Novel Necrosis Inhibitor to prevent Myocardial Ischemia-Reperfusion Injury

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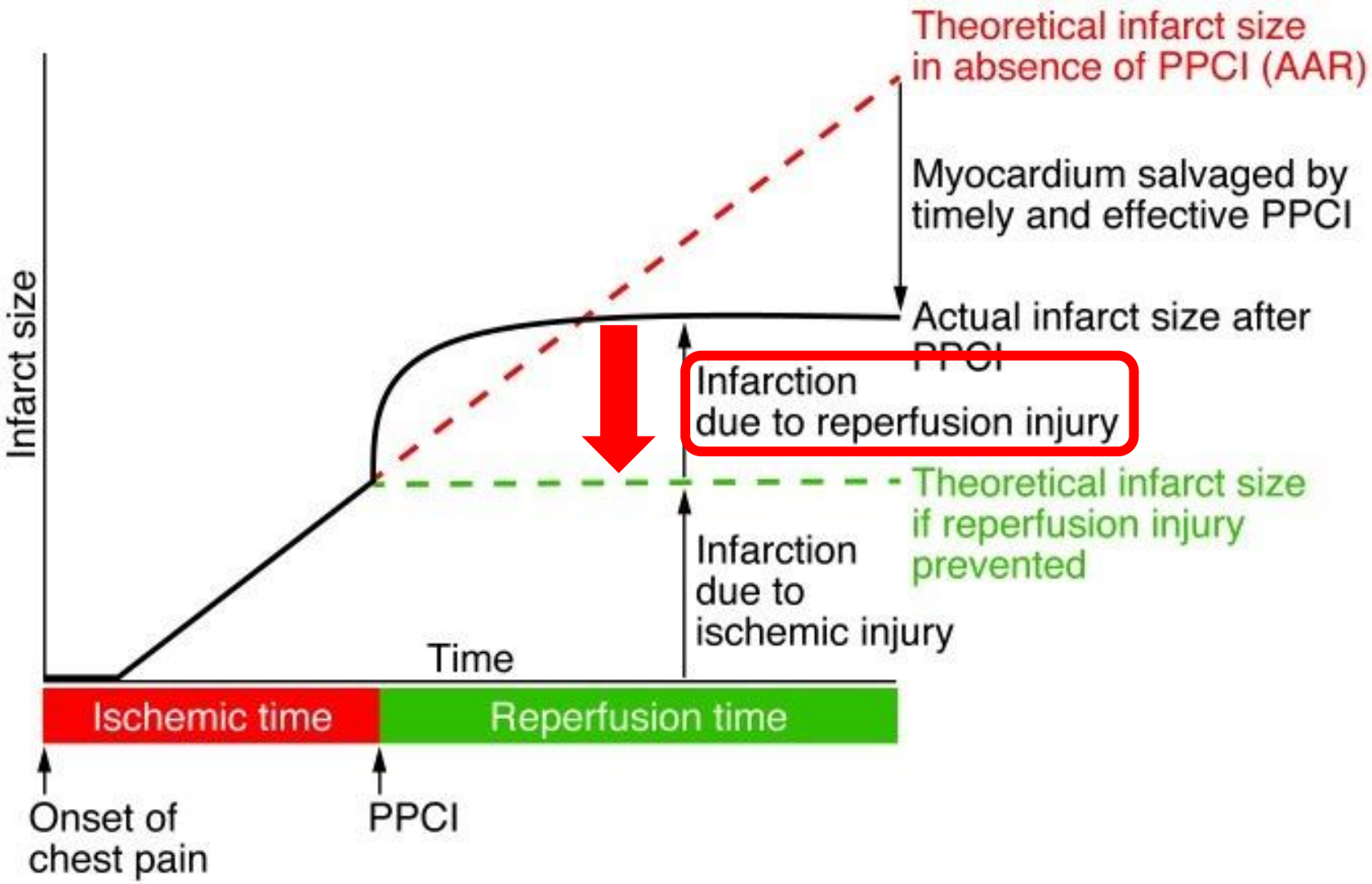
SNUH Cardiovascular Center

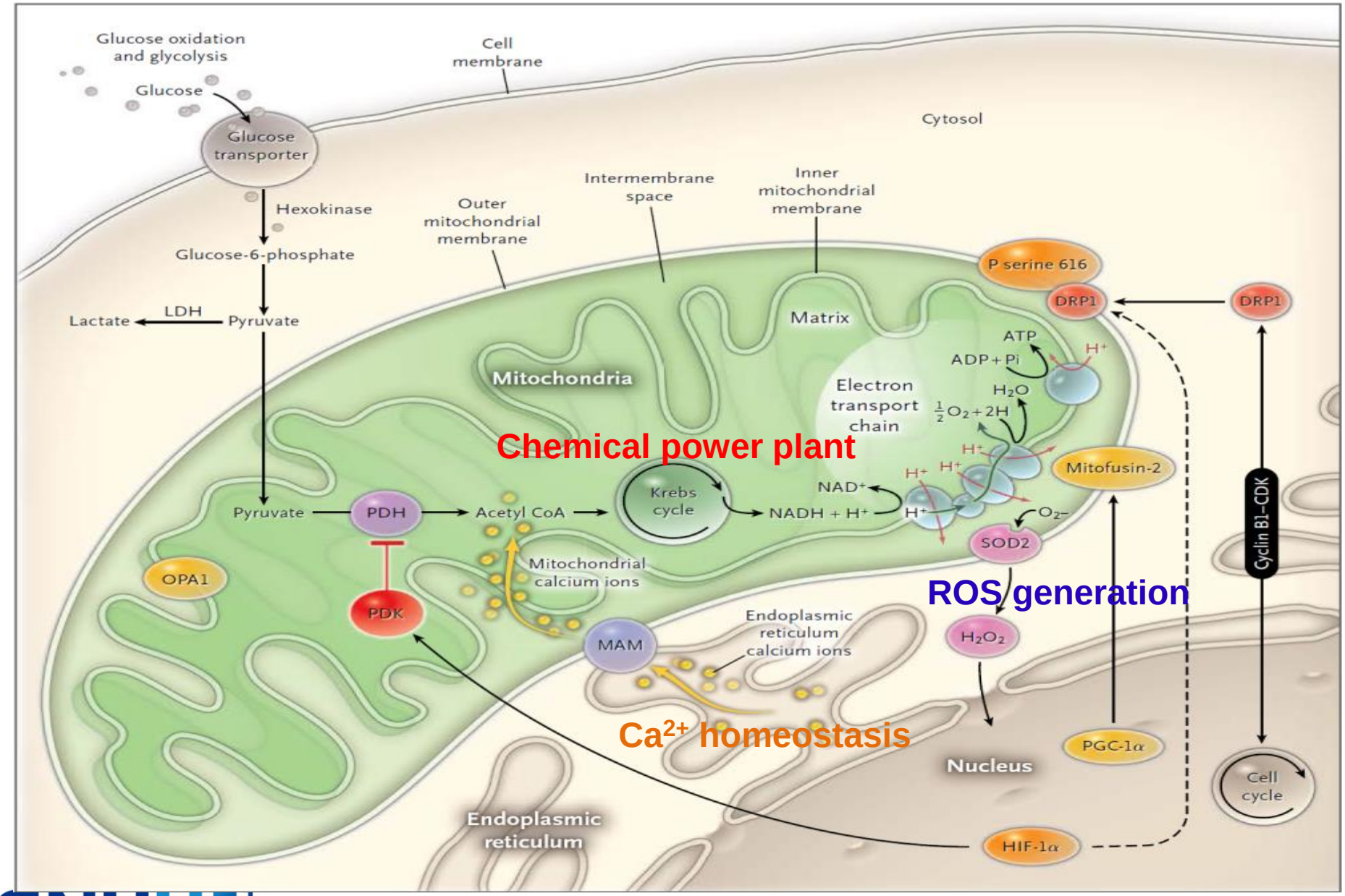
INTRODUCTION

Myocardial I-R Injury: Neglected Therapeutic Target



J Clin Invest. 2013;123(1):92–100.

