Reperfusion Injury: How Can We Reduce It?

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Acute Myocardial Infarction

Rupture of atheromatous plaque in the coronary artery
Platelet aggregation and thrombus formation
Occlusion of the coronary artery

Time is Muscle!
Time is Myocardium!

- Treatment flow for STEMI

Early reperfusion therapy
  - the first priority in the treatment of STEMI
  - thrombolytic therapy
  - Percutaneous coronary intervention (PCI)
Reperfusion immediately causes myocyte necrosis and sarcolemmal disruption, with the leakage of cell contents into the extracellular space.

**Apoptosis** is a coordinated involution of myocytes that circumvents the inflammation associated with **necrotic cell death**. Because apoptosis is an energy-dependent process, cells can be forced to switch to a necrotic pathway if energy levels are depleted below critical levels.

In more chronic settings, autophagy can contribute to the mechanisms of myocyte death.
Myocardial ischemia in absence of reperfusion
Infarct size, 70%

Myocardial ischemia with reperfusion
Reperfusion reduces infarct size by 40%
Part of the remaining 30% infarct is due to lethal reperfusion injury and is therefore preventable

Myocardial ischemia with reperfusion and cardioprotection
Preventing lethal reperfusion injury reduces infarct size by a further 25%, realizing the full benefits of reperfusion
Ischemia-reperfusion injury

1. Oxygen paradox
2. Calcium paradox
3. pH paradox

4. Inflammation due to necrosis

Mitochondrial Permeability Transition Pore (mPTP) opening
[ISCHEMIA]

Oxygen and glucose depletion

ATP depletion, anaerobic glycolysis↑

pH drop. $[Na^+]↑$, $[Ca^{2+}]↑$

Mitochondria $[Ca^{2+}]$ overload

[REPERFUSION]

Reintroduction of oxygen
Rapid correction of acidosis

Burst of reactive oxygen species $Ca^{2+}$ influx

mPTP opening

Mitochondria matrix swelling, Outer mitochondrial membrane rupture
*Hallmark of necrosis

Necrotic cell death
Ischemic conditioning to prevent I/R injury

The 30-year anniversary of the discovery of “ischemic preconditioning”

Preconditioning with ischemia: a delay of lethal cell injury in ischemic myocardium

CHARLES E. MURRY, B.S., ROBERT B. JENNINGS, M.D., AND KEITH A. REIMER, M.D., PH.D.

ABSTRACT We have previously shown that a brief episode of ischemia slows the rate of ATP depletion during subsequent ischemic episodes. Additionally, intermittent reperfusion may be beneficial to the myocardium by washing out catabolites that have accumulated during ischemia. Thus, we proposed that multiple brief ischemic episodes might actually protect the heart from a subsequent sustained ischemic insult. To test this hypothesis, two sets of experiments were performed. In the first set, one group of dogs (n = 7) was preconditioned with four 5 min circumflex occlusions, each separated by 5 min of reperfusion, followed by a sustained 40 min occlusion. The control group (n = 5) received a single 40 min occlusion. In the second study, an identical preconditioning protocol was followed, and animals (n = 9) then received a sustained 3 hr occlusion. Control animals (n = 7) received a single 3 hr occlusion. Animals were allowed 4 days of reperfusion thereafter. Histologic infarct size then was measured and was related to the major baseline predictors of infarct size, including the anatomic area at risk and collateral blood flow. In the 40 min study, preconditioning with ischemia paradoxically limited infarct size to 25% of that seen in the control group (p < .001). Collateral blood flows were not significantly different in the two groups. In the 3 hr study, there was no difference between infarct size in the preconditioned and control groups. The protective effect of preconditioning in the 40 min study may have been due to reduced ATP depletion and/or to reduced catabolite accumulation during the sustained occlusion. These results suggest that the multiple anginal episodes that often precede myocardial infarction in man may delay cell death after coronary occlusion, and thereby allow for greater salvage of myocardium through reperfusion therapy. Circulation 74, No. 5, 1124–1136, 1986.
1) Reperfusion Injury Salvage Kinase (RISK) pathway (PI3K–Akt and Mek1/2–Erk1/2)
2) Survivor Activator Factor Enhancement (SAFE) pathway (TNF and JAK–STAT),
3) cGMP–protein kinase G (PKG) pathway

\[\text{inhibitory effect on mitochondrial permeability transition pore (mPTP) opening}\]

