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# Postconditioning: from experimental proof to clinical concept

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The therapeutic strategies for acute myocardial infarction in the last decade have, among other therapeutic targets, focused on myocardial reperfusion injury, which accounts for a significant part of the final infarct size. Although several experiments in the last 20 years have reported that pharmacological interventions at reperfusion might reduce myocardial reperfusion injury, this could not be consistently confirmed in animal models or human studies. An alternative to chemical modifiers, postconditioning (brief repeated periods of ischemia applied at the onset of reperfusion) is the first method proven to be efficient in different animal models and to be confirmed in a recent human study. This simple method, applied in the first minute of reperfusion, reduces the final infarct size by 30-50%. This review will focus on the postconditioning technique and show how the data from different animal models and experimental settings have advanced our understanding of both the mechanisms and the definition of an accurate protocol that is easily applicable in human patients in the setting of acute myocardial infarction.

## Introduction

In the last 15 years there has been a significant improvement in the outcome of acute myocardial infarction (AMI) patients. This improvement is mainly based on the introduction of efficient reperfusion strategies, such as primary percutaneous coronary intervention (PCI) and thrombolysis (McGovern et al., 1996). Yet, coronary heart disease remains the leading cause of death in Western countries, with a mortality rate of around 10% at one year for all AMI patients.

This remaining high mortality rate is partly a consequence of the myocardial reperfusion injury phenomenon (Yellon and Hausenloy, 2007). This dynamic and complex phenomenon, originally described by Jennings and Kloner in the seventies on experimental models of myocardial infarction, causes additional functional and structural damage to the acutely reperfused myocardium (Jennings et al., 1960; Kloner et al., 1974). Studies of AMI in animal models suggest that lethal reperfusion injury, which starts immediately after the opening of the culprit coronary artery, accounts for up to 50% of the final size of a myocardial infarct (Yellon and Hausenloy, 2007). In human clinical studies, this phenomenon is associated with detrimental myocardial remodeling, ventricular arrhythmias and no-reflow, and predicts a negative prognosis in myocardial infarction patients (Wu et al., 1998). Thus, lethal reperfusion injury has become a major therapeutic target in the search for improvement in AMI patient recovery and numerous strategies have been tested in experimental settings to reduce reperfusion injury.

Several investigations during the last 20 years reported that pharmacological interventions at reperfusion might reduce myocardial reperfusion injury and thus, the final infarct size. Unfortunately, owing to contradictory results, inconsistent data and a failure to translate the experimental results from in vitro to in vivo models, or from animal to human models, the first attempts to reduce reperfusion injury in humans were disappointing. The perfect example of this situation was the use of calcium channel antagonists at the onset of reperfusion. At reperfusion, there is an intracellular and mitochondrial $Ca^{2+}$ overload, and this excess of $Ca^{2+}$ induces cardiomyocyte death by causing hypercontracture of the heart cells and mitochondrial permeability transition pore (mPTP) opening. The use of calcium channel antagonists at reperfusion reduced this intracellular $Ca^{2+}$ overload and decreased myocardial infarct size significantly in experimental studies (Klein et al., 1989). However, the results of the corresponding clinical study in humans have been negative (Boden et al., 2000).

Vinten-Johansen's group was the first to describe the concept of postconditioning, a simple and efficient method that could significantly reduce infarct size when used during reperfusion. These authors first reported that brief episodes of ischemia, performed just at the onset of reperfusion following a prolonged ischemic insult, dramatically reduced infarct size (Hausenloy and Yellon, 2008; Zhao et al., 2003). Those results have been confirmed in several experimental preparations and in different animal species. These beneficial effects have recently been translated to the clinical setting in AMI patients undergoing PCI, with a significant 36% reduction of final infarct size, regenerating interest in the reperfusion phase as a target for cardioprotection (Staat et al., 2005).

From bringing the myocardial lethal reperfusion injury phenomenon to light to assessing and understanding new therapeutic strategies to limit lethal reperfusion injury in AMI patients, animal models have always been essential. In this Commentary, we will review the different fundamental aspects where animal models have helped in demonstrating, exploring and building the basis for clinical experimentation in the area of myocardial postconditioning.

## Proof of postconditioning concept

After demonstrating that preconditioning using brief, repetitive periods of ischemia protected the myocardium from a subsequent

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longer ischemic insult on experimental models (Murry et al., 1986), the idea of mechanical postconditioning emerged as a potential therapeutic strategy applicable to AMI patients. The initial, experimental proof-of-concept study was developed by Zhao et al. on an ischemia-reperfusion model of open-chest dogs (Zhao et al., 2003). In this first experiment, after 60 minutes of left anterior descending coronary artery (LAD) occlusion, reperfusion was initiated for 30 seconds followed by 30 seconds of reocclusion, which was repeated for three cycles (i.e. the total intervention lasted for 3 minutes). Reperfusion was continued for a total of 3 hours in all experiments. The results of this study showed that the infarct size in the postconditioning group was 44% smaller than that in the control group and that there was no statistical difference in infarct size between the preconditioning and postconditioning groups (Fig. 1).