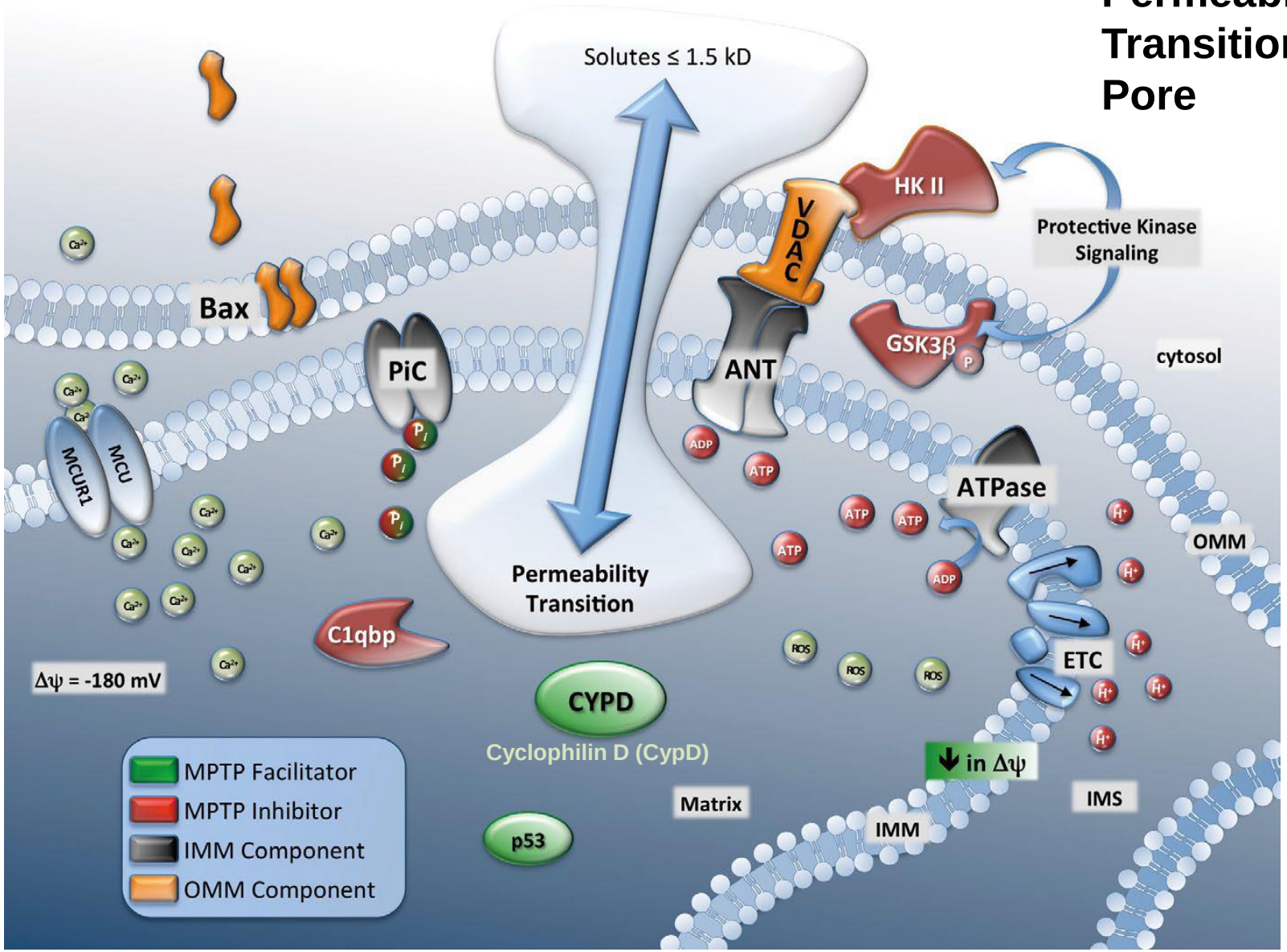
Three Roles of Mitochondria Abundant in CMC



N Engl J Med 2013;369:2236-51.

Structure and Mechanism of MPTP

Mitochondrial Permeability Transition Pore



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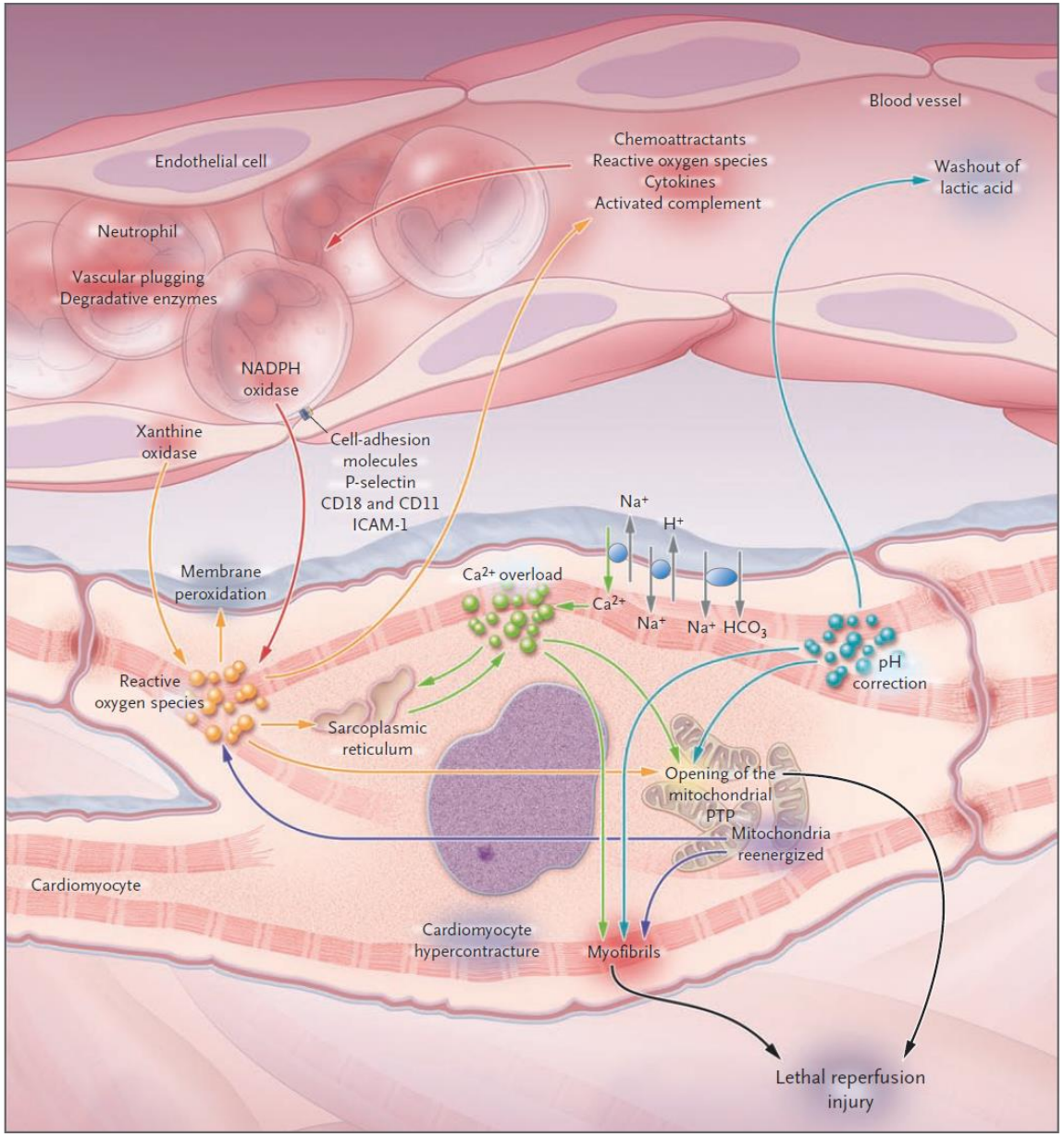
Circ J 2013;77:1111 – 1122.

Mechanism of Ischemia-Reperfusion Injury

Major Mediators

1. Oxygen paradox
2. Calcium paradox
3. pH paradox
4. Inflammation (granulocytes, plts)



N Engl J Med. 2007;357:1121-35

Mechanism of Ischemia-Reperfusion Injury

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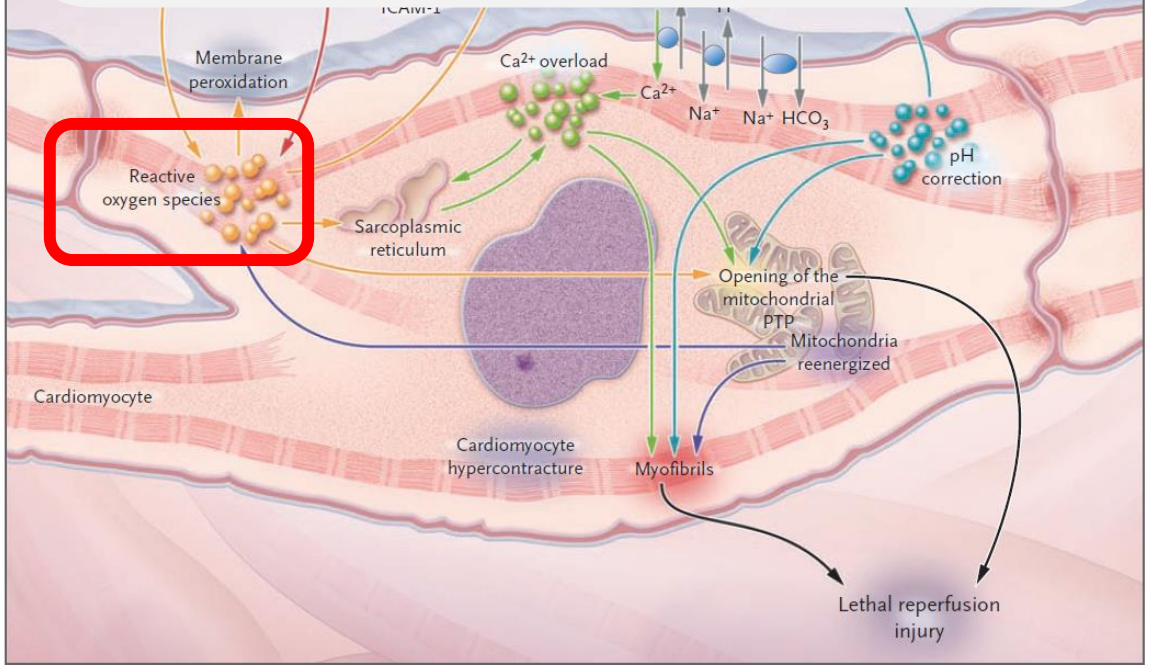
Oxygen paradox

- Reperfusion generates oxidative stress which itself can mediate organ injury
- Oxidative stress reduces the bioavailability of nitric oxide



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Mechanism of Ischemia-Reperfusion Injury