Ischemic conditioning to prevent I/R injury

*Clinical implication
1) Ischemic *in situ* conditioning & post-conditioning
2) Pharmacological cardioprotection

Clinical results of post-conditioning (STEMI)

<table>
<thead>
<tr>
<th>Study</th>
<th>n</th>
<th>Patient selection</th>
<th>IPost protocol</th>
<th>Main outcome</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive studies</td>
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<tr>
<td>Staat et al. (2005)</td>
<td>30</td>
<td>• LAD/RCA only</td>
<td>• 4 × 1 min inflations and deflations of angioplasty balloon upstream of stent</td>
<td>• 36% reduction in MI size (72 h AUC CK)</td>
<td>• First clinical study to translate IPost into clinical setting</td>
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<tr>
<td></td>
<td></td>
<td>• ≤6 h ischaemic time</td>
<td></td>
<td>• Better blush grade</td>
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<tr>
<td></td>
<td></td>
<td>• TIMI 0 pre-PPCI</td>
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<td></td>
<td></td>
<td>• TIMI 2–3 post-PPCI</td>
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<tr>
<td></td>
<td></td>
<td>• No collaterals</td>
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<td></td>
<td></td>
<td>• No angina in 48 h</td>
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<tr>
<td>Ma et al. (2006)</td>
<td>94</td>
<td>• All STEMI</td>
<td>• 3 × 0.5 min inflations and deflations of angioplasty balloon</td>
<td>• 27% and 32% reductions in MI size (peak CK and CK–MB)</td>
<td>• This study showed an alternative IPost protocol to be effective</td>
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<tr>
<td></td>
<td></td>
<td>• ≤12 h ischaemic time</td>
<td></td>
<td>• Better TIMI flow, WMSI, and endothelial function</td>
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<td></td>
<td></td>
<td>• TIMI 3 post-PPCI</td>
<td></td>
<td>• Less malondialdehyde</td>
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<tr>
<td>Yang et al. (2007)</td>
<td>41</td>
<td>• All STEMI</td>
<td>• 3 × 0.5 min inflations and deflations of angioplasty balloon</td>
<td>• 27% reduction in MI size (72 h AUC CK)</td>
<td>• First clinical study to demonstrate MI size reduction on SPECT</td>
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<tr>
<td></td>
<td></td>
<td>• ≤12 h ischaemic time</td>
<td></td>
<td>• 27% reduction in MI size (SPECT at 1 week)</td>
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<tr>
<td></td>
<td></td>
<td>• TIMI 0–1 pre-PPCI</td>
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<tr>
<td></td>
<td></td>
<td>• No collaterals</td>
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<tr>
<td>Thibault et al.</td>
<td>38</td>
<td>• LAD/RCA only</td>
<td>• 4 × 1 min inflations and deflations of angioplasty balloon upstream of stent</td>
<td>• 40% and 47% reductions in MI size (72 h AUC CK and troponin I)</td>
<td>• First clinical study to demonstrate long-term benefit with IPost</td>
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<tr>
<td>(2008)</td>
<td></td>
<td>• ≤6 h ischaemic time</td>
<td></td>
<td>• 39% reduction in MI size (SPECT at 6 months)</td>
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<td></td>
<td></td>
<td>• TIMI 0 pre-PPCI</td>
<td></td>
<td>• 7% increase in LVEF (echo at 1 year)</td>
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<td>• TIMI 2–3 post-PPCI</td>
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<td>Lonborg et al. (2010)</td>
<td>118</td>
<td>• All STEMI</td>
<td>• 4 × 0.5 min inflations and deflations of angioplasty balloon within the stent</td>
<td>• 31% increase in myocardial salvage ratio</td>
<td>• First clinical study to demonstrate MI size reduction on MRI</td>
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<td></td>
<td></td>
<td>• ≤12 h ischaemic time</td>
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<td>• 19% relative reduction in MI size (MRI at 3 months)</td>
<td>• Largest positive study to date</td>
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<td></td>
<td></td>
<td>• TIMI 0–1 pre-PPCI</td>
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<td>• 41% reduction in patients developing heart failure</td>
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<td><strong>Neutral or negative studies</strong></td>
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<td>Dwyer et al. (2013)</td>
<td>102</td>
<td>• All STEMI&lt;br&gt;• &lt;6 h ischaemic time&lt;br&gt;• TIMI 0–1 pre-PPCI&lt;br&gt;• No collaterals</td>
<td>• 4 × 0.5 min inflations and deflations of angioplasty balloon at site of lesion</td>
<td>• No difference in myocardial salvage or MI size (MRI at day 3)</td>
<td>• First neutral study using an alternative IPost protocol</td>
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<tr>
<td>Hahn et al. (2014) POST</td>
<td>700</td>
<td>• All STEMI&lt;br&gt;• &lt;12 h ischaemic time&lt;br&gt;• TIMI 0–1 pre-PPCI</td>
<td>• 4 × 1 min inflations and deflations of angioplasty balloon at site of lesion</td>
<td>• No difference in ST-segment resolution, myocardial blush grade, peak CK–MB levels or MACE (death, MI, severe heart failure, or stent thrombosis)&lt;br&gt;• No difference in MI size or myocardial salvage (MRI at day 3) in substudy of 111 patients</td>
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<td>Eitel et al. (2015) LIPSIA</td>
<td>333</td>
<td>• All STEMI</td>
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<td>• Improved myocardial salvage when IPost combined with RIC</td>
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| **Ongoing studies**          |     |                                                                                    |                                                                                 |                                                                                                   |                                                                                                |
| DANAMI 3                     | 1,252 | • All STEMI<br>• <12 h ischaemic time<br>• TIMI 0–1 pre-PPCI                       | • 4 × 0.5 min inflations and deflations of angioplasty balloon at site of lesion | • Primary outcome is all-cause death and heart failure at 2 years                                 | • Recruitment complete<br>• Currently in follow-up; results available early 2016               |

