA using genetically modified mice or additional drugs	Validation was found in the mice lacking cyclophylin D and with mPTP inhibitors
Efficacy in animals of both sex	Necessary	Limited data
Efficacy in animal models of aging or metabolic impairment	Older animals or models of obesity or diabetes	None available
Clinical studies		
Toxicity in phase I clinical trials	No or limited toxicity	Significant dose-dependent toxicity
Design of phase II clinical trials	Double-blinded, random allocation	Variable design (open label, single blind, double blind), random allocation
Efficacy in phase II clinical trials	Efficacy established on all surrogate endpoints, favorable signal toward reduction of clinical endpoints, no unanticipated adverse events	Discordant results of phase II clinical trials

MoA = mechanism of action; mPTP = mitochondrial permeability transition pore.

ischemia onset (60 min of ischemia, with MTP-131 infusion initiated 30 min prior to reperfusion), but no benefit was seen in the rabbit models (total 30 min of ischemia, with MTP-131 infusion initiated 20 min, 10 min, or 1 min prior to reperfusion) (59). This was contradicted by positive results in later rabbit models where MTP-131 was infused either prior to 180 min of ischemia or 20 min prior to reperfusion (60).

The EMBRACE-STEMI (Evaluation of Myocardial Effects of Bendavia for Reducing Reperfusion Injury in Patients With Acute Coronary Events-ST-Segment Elevation MI) clinical trial was a phase II clinical trial including 297 patients with ST-segment elevation myocardial infarction, designed with a pre-specified per-protocol primary analysis that had strict timing criteria that excluded from the analysis those patients who did not begin the infusion of MTP-131 at least 10 min prior to PCI or who did not receive infusion for at least 45 min after PCI (n = 118). MTP-131 failed to meet its primary endpoint in a reduction in infarct size by biochemical analysis. The reasons for the lack of benefit are not clear; possibly the interval of 10 min prior to PCI was insufficiently long to guarantee full activity of the inhibitor at time of reperfusion. Of note, over 60% of the enrolled and treated subjects were excluded from the primary analysis, most of them due to pre-PCI TIMI flow grade >1 or other exclusion criteria (61).

CsA AND THE "Ideal DRUG" FOR BENCH-TO-BEDSIDE TRANSLATION

The lack of benefit of CsA in the CIRCUS trial has brought into question not only the value of CsA as a therapy for AMI, but the entire process of bench-to-bedside translational research (62) and the value of pilot phase II clinical trials (63). As such, it is worth discussing what constitutes the ideal drug for clinical translation and reviewing whether CsA fits that profile (Table 3).

It is indeed apparent that CsA given as a single dose of 2.5 to 10 mg/kg intravenously 10 to 15 min prior to reperfusion in animal models significantly reduces infarct size, preserving functional myocardium and global systolic function (37,40,43). As such, the phase II pilot study published in 2008 was able to largely reproduce the benefits of CsA in patients with STEMI by administering the drug up to 10 min prior to reperfusion with direct stenting (47). It is worth

considering, once again, the 2 different scenarios that may have occurred in this single-blinded study: 1) direct stenting could have been delayed by 10 min only in the CsA group without delaying reperfusion for any amount of time in the control group (possibly because considered unethical); or a less likely scenario in which 2) direct stenting was delayed also in the control group, which would have introduced harm to the control group while favoring the CsA group.

Pre-clinical studies with CsA in AMI, however, have inconsistently shown a reduction in infarct size and, in particular, have shown a limited effect when CsA is given <10 min prior to reperfusion. In the open-label CYCLE study, CsA treatment was given at least 5 min prior to reperfusion, whereas it is likely that no delay occurred in the "no treatment" arm (a delay in the control arm would be, indeed, difficult to justify ethically) (51). As such, there are 2 differences between the initial phase II study and the CYCLE: 1) a >10-min delay compared with a 5-min delay; and 2) in the initial phase II study there may have been an intentional delay in the control group (favoring the CsA arm), less likely to have occurred in the CYCLE study.