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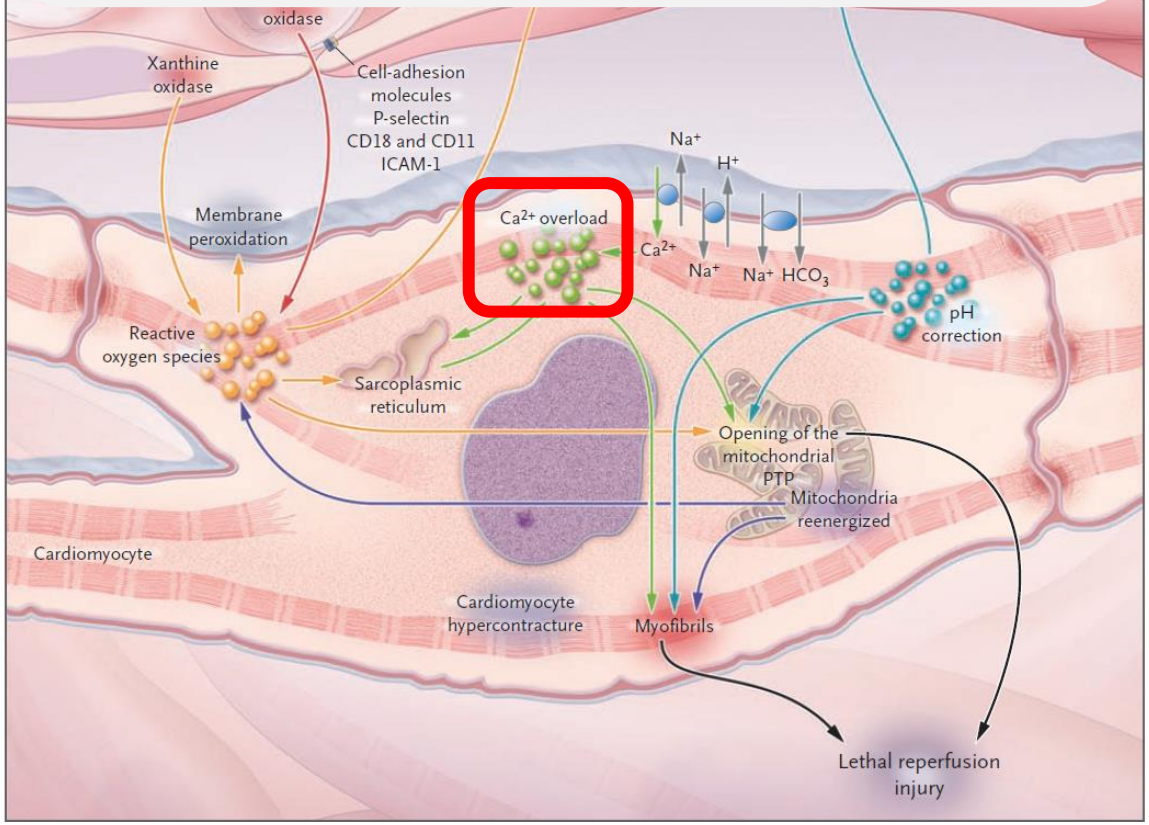
Calcium paradox

✓ Sarcolemmal-membrane damage

✓ Sarcoplasmic reticulum dysfunction

➔ Abrupt increase in intracellular Ca^{2+}



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Mechanism of Ischemia-Reperfusion Injury

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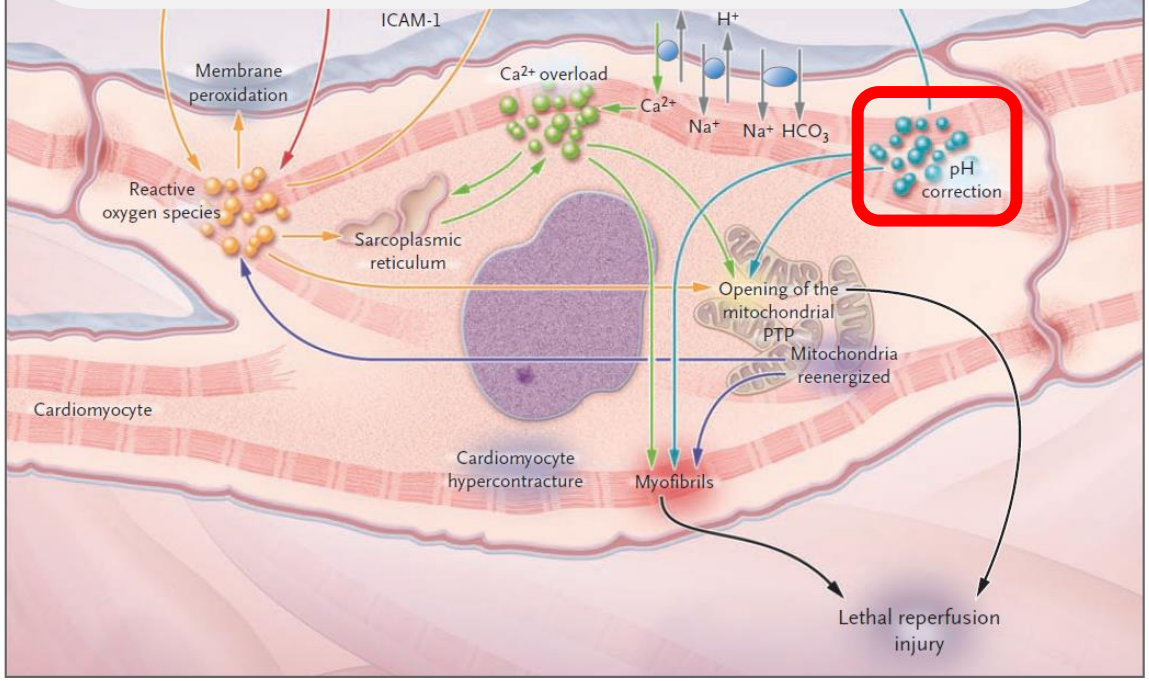
pH paradox

- Reperfusion
- ✓ Wash-out of lactic acid
- ✓ Activation of Na^+/H^+ exchanger & Na^+/HCO_3^- symporter

➔ Rapid restoration of physiologic pH



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Mechanism of Ischemia-Reperfusion Injury

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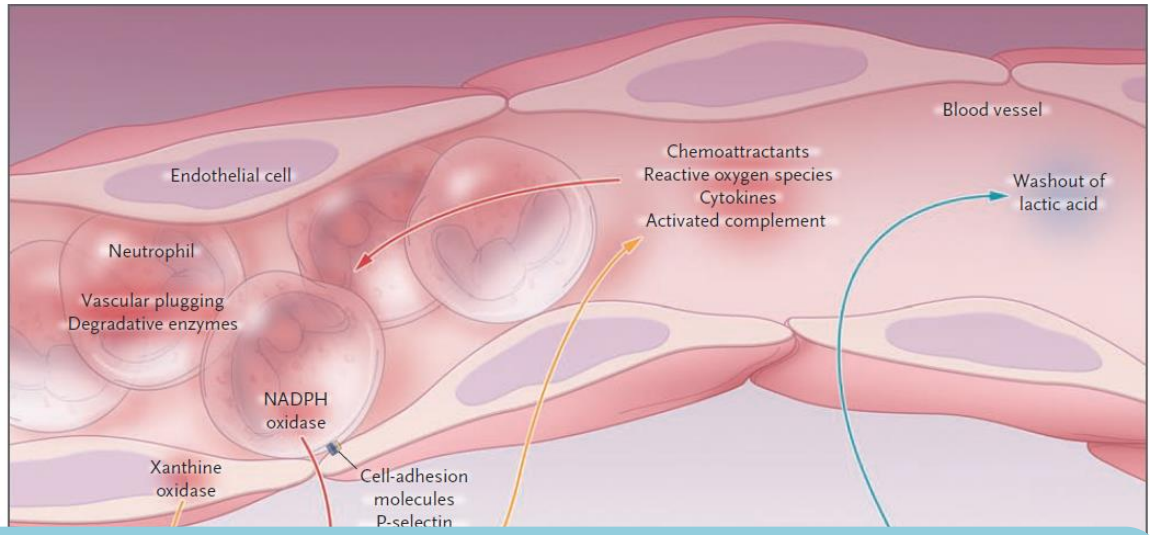
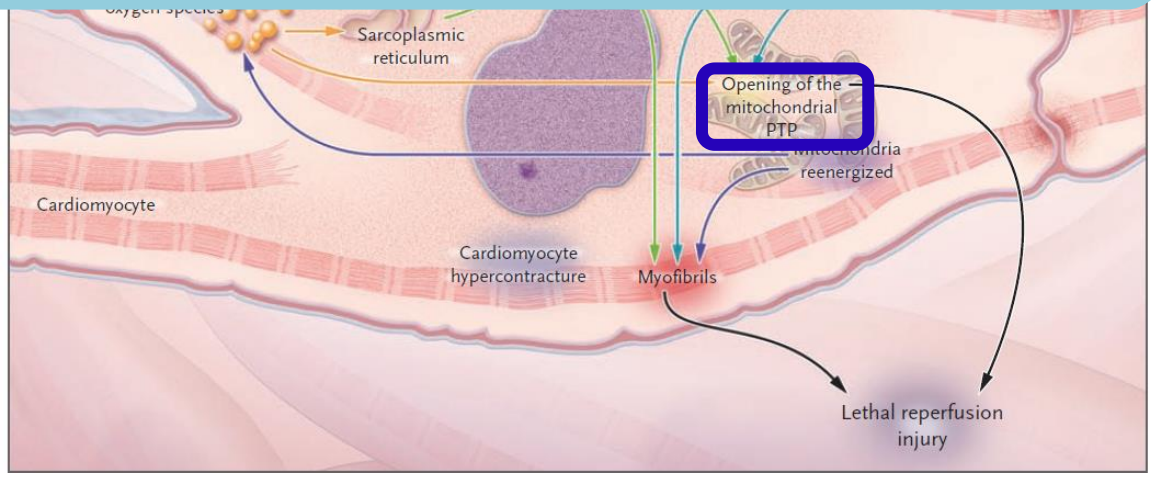