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**ACC 2016, DANish-iPOST (DANAMI 3-iPOST):** DANish Study of Optimal Acute Treatment of Patients with ST-segment Elevation Myocardial Infarction: iPOST conditioning during primary PCI, Denmark

• Results: Ischemic post-conditioning after STEMI did not result in a reduction in death rates or HF hospitalization.
Objective: determine whether cyclosporine A can reduce infarct size in STEMI patients

**Cyclosporine A (or saline)**

- **(2.5 mg/kg, IV bolus)**

**Direct stenting**

- **t₀:** Direct stenting
- **t₋10 min:** Time before stenting

**Coronary artery occlusion**

**Reperfusion**

**Day 1-3:**
- CK / TnI release
- STEMI < 12 hrs
- PCI treatment
- LAD TIMI flow grade 0-1
- No visible collaterals

**Day 5:**
- MRI

**Infarct size**
Objective: determine whether cyclosporine A can reduce infarct size in STEMI patients

Direct stenting
Coronary artery occlusion
Reperfusion

**Objective:**

Effect of Cyclosporine on Reperfusion Injury in Acute Myocardial Infarction

Christophe Piot, M.D., Pierre Crosillé, M.D., Patrick Strat, M.D., Hélène Thibault, M.D., Gilles Roucou, M.D., Ph.D., Nathan Newton, M.D., Rachid Elbelghiti, M.D., Thierry Tri Cong, M.D., Eric Bonnery, M.D., Ph.D., Denis Angoulvant, M.D., Christophe Maccia, M.D., Franck Racza, M.D., Catherine Spottouch, M.D., Gérard Gahide, M.D., Gérard Tinet, M.D., Ph.D., Xavier André-Fouet, M.D., Didier Revel, M.D., Ph.D., Gilbert Kirkorian, M.D., Ph.D., Jean-Pierre Monassier, M.D., Génévieve Derenux, M.D., Ph.D., and Michel Ovize, M.D., Ph.D.