In this same experimental study, the authors found that postconditioning was associated with a significant reduction of myocardial edema at the myocardial area at risk. Postconditioning reduced the accumulation of polymorphonuclear neutrophils (PMNs) in the same area and attenuated PMN adherence to the endothelium by reducing P-selectin expression on coronary vascular endothelium. Postconditioning also significantly reduced the lipid peroxidation in the myocardial area at risk compared with the control group. All those results showed that postconditioning significantly reduced the reperfusion injury phenomenon.

This observation has since been confirmed in several experimental preparations and with different animal species (rats, rabbits and mice), showing that, when applied at the time of reperfusion, postconditioning significantly reduced infarct size with results comparable to preconditioning (Bopassa et al., 2006; Bopassa et al., 2005; Kin et al., 2005; Kin et al., 2004; Tsang et al., 2004).

At first glance, the underlying mechanisms of cardioprotection seem to be complex and varied. The mechanisms involve activation of the prosurvival kinases phosphoinositide 3-kinase (PI3K)-Akt and endothelial nitric oxide synthase (eNOS) of the reperfusion injury salvage kinase (RISK) pathway (Tsang et al., 2004), interaction with the mPTP (Hausenloy et al., 2002; Bopassa et al., 2005), and activation of the endogenous receptors of adenosine (Kin et al., 2005). These underlying cellular mechanisms of postconditioning were identified using animal models of ischemia-reperfusion that are now leading progress towards direct clinical applications.

## Pathophysiology of lethal reperfusion injury

Lethal reperfusion injury of the myocardium is a complex phenomenon with several etiologies. Most of the detrimental effects of reperfusion are triggered within the first minutes following the re-opening of the occluded coronary artery (Piper et al., 2004). However, most of the cellular disturbances that occur at the time of reperfusion are determined or triggered by the abnormalities induced during the ischemic period. During ischemia, the increase in anaerobic glycolysis results in the progressive accumulation of protons and lactic acid, eventually inhibiting glycolytic flux and synthesis of ATP. As the cardiomyocyte attempts to correct acidosis through the $Na^+/H^+$ exchanger, it consequently becomes loaded with $Na^+$, which cannot be extruded from the cytosol since the $Na^+/K^+$ ATPase progressively fails without sufficient ATP. Secondary activation of the $Na^+/Ca^{2+}$ exchanger, in its reverse mode, helps pump $Na^+$ out of the cell, but favors cytosolic accumulation of $Ca^{2+}$. Prolonged ischemia induces a progressive failure of ionic homeostasis, which ultimately causes accumulation of intracellular $Na^+$ and $Ca^{2+}$, and a decline in ATP levels.

In the first minutes of reperfusion, rapid correction of acidosis through the $Na^+/H^+$ exchanger, the $Na/HCO_3$ symporter, and the washout of lactate causes secondary activation of the $Na^+/Ca^{2+}$ exchanger in the reverse mode and aggravates cytosolic $Ca^{2+}$ accumulation. Abrupt re-exposure of the ischemia-inhibited mitochondrial respiratory chain to oxygen generates a membrane potential to drive ATP synthesis, which leads to a rapid overload of $Ca^{2+}$ in the matrix and massive production of oxygen-derived