Mitochondrial permeability transition pore (MPTP) plays a key role in Ischemia-Reperfusion Injury

Opening of MPTP

- Uncoupling oxidative phosphorylation
- Mitochondrial swelling

➔ **Cell death**



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N Engl J Med. 2007;357:1121-35

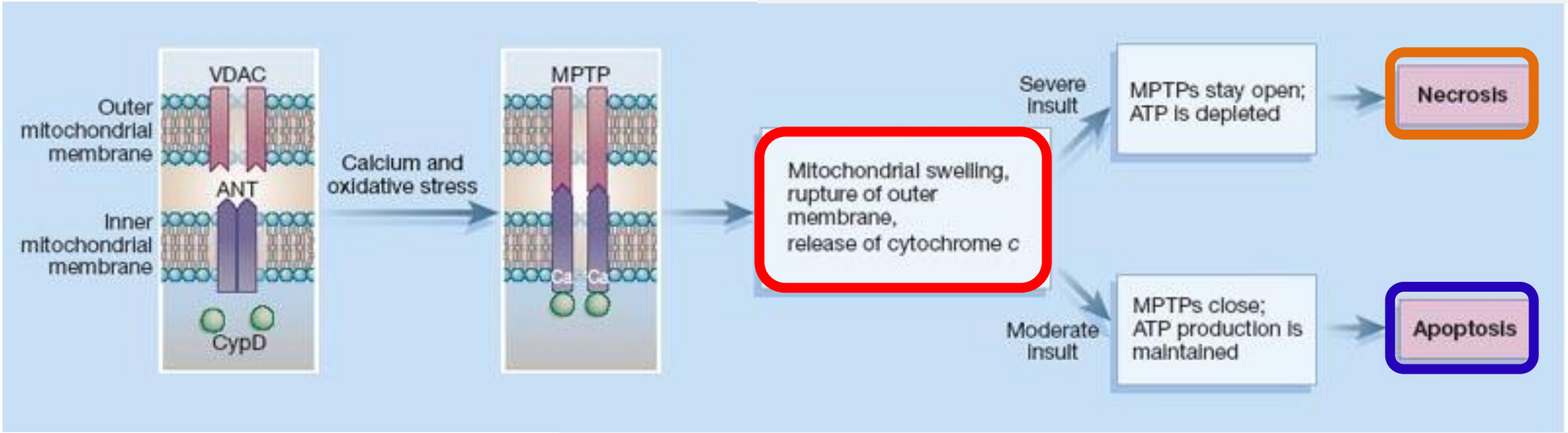
APOPTOSIS vs. NECROSIS

- **Mechanisms of mitochondrial membrane permeabilization**

Ischemia-Reperfusion

- **Mitochondrial ROS generation**
- **Mitochondrial Ca^{2+} overload**
- **Normalization of pH**
- **MPTP opening**

Severe insult ➔ MPTPs stay open ➔ Necrosis



Moderate insult ➔ MPTPs transient opening ➔ Apoptosis

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Nature. 2005;434:578-579.

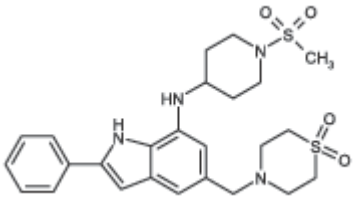
Novel Necrosis Inhibitor, NecroX, Prevents myocardial Ischemia-Reperfusion Injury

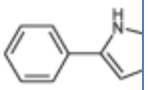
A Novel Necrosis In

- INTRODUCTION

NecroX-7; from LG life scienc

Derivative and combination of p





Anti-necrotic effect by some mec

via **1) Strong antioxidant**

- Mitochondrial ROS and O

- Inhibition of ROS-generati

2) Inhibition of HMGB1

朝鮮日報 2010년 01월 29일 금요일 D04면

대전=신현종 기자 shin@chosun.com

LG생명과학 김순하 박사가 세포보호물질의 효과를 보여주는 현미경 사진을 보여주고 있다. 김 박사는 "화장품에서 시약, 치료제 등으로 다양한 세포보호제의 상용화가 꼬리에 꼬리를 물 것"이라고 말했다. ☞동영상 chosun.com

LG생명과학 김순하 박사 인터뷰

"세포를 보호하면 응용할 곳은 무궁무진"

지난해 10월 LG생명과학은 새로운 세포보호제 개발에 대한 기자설명회를 열었다. 2003년 자사의 항생제 신약 '팩티브'가 국산 신약 최초로 미국 식품의약청(FDA) 승인을 받은 이후 처음 있는 기자설명회였다. 그만큼 회사가 세포보호제를 중요하게 여기고 있다는 뜻이었다.

세포보호제 개발의 주역은 LG생명과학 의약연구소 김순하 박사. 지난 26일 대전 연구소에 만난 김 박사는 "현재 국내외 10개사와 다양한 분야의 응용제품 개발을 위한 논의를 하고 있다"며 "자료는 쏟아지는데 논문 쓸 틈도 없이 날마다 회의의 연속"이라고 말했다.

기자설명회 당시 LG생명과학 김인철 대표는 "신약개발엔 시간이 오래 걸릴 뿐만 아니라 개발되더라도 한 가지 효능만으로는 한계가 있다"면서 "세포보호물질은 여러 산업에 기여할 수 있는 파괴적 기술"이라고 밝혔다. 김 박사는 "처음엔 뭔가 재미있는 일이라고만 생각했지 이 정도로 일이 커질지 몰랐다. 모든 분야에 다 적용하고 싶지만 감당할 수 있는 만큼만 하려고 욕심을 누르고 있다"고 말했다.

시작은 우연이었다. 2004년 8월 김 박사는 새로운 당뇨병 치료제 후보물질의 독성(毒性)을 시험했다. 보통 신약 후보물질의 농도가 짙어질수록 세포가 급격히 죽는다. 그런데 이 물질은 처음 시작이 달랐다. 세포가 100에서 시작해 점점 수가 줄어야 하는데 시험기기에 처음에

세포 괴사 막는 유일 물질 실험용 시약으로 출시 후 치료제로 개발하는 연구도

140이란 숫자가 나온 것이다. 시험을 담당한 연구원은 "기기 이상인지 이해가 안 된다"고만 했다.

하지만 김 박사는 그냥 지나치지 않았다. "사진을 보니 세포가 더 싱싱해졌다는 느낌을 받았어요. 분석을 해보니 실제로 세포보호 효과가 있었습니다." 시험이 시작되면서부터 바로 세포가 손상되기 시작하는데, 이 물질이 들어가면서 세포를 보호해 초기 손상이 거의 없었던 것이다. 그래서 100이 140으로 나온 것.

김 박사는 이후 세포보호 메커니즘을 밝혀냈다. 세포는 혈액을 공급받지 못하면 죽는다. 이른바 괴사(壞死·necrosis)다. 분석 결과 이 물질은 세포에서 에너지를 만드는 미토콘드리아에 들어가 세포를 해치는 활성산소를 억제하는 것으로 나타났다. 미토콘드리아는 세포가 받는 산소의 98%를 소비한다. 활성산소의 95%도 여기서 나온다. 결국 세포 손상의 주범을 공격하는 물질인 셈이다. 현재로선 세포 괴사를 막는 유일한 물질이다.

"세포를 보호하면 응용할 곳이 무궁무진입니다. 예를 들어 세포치료용 줄기세포를 오랫동안 생생하게 할 수도 있고, 간암수술에서 혈액 공급이 중단될 때 일어나는 괴사도 막을 수 있습니다. 피부 노화를 막는 화장품으로도 개발할 수 있습니다."

LG생명과학은 먼저 '네크록스(NecroX)'란 이름의 실험용 세포보호 시약을 출시했다. 현재 국내에 5mL당 50만원에 공급하고 있으며 외국 회사와 해외 판매권 협상을 진행 중이다. 또 국내 화장품 업체와 피부 화장품으로 개발하는 문제를 논의 중이다. 해외 화장품 회사와도 논의를 진행 중이다.

치료제로 개발하는 연구도 시작했다. 김 박사는 서울아산병원과 손을 잡고 50마리의 개를 대상으로 간 수술 시 세포 괴사 방지 효과가 있는지를 시험하고 있다. 간 수술에서는 조직 일부를 제거하거나 혈관을 막는 일이 다반사다. 이때 세포에 혈액이 공급되지 못하면 괴사가 일어난다. 김 박사는 "수술 도중 의사가 혈관을 풀었다 조이기를 반복하는 것도 혈액 공급 중단 시간을 줄이기 위해서다"며 "세포보호물질이 효과를 발휘하면 그런 염려 없이 수술을 해 성공률을 높일 수 있을 것"이라고 말했다.

최근에는 서울대 의대 세포치료사업단 김효수 교수와 혈관이 막혀 일어나는 심근경색에 대한 치료 효과를 알아보는 동물시험을 시작했다. 이 분야도 마땅한 치료제가 없는 것은 마찬가지다.