**Abstract**

**Background**

Experimental evidence suggests that cyclosporine, which inhibits the opening of mitochondrial permeability-transition pores, attenuates lethal myocardial injury that occurs at the time of reperfusion. In this pilot trial, we sought to determine whether the administration of cyclosporine at the time of percutaneous coronary intervention (PCI) would limit the size of the infarct during acute myocardial infarction.

**Methods**

We randomly assigned 82 patients who presented with acute ST-segment elevation myocardial infarction to receive either an intravenous bolus of 2.5 mg per kilogram of body weight cyclosporine group or normal saline (control group) immediately before undergoing PCI. Infarct size was assessed in all patients by measuring the release of creatine kinase and troponin I and in a subgroup of 27 patients by performing magnetic resonance imaging (MRI) on day 5 after infarction.

**Results**

The cyclosporine and control groups were similar with respect to ischemia time, the size of the area at risk, and the ejection fraction before PCI. The release of creatine kinase was significantly reduced in the cyclosporine group as compared with the control group (P = 0.04). The release of troponin I was not significantly reduced (P = 0.15). On day 5, the absolute mass of the area of hyperenhancement (i.e., infarcted area) on MRI was significantly reduced in the cyclosporine group as compared with the control group, with a median of 37 g (interquartile range, 21 to 51) versus 46 g (interquartile range, 20 to 65; P = 0.04). No adverse effects of cyclosporine administration were detected.

**Conclusion**

In our small, pilot trial, administration of cyclosporine at the time of reperfusion was associated with a smaller infarct by some measures than that seen with placebo. These data are preliminary and require confirmation in a larger clinical trial.

STEMI < 12 hrs
PCI treatment
AD TIMI flow grade 0-1
No visible collaterals

Day 1-3
CK / TnI release

Infarct size

Day 5
MRI

Coronary artery obstruction
Reduction of cardiac enzymes release by cyclosporine A
Piot et al. NEJM 2008

CK release

TnI release

Reduction of cardiac enzymes release by cyclosporine A
Piot et al. NEJM 2008
Clinical results of pharmacological cardioprotection

Cyclosporine before PCI in Patients with Acute Myocardial Infarction


CONCLUSIONS

In patients with anterior STEMI who had been referred for primary PCI, intravenous cyclosporine did not result in better clinical outcomes than those with placebo and did not prevent adverse left ventricular remodeling at 1 year. (Funded by the French Ministry of Health and NeuroVive Pharmaceutical; CIRCUS ClinicalTrials.gov number, NCT01502774; EudraCT number, 2009-013713-99.)

Cyclosporine A in Reperfused Myocardial Infarction

Filippo Ottani, MD,1 Roberto Latini, MD,1 Lidia Stasiewsky, MD,2 Luigi La Vecchia, MD,2 Nicola Locuratolo, MD,2 Marco Sicuro, MD,2 Serge Masson, PdT,3 Simona Barlera, MS,2 Valentina Milanzi, PdT,4 Mario Lombardi, MD,2 Alessandra Costantini, MD,2 Nadia Mollicelli, MD,1 Andrea Santarelli, MD,1 Nicoletta De Cesare, MD,1 Paolo Spanzerla, MD,1 Alberto Boi, MD,1 Aldo Pietro Maggioni, MD,1 Ugo Limbruno, MD,1 on behalf of the CYCLE Investigators

CONCLUSIONS

In the CYCLE (CYClOsporinE A in Reperfused Acute Myocardial Infarction) trial, a single intravenous CsA bolus just before primary percutaneous coronary intervention had no effect on ST-segment resolution or hs-cTnT, and did not improve clinical outcomes or LV remodeling up to 6 months. (CYClOsporinE A in Reperfused Acute Myocardial Infarction [CYCLE]; NCT01650662; EudraCT number 2011-002876-18) (J Am Coll Cardiol 2016;67:365-74)
Targeting Myocardial IR Injury — The Search Continues

Cardioprotective Effects of Exenatide in Patients With ST-Segment–Elevation Myocardial Infarction Undergoing Primary Percutaneous Coronary Intervention

Results of Exenatide Myocardial Protection in Revascularization Study

Jong Shin Woo, Weon Kim, Sang Jin Ha, Jin Bae Kim, Soo-Joong Kim, Woo-Shik Kim, Hyun Ju Seon, Kwon Sam Kim

58 patients: acute STEMI within the 12 h of the onset of symptoms
*exenatide: 10 μg SC and IV bolus 10 μg injection of BYETTA® (Amylin-Lilly) 5 min before the onset of reperfusion. In addition, 10 μg SC bid on the following 2 days.