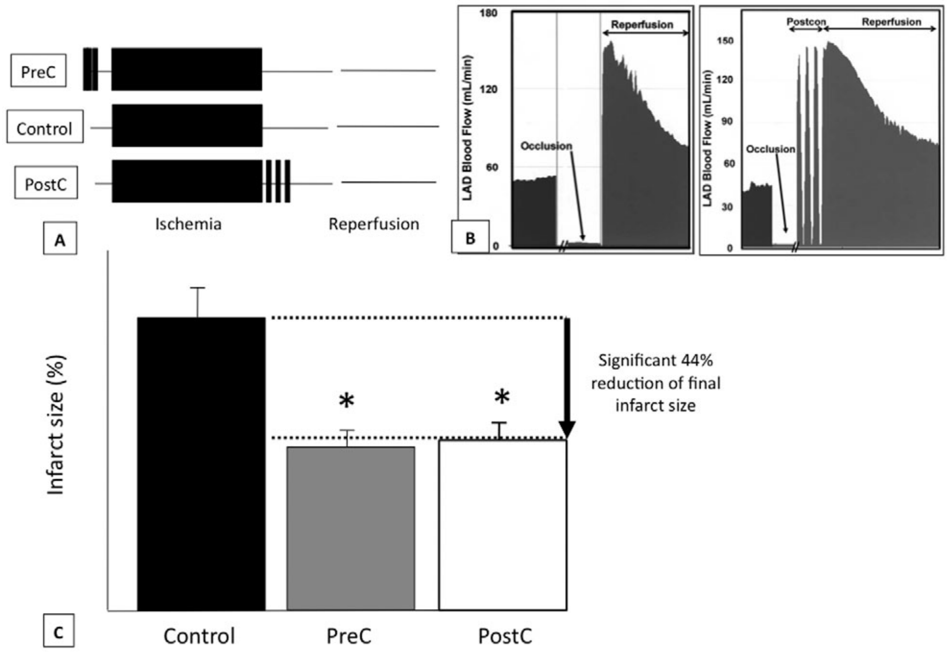

**Fig. 1. Postconditioning: proof of concept.** (A) Experimental protocol used to determine the effect of one possible variation in ischemic postconditioning (PostC) on myocardium after ischemia (I) and reperfusion (R). In the control group there was no intervention; ischemic preconditioning (PreC) was elicited by 5 minutes of ischemia followed by 10 minutes of reperfusion, before 60 minutes of ischemia; ischemic postconditioning was performed by three cycles of 30 seconds of reperfusion followed by 30 seconds of ischemia, before 3 hours of reperfusion. (B) Effect of postconditioning on regional myocardial blood flow compared with the control group and interaction with the hyperemic phase of the first minutes of reperfusion. (C) Determination of infarct size by triphenyltetrazolium chloride (TTC) staining. Postconditioning significantly reduces the final infarct size by 44% compared with the control group, showing equivalent cardioprotection to that of preconditioning; *$P<0.05$ vs control. Values are group means ± standard error (SE).

free radicals. These two factors trigger the mPTP to open (Crompton, 1999).

Under normal physiological conditions, the mitochondrial inner membrane is impermeable to almost all metabolites and ions, and the mPTP is in a closed conformation. Under some stress conditions, the mPTP may open and allow the equilibration of molecules that are smaller than approximately 1500 daltons. Osmotic force generated by matrix proteins results in matrix swelling, leading to further rupture of the outer membrane and the release of proapoptotic factors, such as cytochrome c, into the cytosol. In addition, disruption of the mitochondrial membrane potential also causes the ATP synthase to behave as an ATPase and accelerate energy depletion secondary to the ischemic insult (Halestrap et al., 2004; Kroemer et al., 1998). In the isolated rat heart model, Di Lisa et al. demonstrated that the cytosolic release of $NAD^+$, presented as a surrogate marker of mPTP opening, occurs at the time of reperfusion following a prolonged ischemic insult (Di Lisa et al., 2001). Griffiths and Halestrap used the $[^3H]$2-deoxyglucose ($[^3H]$2-DG) entrapment technique to investigate the kinetics of in situ mPTP opening, and demonstrated that mPTP opening does not happen during ischemia, but occurs within the first 5 minutes of reflow following a 30-minute period of ischemia in the isolated rat heart (Griffiths and Halestrap, 1995). Importantly, the time course of mPTP opening appeared to match the rapid correction of pH that occurs at reperfusion. Recent in vivo studies support this concept by showing that postconditioning may mediate its cardioprotective effects through prolonged transient acidosis during the early reperfusion phase (Cohen et al., 2008).

## Exploring the mechanisms of postconditioning

Numerous experimental studies demonstrate a central role for mitochondria and its mPTP in the process of lethal myocardial reperfusion injury. All these studies have assessed the interaction between the mPTP opening, together with the cascade of cytotoxic events that it induces, and the postconditioning interventions.

In a rabbit model of ischemia-reperfusion, Argaud et al. found that both mechanical postconditioning and chemical postconditioning with cyclosporin A (CsA) or NIM811, a specific inhibitor of the mPTP that was given 1 minute before reperfusion, limit infarct size by at least 45% compared with control animals. They also showed that both mechanical and chemical postconditioning increase the $Ca^{2+}$ load that is required to open the mPTP (Argaud et al., 2005a; Argaud et al., 2005b). Thus, specific inhibition of mPTP opening at reperfusion provided powerful antinecrotic and anti-apoptotic protection to the ischemic myocardium (Fig. 2).