그다음엔 역시 뇌혈관이 막혀 일어나는 뇌졸중을 시험할 계획이다. 꼬리에 꼬리를 물고 신약들이 쏟아질 날이 머지 않았다.

대전=이영완 기자 ywlee@chosun.com

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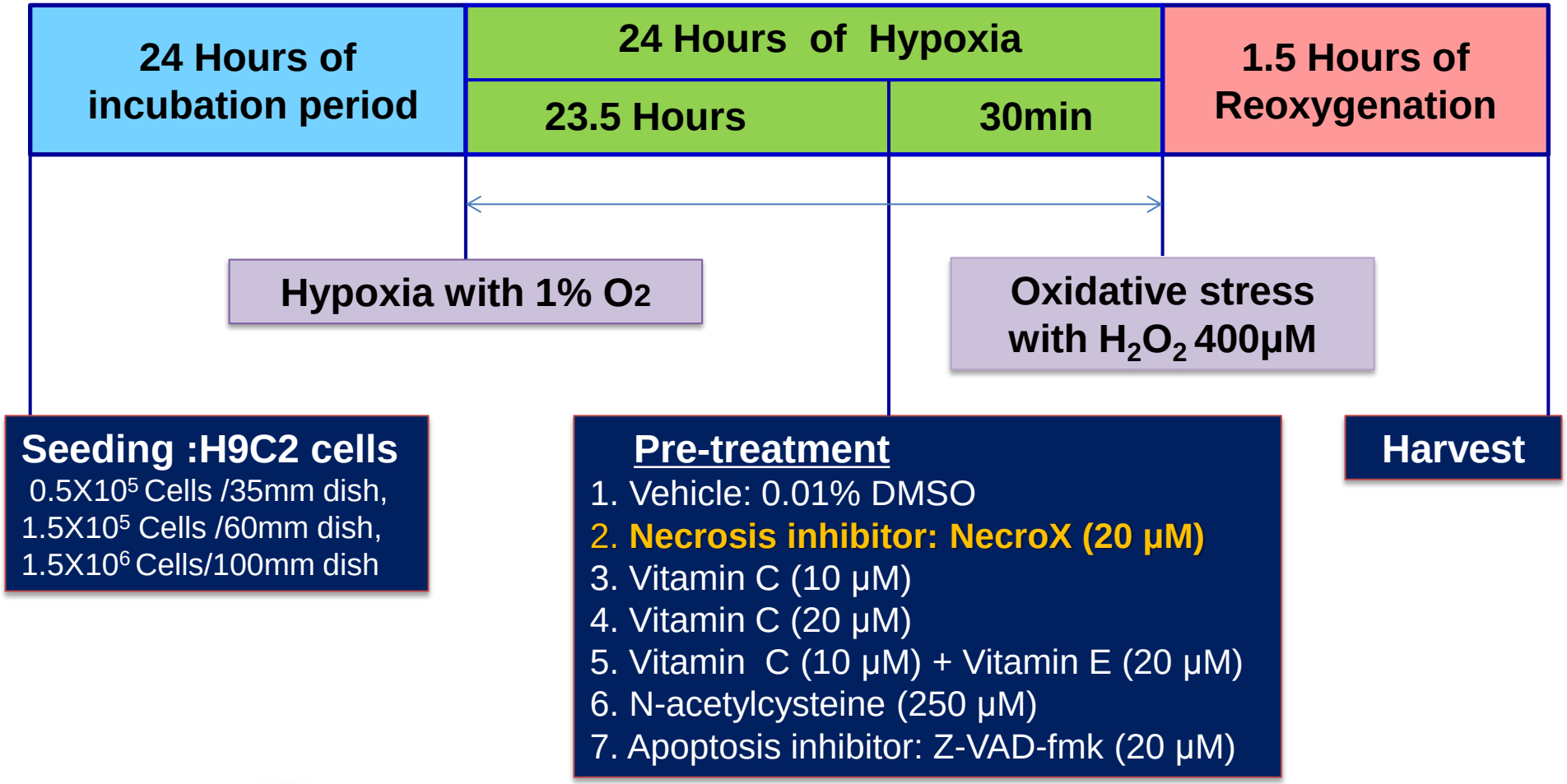
in vitro study

in vitro protocol

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



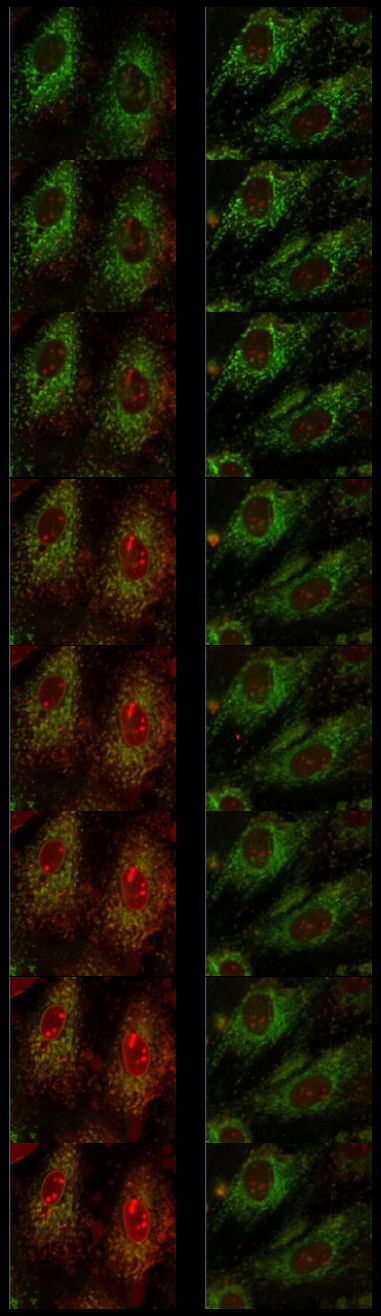
Measurement of mitochondrial Ca^{2+} influx

Vehicle NecroX

0

Time (minutes)