Conclusions—In patients with ST-segment–elevation myocardial infarction, adjunctive exenatide therapy with primary percutaneous coronary intervention was associated with reduction of infarct size and improvement of subclinical left ventricular function. (Arterioscler Thromb Vasc Biol. 2013;33:2252-2260.)
Targeting Myocardial IR Injury — The Search Continues

Necrosis inhibitor, NecroX?

NecroX-7; from LG life sciences (SH Kim, PhD)

Derivative and combination of products

Anti-necrotic effect by some mechanisms
via 1) Strong antioxidant
2) Inhibition of HMGB1
3) Mitochondrial ROS and ONOO⁻ scavenger

Well-known the effect on hepatic ischemia/reperfusion injury
Transplant Proc 2010
Mechanism of necrosis inhibitor, NecroX

Ischemia/Reperfusion

Necrotic cardiomyocyte

Mitochondrial swelling

mPTP

H$_2$O + solute

NecroX

Ca$^2+$

TCA cycle & ETC stimulation

ROS burst
## Necrosis inhibitor

### In vitro Design

Hypoxia-Oxidative stress/Reoxygenation (H-O/R) model using H9C2 cell (myoblast cell line)

<table>
<thead>
<tr>
<th>24 Hours of incubation period</th>
<th>24 Hours of Hypoxia</th>
<th>1.5 Hours of Reoxygenation</th>
</tr>
</thead>
<tbody>
<tr>
<td>23.5 Hours</td>
<td>30min</td>
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</table>

- Hypoxia with 1% O₂
- Oxidative stress With H₂O₂ 400μM and Reoxygenation with 20% O₂

**Seeding :** H9C2 cells  
- 0.5x10⁵ Cells /35mm dish,  
- 1.5x10⁵ Cells /60mm dish,  
- 1.5x10⁶ Cells/100mm dish

**Pre-treatment**

1. Vehicle : 0.01% DMSO  
2. Necrosis inhibitor: NecroX 20 μM  
3. Antioxidant: Vitamin C 10 μM  
4. Apoptosis inhibitor: Z-vad 20 μM

**Harvest**
Measurement of mitochondrial Ca$^{2+}$ influx

Vehicle treated group showed prominent calcium influx (red stain) in the swollen mitochondria via mPTP opening.

Necrosis Inhibitor revealed protective effect on mPTP opening under I/RI.
Measurement of mitochondrial Ca$^{2+}$ influx

Under hypoxia and oxidative stress condition

Vehicle (3~5 minutes)

Necrosis Inhibitor (over 30 minutes)

[ Vehicle-treated H9C2 cells ]

[ NecX-treated H9C2 cells ]

* Condition: Hypoxia (24hrs) - Reoxygenation (90mins)
* Red (Rhod-2 fluorescence): free Ca$^{2+}$ level
* Green (Mitotracker): mitochondrial shape
H9C2 cells pretreated with vehicle (DMSO) exhibiting rapid increase in Ca$^{2+}$ influx and mitochondrial swelling.