Additional evidence for a major role of the mPTP in lethal reperfusion injury recently came from the use of transgenic mice lacking cyclophilin D (CypD) (Baines et al., 2005; Nakagawa et al., 2005). CypD, which is recognized as a key molecular component of the mPTP, is a mitochondrial member of the family of peptidyl-prolyl cis-trans isomerases (PPIases). Although still debated, it was reported that, in the presence of a high matrix $Ca^{2+}$ concentration, CypD modifies the conformation of the inner membrane proteins to form a mega-channel. The molecular structure of the mPTP remains poorly known and, besides CypD, might involve various proteins including voltage-dependent anion channel (VDAC) or adenine nucleotide translocator (ANT); unfortunately, their precise role is still elusive and no pharmacological agent targeting these proteins is currently available for clinical trials (Leung and Halestrap, 2008; Rasola and Bernardi, 2007). CsA is considered to inhibit mPTP opening by preventing the binding of CypD to the inner membrane. In vivo, CypD-deficient mice develop smaller infarcts after a prolonged coronary artery occlusion followed by reperfusion (Baines et al., 2005; Nakagawa et al., 2005). Recently, Lim et al. reported that CypD-deficient mice cannot be postconditioned, further suggesting that lethal reperfusion injury is mediated by mPTP opening (Lim et al., 2007). These results strongly support the proposal that mPTP opening, triggered by mitochondrial $Ca^{2+}$ overload and overproduction of reactive oxygen species, plays a central role in lethal reperfusion injury.

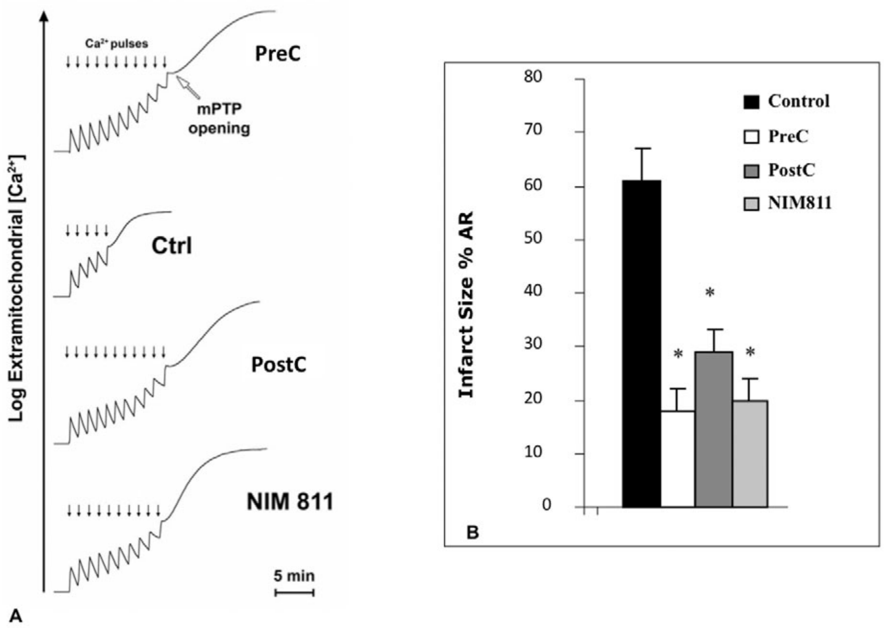

**Fig. 2. Postconditioning inhibits $Ca^{2+}$-induced mPTP opening.** (A) A typical example of $Ca^{2+}$-induced mPTP opening recordings in mitochondria that were isolated from control (Ctrl), preconditioned (PreC) and postconditioned (PostC) animals. mPTP opening was defined as a massive release of $Ca^{2+}$ by mitochondria after a progressive $Ca^{2+}$ overload of the suspension medium. The $Ca^{2+}$ required for mPTP opening was significantly increased compared with animals in the control group. Postconditioning, preconditioning and NIM811 (a nonimmunosuppressive derivative of cyclosporin A that specifically inhibits opening of the mPTP) inhibited mPTP opening equally. (B) Inhibition of the mPTP resulted in a significant reduction of infarct size. The area of necrosis was reduced by a comparable extent in the preconditioned, postconditioned and NIM811 groups. *$P<0.05$ vs control.