20

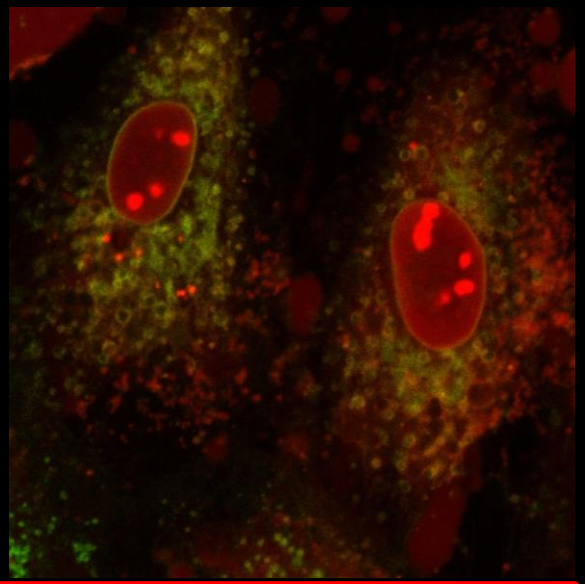


Hypoxia 24h + Oxidative stress with H_2O_2

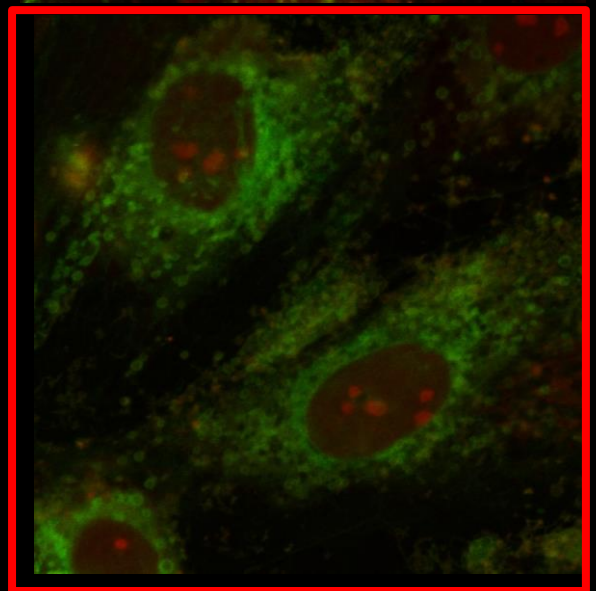
Mitotracker : mitochondria

Rhod-2 : Ca^{2+} influx

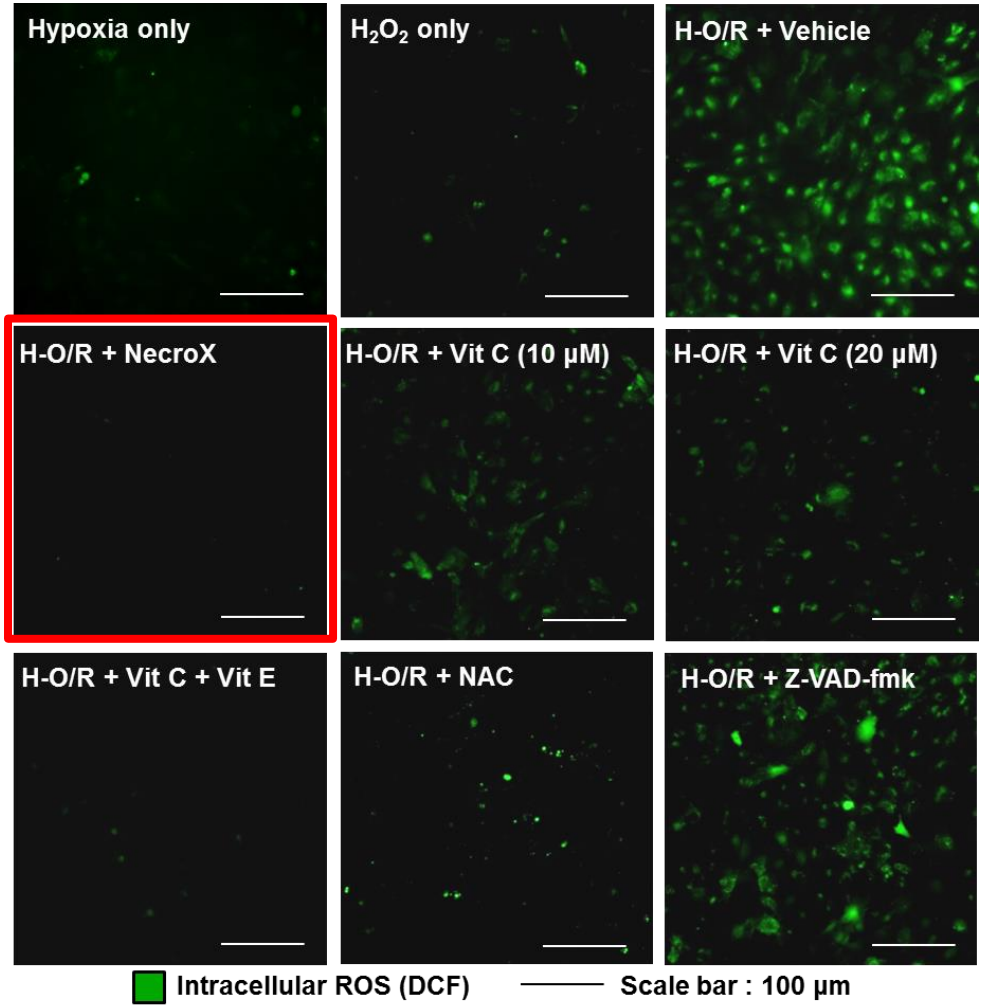
Vehicle treated group showed prominent calcium influx (red stain) in the swollen mitochondria via mPTP opening

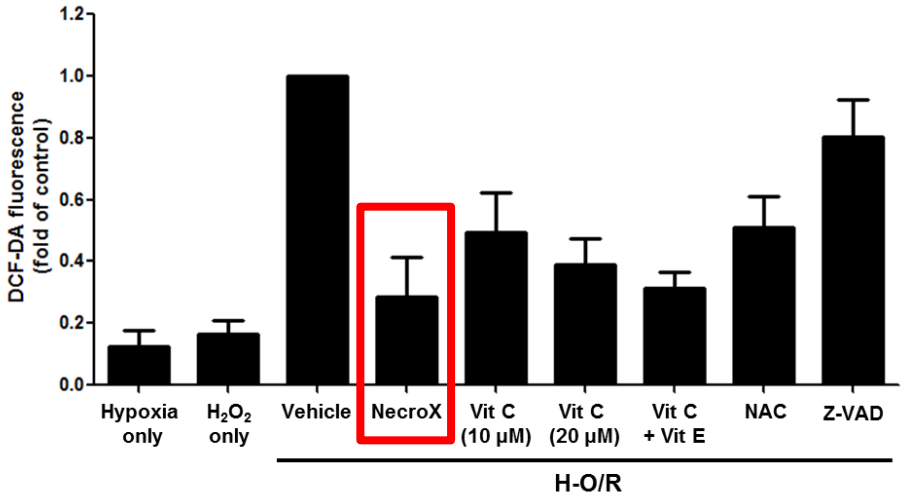


Necrosis Inhibitor revealed protective effect on mPTP opening under I/RI



Action Mechanism of NecroX : ROS scavenging activity





H_2DCF-DA assay

- Intracellular ROS probe
- Oxidization by ROS
  - ➔ DCF : highly fluorescent

NecroX has potent scavenging activity on intracellular ROS.

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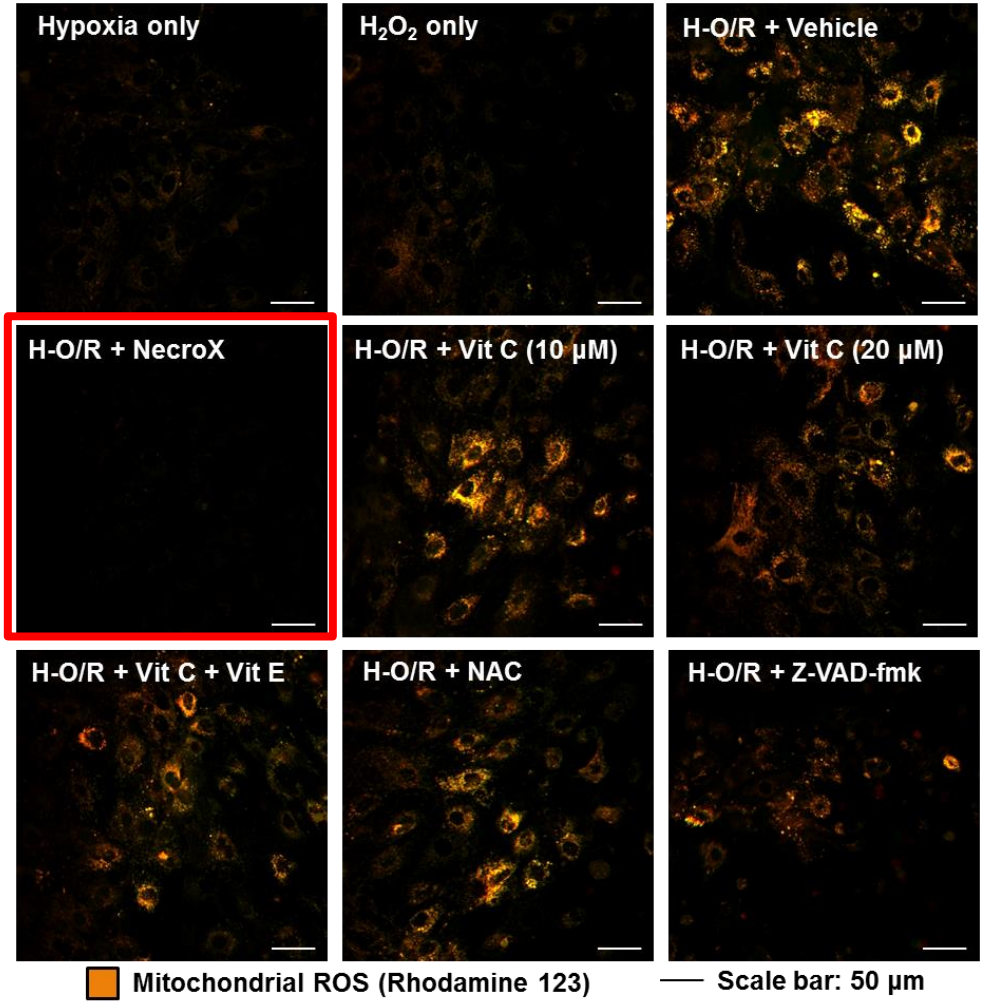
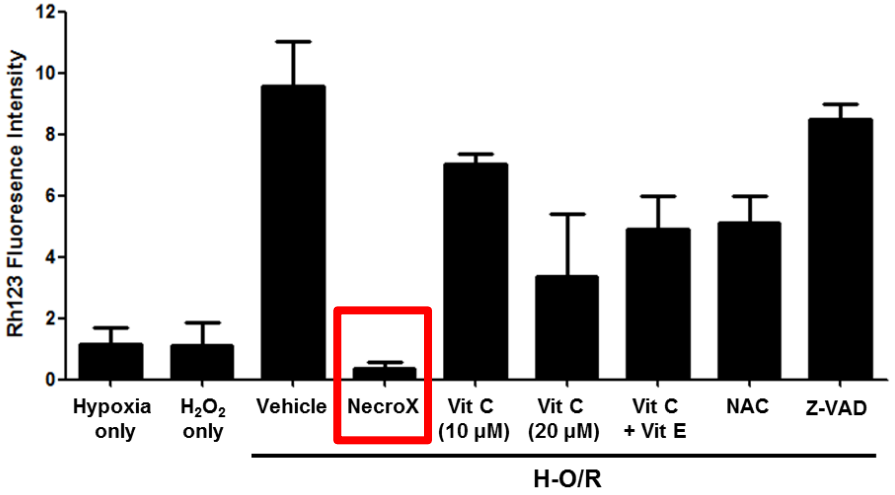
H-O/R : hypoxia-oxidative stress/reoxygenation.
NAC : N-acetylcysteine

Action Mechanism of NecroX : ROS scavenging activity





Dihydrorhodamine 123 (DHR 123) assay

- **Mitochondria-specific ROS probe**
- **Oxidization by ROS**
  - ➔ **Rhodamine 123 : highly fluorescent**

NecroX has potent mitochondria-specific ROS scavenging activity.