* Condition: Hypoxia (24hrs) - Reoxygenation (90mins)
* Red (Rhod-2 fluorescence): free Ca$^{2+}$ level
* Green (Mitotracker): mitochondrial shape
H9C2 cells pretreated with necrosis inhibitor (NecX) showing minimal increase in Ca$^{2+}$ influx and preserved mitochondrial shapes.
In vivo Design

IV continuous infusion for 20min. Like as Preconditioning (N=13)
1. Vehicle
2. Cyclosporin A (5 mg/kg) for positive control
3. NecroX-7 (1 mg/kg)

Ischemia by Ligation of LAD

45min. of Ischemia

25min. 20min. 3 Days

14 days after I/RI injury

NecroX-7 (30 mg/kg) P.O. daily

Reperfusion
EchoCG under anesthesia

Harvest
After EchoCG & blood sampling
Formalin fixed tissue

EchoCG under anesthesia At 7 days after I/RI
Necrosis inhibitor

Day 14 harvested heart

Areas of Fibrosis

- Control (N=13)
- Cyclosporin A (N=13)
- NecroX (N=13)

Lengths of Fibrosis

- Control (N=13)
- Cyclosporin A (N=13)
- NecroX (N=13)

Percents of Fibrosis at 14 days

- Control (N=13)
- Cyclosporin A (N=13)
- NecroX (N=13)

P-values:
- Areas of Fibrosis: P = 0.16, P < 0.01, P < 0.01
- Lengths of Fibrosis: P = 0.23, P < 0.01, P < 0.01
- Percents of Fibrosis: P = 0.04, P < 0.01, P < 0.01
Necrosis inhibitor

Quantification for Necrosis

Control  Cyclosporin A  Necrosis inhibitor

Black arrow indicated necrotic myocyte.

Control  Cyclosporin A  Necrosis inhibitor

WGA (Sarcolemma)  Nucleus

Necrotic myocyte (MHC+)

Myosin positive myocytes (%)

Necrosis of cardiomyocyte (% area)

P < 0.01

Why the anti-MHC Ab get injected before the ligation?
Necrosis inhibitor: dose finding preclinical study

- NecroX prevented myocardial necrosis from I/R injury.
- The minimal effective dose (MED) in Rat MI/R model was 0.3 mg/kg.
Necrosis inhibitor: clinical trials

Phase 1. 건강한 남성 피험자를 대상으로 LC28-0126의 안전성, 내약성 및 약동학적 특성을 평가하기 위한 용량군별 무작위배정, 이중노가림, 위약대조, 단회투여, 단계적 증량 제 1상 임상시험

Phase 2a. ST분절 상승 심근경색(ST-segment Elevation Myocardial Infarction) 환자를 대상으로 경피적 관상동맥 중재술 (Percutaneous Coronary Intervention, PCI) 전, LC28-0126을 단회 정맥 주사 시 효능, 안전성 및 약동학적 특성을 평가하기 위한 다기관, 무작위 배정, 병행설계, 이중노가림, 위약 대조, 임상 2상 시험

Phase 2b. Ongoing

Chest pain
- ECG
- Cardiac enzyme
- History taking

STEMI

F/U at 30 days
- EchoCG F/U for LVEF
- CMR evaluation for necrosis

응급실 | 심혈관조영실 | Reperfusion | Stenting & CCU adm | Echo/CMR F/U

NecroX (5분)
IV infusion during the preparation of patient for CAG (Before reperfusion injury)

Early phase evaluation
CK-MB AUC
Prevalence of MACE (Death, VT/Vf, Cardiogenic shock (HF), Re-infarction)

Before discharge, Baseline EchoCG, CMR
Benefit of Reperfusion & Prevention of IR injury

- Solid line: the benefit of reperfusion in terms of lives saved at 35 days
- Dotted line: relation between an effective cardioprotective intervention and reperfusion

Cardio-protection **before** reperfusion

The mitochondrial PTP is a nonselective channel of the inner mitochondrial membrane. Opening the channel collapses the mitochondrial membrane potential and uncouples oxidative phosphorylation, resulting in ATP depletion and cell death.

During myocardial ischemia, the mitochondrial PTP remains closed, only to open within the first few minutes after myocardial reperfusion in response to mitochondrial Ca2+ overload, oxidative stress, restoration of a physiologic pH, and ATP depletion. Therefore, the mitochondrial PTP is a critical determinant of lethal reperfusion injury, and as such it is an important new target for cardioprotection.
Thank you for your attention
경청해 주셔서 감사합니다
Reperfusion Injury: How Can We Reduce It?

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