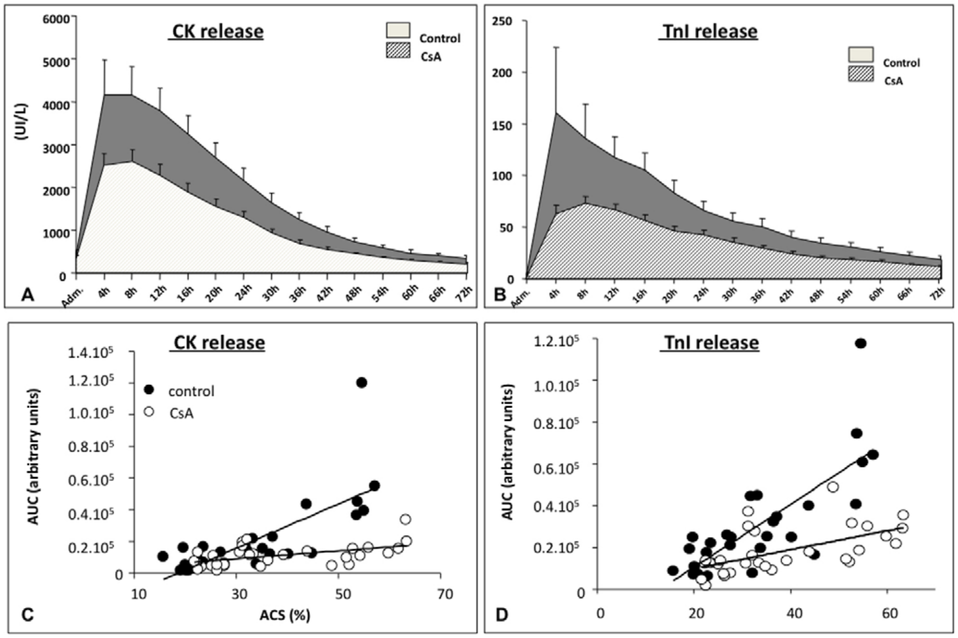

**Fig. 3. Reduction of cardiac enzyme release by CsA in humans.** (A) Serum creatine kinase (CK) was measured every 4 hours on day 1, and every 6 hours on days 2 and 3, after coronary reperfusion. Curves for the control and CsA groups are shown in A. CsA administration (Adm.) resulted in a significant reduction in infarct size of approximately 40%, as measured by creatine kinase release. (B) Serum troponin I (TnI) was measured every 4 hours on day 1, and every 6 hours on days 2 and 3, after coronary reperfusion. Curves for the control and CsA groups are shown in B. CsA administration did not result in a significant reduction in infarct size as measured by troponin I release (the bars denote the SE). (C) The area under the curve (AUC) for serum creatine kinase release was expressed as a function of the circumferential extent of abnormally contracting segments (ACS), an estimate of the area at risk. There was a significant correlation between the two variables in the control group (r2=0.60). Data points for the CsA group (r2=0.34) lie below the regression line for the control group. These data indicate that, for any given area at risk, CsA administration was associated with a reduction in the resulting infarct size, as measured by creatine kinase release. This difference was significant by analysis of covariance (*P*=0.006). (D) There was also a significant correlation between the AUC for troponin I release and the area at risk in the control group (r2=0.54). Data points for the CsA group (r2=0.26) lie below the regression line for the control group. These data indicate that, for any given area at risk, CsA administration was associated with a reduction in the resulting infarct size, as measured by troponin I release. This difference was confirmed to be significant by analysis of covariance (*P*=0.002).

Hausenloy et al. first reported that CsA, given at the time of reperfusion, could limit infarct size in the isolated rat heart (Hausenloy et al., 2002). Furthermore, Argaud et al. reported that the mPTP of mitochondria that were isolated from the risk region of postconditioned hearts displayed an enhanced resistance to $Ca^{2+}$ overload (Argaud et al., 2005a). This pattern of inhibition of mPTP opening by postconditioning was very similar to that observed in hearts treated with the mPTP inhibitor NIM811 at the onset of reperfusion, as well as to that of preconditioned rabbits.

## Developing practical aspects of postconditioning

Work in animal models set the therapeutic time frame for postconditioning. The mPTP opening triggered by the $Ca^{2+}$ overload occurs in the first minute following reperfusion, and postconditioning strategies need to be applied at this crucial time. During the whole ischemic period, the mPTP remains closed and preconditioning does not influence its opening (Argaud et al., 2008). In a rabbit model of infarction, postconditioning cycles that were initiated 10 minutes after the onset of reperfusion no longer offered protection against injury (Yang et al., 2004). This was confirmed in another rat model of infarction, where postconditioning that was delayed until one minute after the onset of reperfusion did not have a significant effect on the final infarct size when compared with controls (Kin et al., 2004). In rat and rabbit models of myocardial ischemia-reperfusion, infarct size was optimally reduced by three cycles of mechanical postconditioning that were applied during the first minute of reperfusion, and extension of the postconditioning algorithm to six cycles over the first two minutes of reperfusion did not further reduce the infarct size (Kin et al., 2004; Yang et al., 2004). This suggests that, in a clinical setting, the postconditioning procedure must be applied very shortly after the onset of reperfusion and that a postconditioning algorithm of four cycles should be sufficient in patients.