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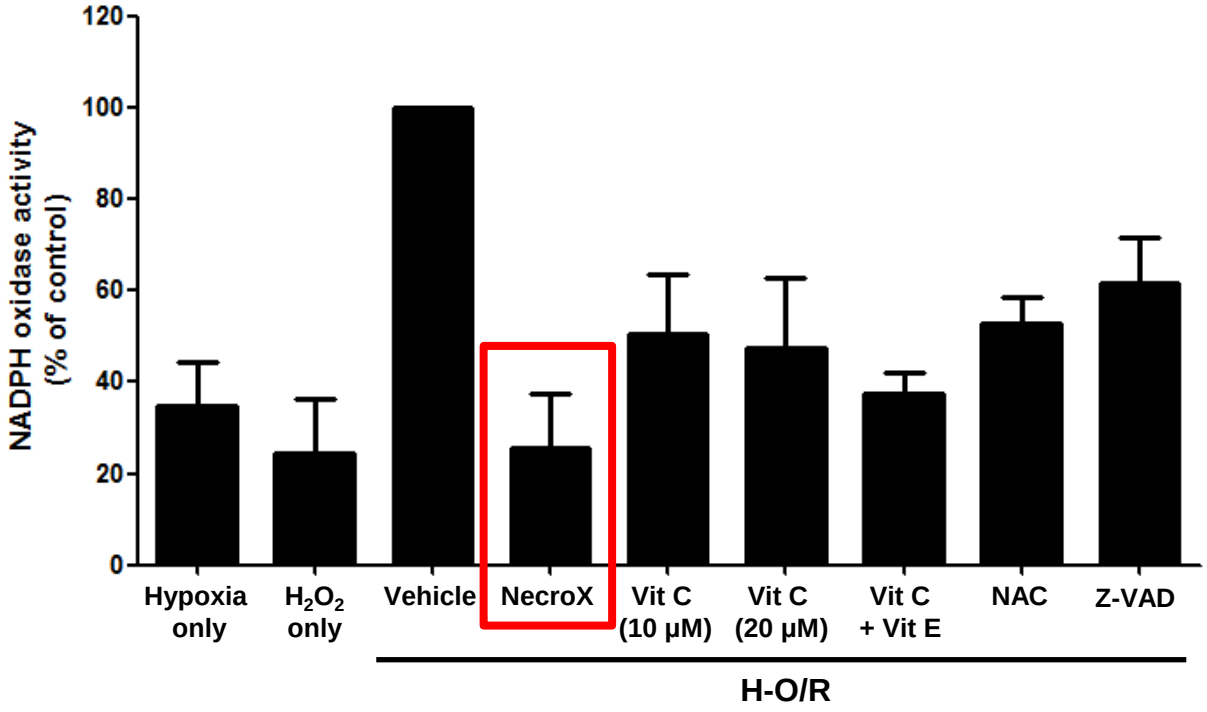
H-O/R : hypoxia-oxidative stress/reoxygenation.
NAC : N-acetylcysteine

Action Mechanism of NecroX
: Inhibition of ROS generating enzyme



NecroX has inhibitory effect on NADPH oxidase, an important ROS-generating enzyme.

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H-O/R : hypoxia-oxidative stress/reoxygenation.
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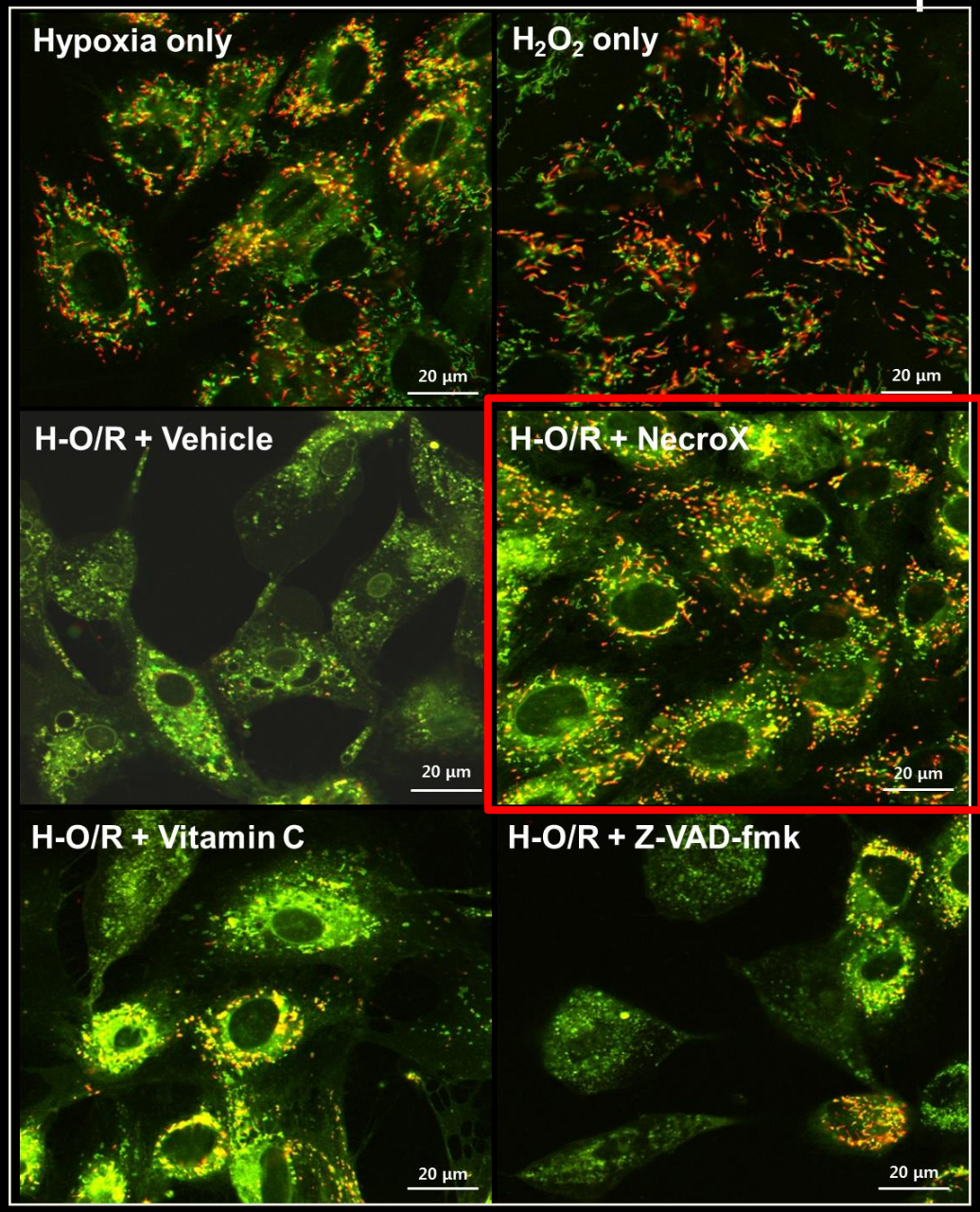
Mitochondrial membrane potential measurement



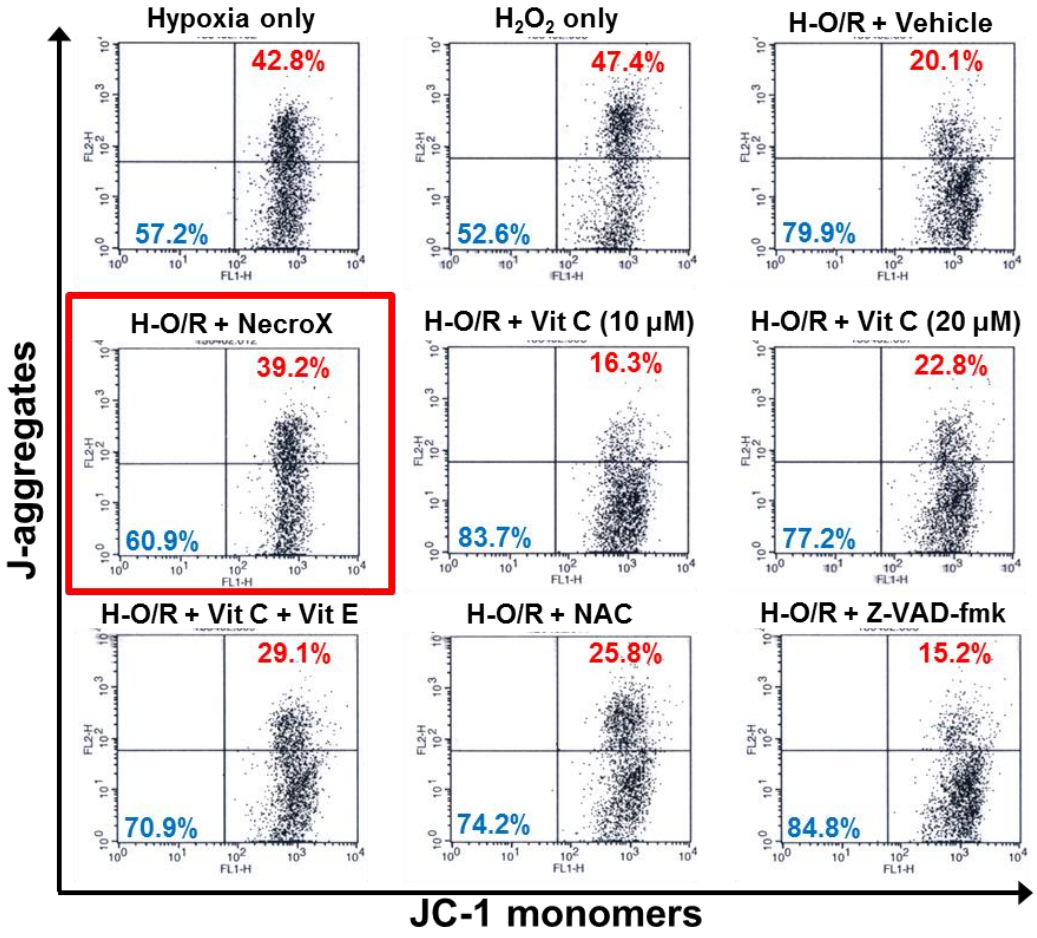
NecroX preserved mitochondrial membrane potential (Δψ) under H-O/R stress.