The CIRCUS study, a larger double-blinded phase III study, would not have been able to accommodate a delay in PCI, and hence CsA was given shortly before reperfusion, thus jeopardizing the efficacy of the CsA treatment (on the basis of the data derived from pre-clinical studies). The exquisite dose-dependent and time-dependent efficacy of CsA presents a unique challenge to the performance of a clinical trial. Indeed, having both a 10-min pretreatment or delay to allow for optimal CsA distribution and no delay in the control arm to prevent harm from delaying reperfusion is virtually impossible in a double-blinded clinical trial. As such, an open-label design with PROBE trial endpoint assessment, as in the CYCLE study, but with a clearly specified treatment delay of 10 min in the CsA arm and no delay in the control arm, would be advisable, with the understanding that this model was never tested in pre-clinical studies. In the animal models, CsA was given 10 min before reperfusion, but not at the cost of prolonging ischemia; therefore, both arms had the same duration of ischemia. In the clinical setting, either a shift would be made such that CsA is given before angiography, or it must necessarily prolong ischemia by 10 min in the CsA arm. Therefore, a question that could be tested in animal models would be whether extending ischemia to allow for CsA steady states is superior to immediate reperfusion.

As outlined in **Table 3**, though promising, CsA did not show all the characteristics of an ideal drug for bench-to-bedside clinical translation.

Moreover, in this review we focused on potential explanations for failure to translation for this class of drug addressing specifically what is characteristic of this class and not discussing other factors that are common to the entire field of ischemia-reperfusion injury studies. First, the process of an AMI in humans profoundly differs from that in experimental animals: in pre-clinical settings, ischemia or reperfusion is performed using a specific coronary occluder localized to a specific segment of a coronary artery whereas in humans, the coronary occlusion may occur anywhere in the coronary circulation and may be acute and complete (100%) or gradual and incomplete, or intermittent by undergoing several occlusion or reperfusion episodes which may significantly change the signaling in the myocardium and potentially the response to drug(s). Moreover, the duration of coronary occlusion may vary widely in humans whereas it is always precise in pre-clinical studies. Second, the process of re-establishing coronary blood flow in humans is different than in animals: in the latter, the "complete" occlusion is removed with precision and at a very specific time point after occlusion, and invariably full reperfusion is guaranteed, whereas in humans, the reperfusion is established via medications, guidewires, balloons, or stents. Each of these reperfusion modes can itself limit reperfusion and in some cases embolization with obstruction of the flow downstream, conditions that cannot be reproduced in the pre-clinical study. Third, animal experiments are classically characterized by homogeneity of the cases and, in general, lack of comorbidities, whereas humans with AMI classically have heterogeneous clinical conditions with many comorbidities (i.e., diabetes, hypertension), which may affect signaling and efficacy of the drug(s). Moreover, patients with AMI are generally treated with multiple medications or treatments and the novel strategy is tested on top of the standard of care, whereas in pre-clinical research the novel strategy is generally tested against an inactive treatment or no treatment at all. These and other factors may explain why drugs or treatments that are promising in pre-clinical studies may fail in clinical trials (62).

CONCLUSIONS

Reperfusion injury limits the benefit of reperfusion; thus, prevention of this process is a potentially valuable therapeutic target. Despite some encouraging pre-clinical data with CsA and other

mitochondrial membrane permeability inhibitors, the overall results show inconsistent efficacy of the inhibitors across different animal models and exquisite dose- and time-dependent limitations. The phase II clinical trials with CsA in AMI also showed inconsistent results, possibly related to differences in study design. Finally, a phase III study with CsA given with reperfusion in patients with STEMI failed to provide clinically significant benefits. Similar challenges have been seen with 2 other drugs targeting the mitochondria (TRO40303 and MTP-131). To improve on the ability to translate novel concepts and therapies from bench to bedside, it is necessary that the preclinical studies are designed, conducted, and interpreted with future clinical trials in mind, should those tested interventions show promising results. Clinical relevance of any benefit must always be considered, even in pre-clinical settings. On the other hand, clinical trialists must also carefully review all available pre-clinical data, and that phase III clinical trials should be on the basis of the careful reviewed data obtained from phase II studies, to assure that the larger studies are adequately designed to test the current understanding of any intervention and thus optimize the chances of success for the treatment, and ultimately determine whether the beneficial effects on surrogate endpoints seen in phase II trials translates in a reduction in adverse clinical events in phase III trials. For CsA and other mitochondrial membrane permeability inhibitors, timing of treatment appears to be a fundamental determinant, To what extent this applies to other classes of drugs or treatments aimed at reducing ischemia-reperfusion injury is, however, unknown.

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