## Translation to patients

Ischemic postconditioning is an unmet opportunity to determine whether lethal reperfusion injury might exist in the human heart, and whether it might represent a new therapeutic target to further decrease infarct size and improve clinical outcome. Between 2004 and 2007, we performed three small Phase II clinical trials aimed at demonstrating: (1) whether ischemic postconditioning could reduce infarct size and improve myocardial functional recovery several months after PCI, and (2) whether pharmacological inhibition of mPTP opening by the commercially available mPTP inhibitor CsA could represent a pharmacological alternative to ischemic postconditioning in AMI patients. We found that PCI postconditioning reduced infarct size by 30-40%, and that this protection persisted for 6 months post-AMI, with a significant improvement in contractile function continuing for 1 year after infarction (Staat et al., 2005; Thibault et al., 2008). More recently, we reported that CsA attenuates infarct size, as indicated by cardiac enzyme release during the first 3 days of reperfusion and by magnetic resonance imaging (MRI) at day 5 after reflow (Fig. 3) (Piot et al., 2008).

All of the positive results in the small Phase II trials have applied, and confirmed, the findings of the numerous experimental animal studies. However, the widespread use of these cardioprotective therapies in humans requires a demonstration showing that their application to a large number of patients improves clinical outcomes.

## Potential limitations of postconditioning in the clinical setting

The principal limitation of the mechanical postconditioning technique is that it can only be applied in the setting of primary PCI. In patients where reperfusion is applied and obtained by pharmacological thrombolysis, mechanical postconditioning cannot be performed.

Another limitation to the mechanical postconditioning procedure is the recent emergence of the thromboaspiration technique in the primary PCI strategy. This technique consists of aspirating the culprit coronary thrombus with a specific dedicated catheter just before coronary reopening with balloon dilation and stenting. Thromboaspiration has recently been shown to improve both the final myocardial blood flow and the 1-year clinical outcome in patients with AMI compared with conventional treatment (Svilaas et al., 2008; Vlaar et al., 2008). There is no published evidence that this technique might interfere with the mechanical postconditioning, yet the thromboaspiration procedure usually re-establishes a significant blood flow in the culprit coronary artery, and the delay between applying thromboaspiration and initiating the postconditioning ischemia-reperfusion cycle might exceed the very short time frame in which postconditioning has been shown to be efficient. Further experimental and clinical studies need to explore this question. However, pharmacological postconditioning does not suffer from those limitations, and could be applied with either thrombolysis or thromboaspiration without any evident interference. This still needs to be assessed in further clinical studies.

## Conclusion

Animal models of ischemia-reperfusion have allowed us to explore the mechanisms of postconditioning and to develop a practical, applicable and, above all, successful reperfusion strategy to our patients. Postconditioning might be the next therapy to improve cardiovascular outcome in AMI after primary PCI, although this has to be confirmed in larger clinical trials. In the future, animal models will be essential to improve the practical aspects of the different postconditioning techniques, to explore new potential therapeutic targets, and to broaden our therapies to new groups of ischemic patients.

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### COMPETING INTERESTS

The authors declare no competing financial interests.

### REFERENCES

**Argaud. L., Gateau-Roesch, O., Raisky, O., Loufouat, J., Robert, D. and Ovize, M.** (2005a). Postconditioning inhibits mitochondrial permeability transition. *Circulation* **111**, 194-197.

**Argaud, L., Gomez, L., Gateau-Roesch, O., Couture-Lepetit, E., Loufouat, J., Robert, D. and Ovize, M.** (2005b). Trimetazidine inhibits mitochondrial permeability transition pore opening and prevents lethal ischemia-reperfusion injury. *J. Mol. Cell Cardiol*. **39**, 893-899.

**Argaud, L., Loufouat, J., Gateau-Roesch, O., Gomez, L., Robert, D. and Ovize, M.** (2008). Persistent inhibition of mitochondrial permeability transition by preconditioning during the first hours of reperfusion. *Shock* **30**, 552-556.

**Baines, C. P., Kaiser, R. A., Purcell, N. H., Blair, N. S., Osinska, H., Hambleton, M. A., Brunskill, E. W., Sayen, M. R., Gottlieb, R. A., Dorn, G. W. et al.** (2005). Loss of cyclophilin D reveals a critical role for mitochondrial permeability transition in cell death. *Nature* **434**, 658-662.

**Boden, W. E., van Gilst, W. H., Scheldewaert, R. G., Starkey, I. R., Carlier, M. F., Julian, D. G., Whitehead, A., Bertrand, M. E., Col, J. J., Pedersen, O. L. et al.** (2000). Diltiazem in acute myocardial infarction treated with thrombolytic agents: a randomised placebo-controlled trial. Incomplete infarction trial of European research collaborators evaluating prognosis post-thrombolysis (INTERCEPT). *Lancet* **355**, 1751-1756.

**Bopassa, J. C., Michel, P., Gateau-Roesch, O., Ovize, M. and Ferrera, R.** (2005). Low-pressure reperfusion alters mitochondrial permeability transition. *Am. J. Physiol. Heart Circ. Physiol*. **288**, H2750-H2755.

**Bopassa, J. C., Ferrera. R., Gateau-Roesch, O., Couture-Lepetit, E. and Ovize, M.** (2006). PI 3-kinase regulates the mitochondrial transition pore in controlled reperfusion and postconditioning. *Cardiovasc. Res*. **69**, 178-185.

**Cohen, M. V., Yang, X. M. and Downey, J. M.** (2008). Acidosis., oxygen., and interference with mitochondrial permeability transition pore formation in the early minutes of reperfusion are critical to postconditioning's success. *Basic Res. Cardiol*. **103**, 464-471.

**Crompton, M.** (1999). The mitochondrial permeability transition pore and its role in cell death. *Biochem. J*. **341**, 233-249.

**Di Lisa, F., Menabo, R., Canton, M., Barile, M. and Bernardi, P.** (2001). Opening of the mitochondrial permeability transition pore causes depletion of mitochondrial and cytosolic NAD+ and is a causative event in the death of myocytes in postischemic reperfusion of the heart. *J. Biol. Chem*. **276**, 2571-2575.

**Griffiths, E. J. and Halestrap, A. P.** (1995). Mitochondrial non-specific pores remain closed during cardiac ischaemia., but open upon reperfusion. *Biochem. J*. **307**, 93-98.

**Halestrap, A. P., Clarke, S. J. and Javadov, S. A.** (2004). Mitochondrial permeability transition pore opening during myocardial reperfusion – a target for cardioprotection. *Cardiovasc. Res*. **61**, 372-385.

**Hausenloy, D. J. and Yellon, D. M.** (2008). Preconditioning and postconditioning: Underlying mechanisms and clinical application. *Atherosclerosis* **204**, 334-341.

**Hausenloy, D. J., Maddock, H. L., Baxter, G. F. and Yellon, D. M.** (2002). Inhibiting mitochondrial permeability transition pore opening: a new paradigm for myocardial preconditioning? *Cardiovasc. Res*. **55**, 534-543.

**Jennings, R. B., Sommers, H. M., Smyth, G. A., Flack, H. A. and Linn, H.** (1960). Myocardial necrosis induced by temporary occlusion of a coronary artery in the dog. *Arch. Pathol*. **70**, 68-78.

**Kin, H., Zhao, Z. Q., Sun, H. Y., Wang, N. P., Corvera, J. S., Halkos, M. E., Kerendi, F., Guyton, R. A. and Vinten-Johansen, J.** (2004). Postconditioning attenuates myocardial ischemia-reperfusion injury by inhibiting events in the early minutes of reperfusion. *Cardiovasc. Res*. **62**, 74-85.

**Kin, H., Zatta, A. J., Lofye, M. T., Amerson, B. S., Halkos, M. E., Kerendi, F., Zhao, Z. Q., Guyton, R. A., Headrick, J. P. and Vinten-Johansen, J.** (2005). Postconditioning reduces infarct size via adenosine receptor activation by endogenous adenosine. *Cardiovasc. Res*. **67**, 124-133.

**Klein, H. H., Pich, S., Lindert, S., Nebendahl, K., Warneke, G. and Kreuzer, H.** (1989). Treatment of reperfusion injury with intracoronary calcium channel antagonists and reduced coronary free calcium concentration in regionally ischemic, reperfused porcine hearts. *J. Am. Coll. Cardiol*. **13**, 1395-1401.

**Kloner, R. A., Ganote, C. E. and Jennings, R. B.** (1974). The "no-reflow" phenomenon after temporary coronary occlusion in the dog. *J. Clin. Invest*. **54**, 1496-1508.

**Kroemer, G., Dallaporta, B. and Resche-Rigon, M.** (1998). The mitochondrial death/life regulator in apoptosis and necrosis. *Annu. Rev. Physiol*. **60**, 619-642.

**Leung, A. W. and Halestrap, A. P.** (2008). Recent progress in elucidating the molecular mechanism of the mitochondrial permeability transition pore. *Biochim. Biophys. Acta.* **1777**, 946-952.

**Lim, S. Y., Davidson, S. M., Hausenloy, D. J. and Yellon, D. M.** (2007). Preconditioning and postconditioning: the essential role of the mitochondrial permeability transition pore. *Cardiovasc. Res*. **75**, 530-535.

**McGovern, P. G., Pankow, J. S., Shahar, E., Doliszny, K. M., Folsom, A. R., Blackburn, H. and Luepker, R. V.** (1996). Recent trends in acute coronary heart disease-mortality, morbidity, medical care, and risk factors. The Minnesota Heart Survey Investigators. *N. Engl. J. Med*. **334**, 884-890.

**Murry, C. E., Jennings, R. B. and Reimer, K. A.** (1986). Preconditioning with ischemia: a delay of lethal cell injury in ischemic myocardium. *Circulation* **74**, 1124-1136.

**Nakagawa, T., Shimizu, S., Watanabe, T., Yamaguchi, O., Otsu, K., Yamagata, H., Inohara, H., Kubo, T. and Tsujimoto, Y.** (2005). Cyclophilin D-dependent mitochondrial permeability transition regulates some necrotic but not apoptotic cell death. *Nature* **434**, 652-658.

**Piot, C., Croisille, P., Staat, P., Thibault, H., Rioufol, G., Mewton, N., Elbelghiti, R., Cung, T. T., Bonnefoy, E., Angoulvant, D. et al.** (2008). Effect of cyclosporine on reperfusion injury in acute myocardial infarction. *N. Engl. J. Med*. **359**, 473-481.

**Piper, H. M., Abdallah, Y. and Schafer, C.** (2004). The first minutes of reperfusion: a window of opportunity for cardioprotection. *Cardiovasc. Res*. **61**, 365-371.

**Rasola, A. and Bernardi, P.** (2007). The mitochondrial permeability transition pore and its involvement in cell death and in disease pathogenesis. *Apoptosis* **12**, 815-833.

**Staat, P., Rioufol, G., Piot, C., Cottin, Y., Cung, T. T., L'Huillier, I., Aupetit, J. F., Bonnefoy, E., Finet, G., André-Fouët, X. et al.** (2005). Postconditioning the human heart. *Circulation* **112**, 2143-2148.

**Svilaas, T., Vlaar, P. J., van der Horst, I. C., Diercks, G. F., de Smet, B. J., van den Heuvel, A. F., Anthonio, R. L., Jessurun, G. A., Tan, E. S., Suurmeijer, A. J. et al.** (2008). Thrombus aspiration during primary percutaneous coronary intervention. *N. Engl. J. Med*. **358**, 557-567.

**Thibault, H., Piot, C., Staat, P., Bontemps, L., Sportouch, C., Rioufol, G., Cung, T. T., Bonnefoy, E., Angoulvant, D., Aupetit, J. F. et al.** (2008). Long-term benefit of postconditioning. *Circulation* **117**, 1037-1044.

**Tsang, A., Hausenloy, D. J., Mocanu, M. M. and Yellon, D. M.** (2004). Postconditioning: a form of "modified reperfusion" protects the myocardium by activating the phosphatidylinositol 3-kinase-Akt pathway. *Circ. Res*. **95**, 230-232.

**Vlaar, P. J., Svilaas, T., van der Horst, I. C., Diercks, G. F., Fokkema, M. L., de Smet, B. J., van den Heuvel, A. F., Anthonio, R. L., Jessurun, G. A., Tan, E. S. et al.** (2008). Cardiac death and reinfarction after 1 year in the Thrombus Aspiration during Percutaneous coronary intervention in Acute myocardial infarction Study (TAPAS): a 1-year follow-up study. *Lancet* **371**, 1915-1920.

**Wu, K. C., Zerhouni, E. A., Judd, R. M., Lugo-Olivieri, C. H., Barouch, L. A., Schulman, S. P., Blumenthal, R. S. and Lima, J. A.** (1998). Prognostic significance of microvascular obstruction by magnetic resonance imaging in patients with acute myocardial infarction. *Circulation* **97**, 765-772.

**Yang, X. M., Proctor, J. B., Cui, L., Krieg, T., Downey, J. M. and Cohen, M. V.** (2004). Multiple, brief coronary occlusions during early reperfusion protect rabbit hearts by targeting cell signaling pathways. *J. Am. Coll. Cardiol*. **44**, 1103-1110.

**Yellon, D. M. and Hausenloy, D. J.** (2007). Myocardial reperfusion injury. *N. Engl. J. Med*. **357**, 1121-1135.

**Zhao, Z. Q., Corvera, J. S., Halkos, M. E., Kerendi, F., Wang, N. P., Guyton, R. A. and Vinten-Johansen, J.** (2003). Inhibition of myocardial injury by ischemic postconditioning during reperfusion: comparison with ischemic preconditioning. *Am. J. Physiol. Heart Circ. Physiol.* **285**, H579-H588.