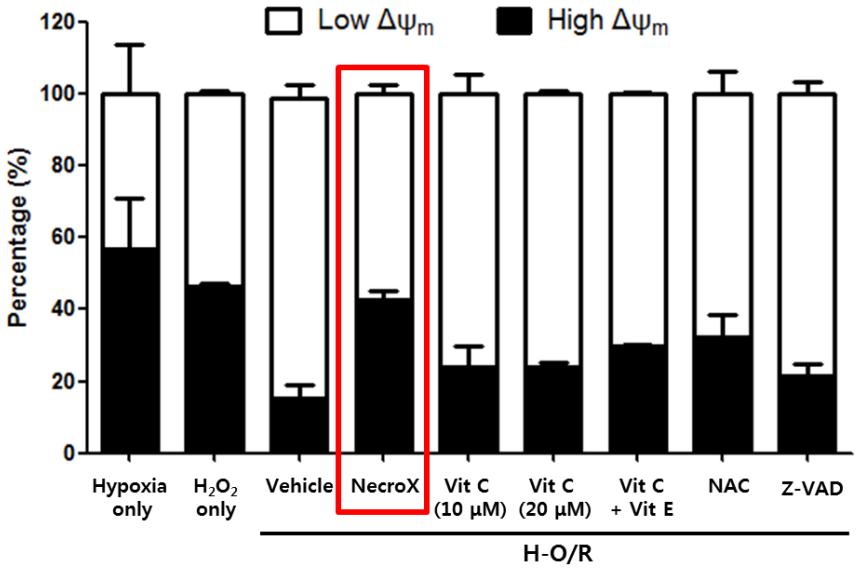
High $\Delta\psi_m$ (J-aggregate)

Low $\Delta\psi_m$ (JC-1 monomer)

H-O/R denotes hypoxia-oxidative stress/reoxygenation.

Mitochondrial membrane potential





During H-O/R injury, mitochondrial transmembrane potential changed from high to low gradient (Δψ), except NecroX treated cells.

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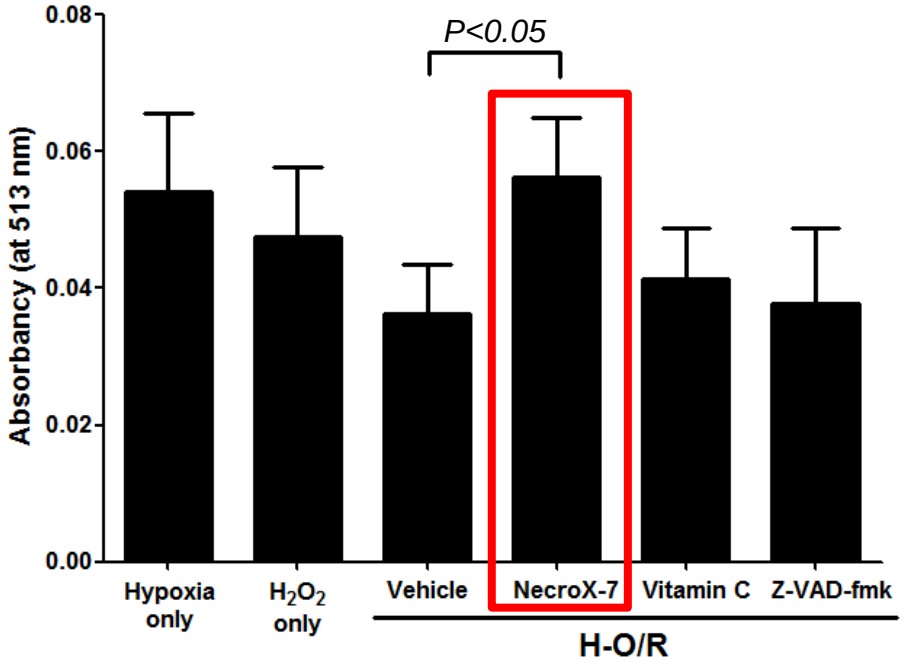
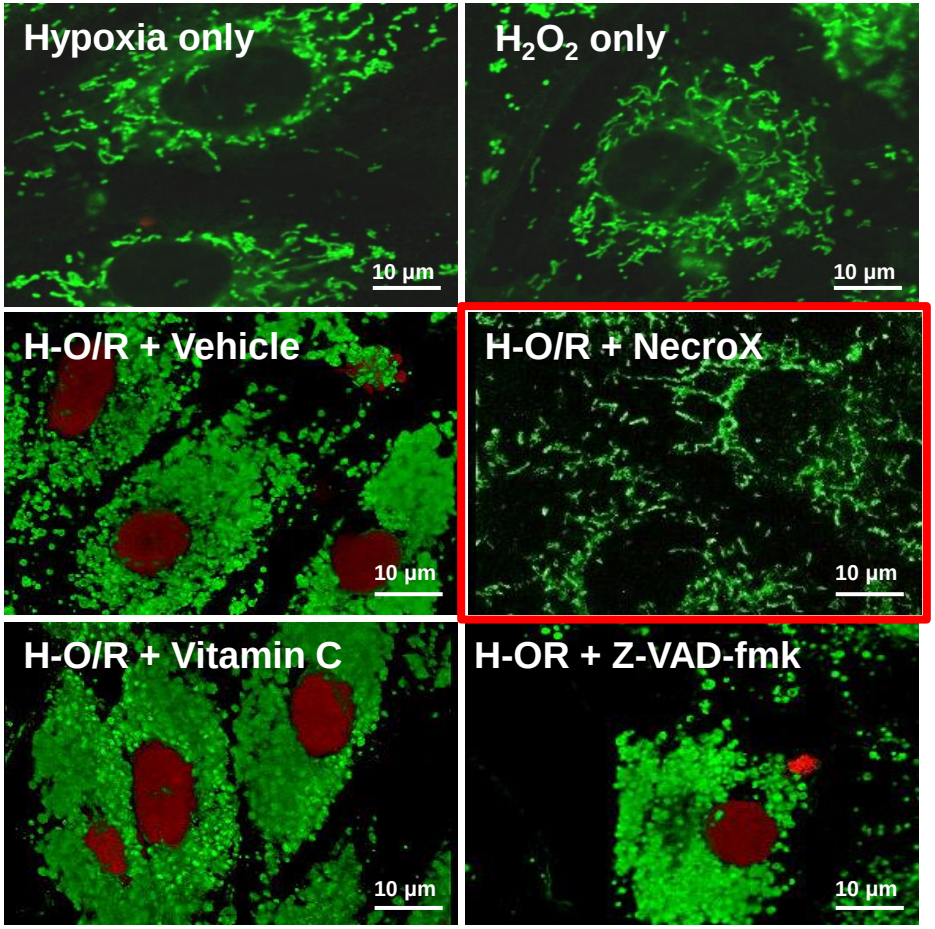
H-O/R : hypoxia-oxidative stress/reoxygenation.
NAC : N-acetylcysteine

Evaluation of mitochondrial swelling

1. Turbidity



2. Confocal imaging for mitochondria



Arrow indicated the swollen mitochondria after H-O/R stress.

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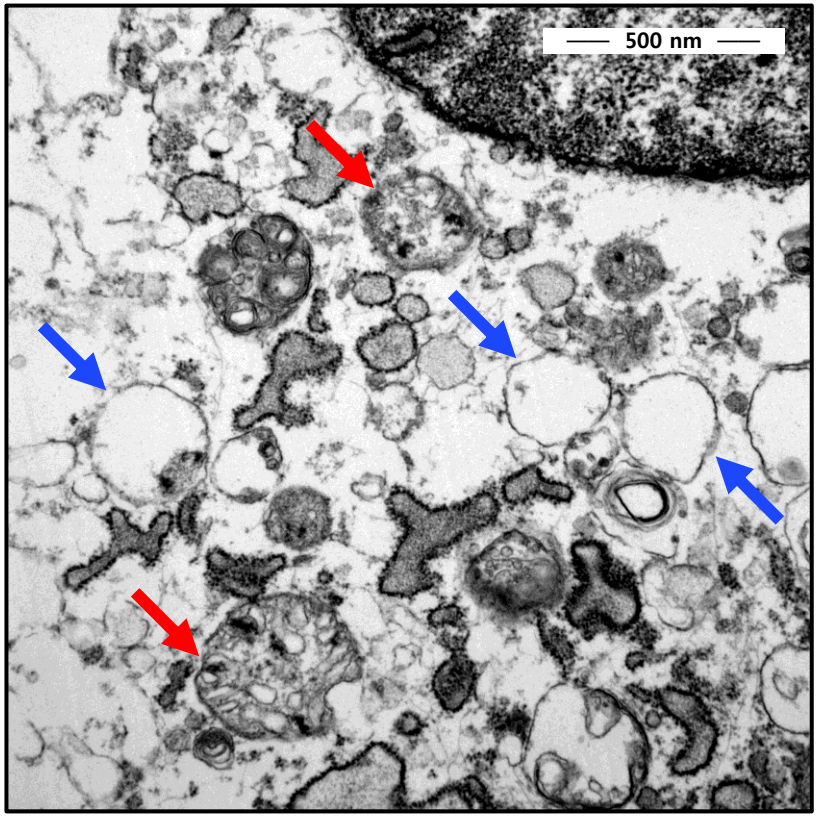
H-O/R denotes hypoxia-oxidative stress/reoxygenation.

TEM for mitochondrial swelling

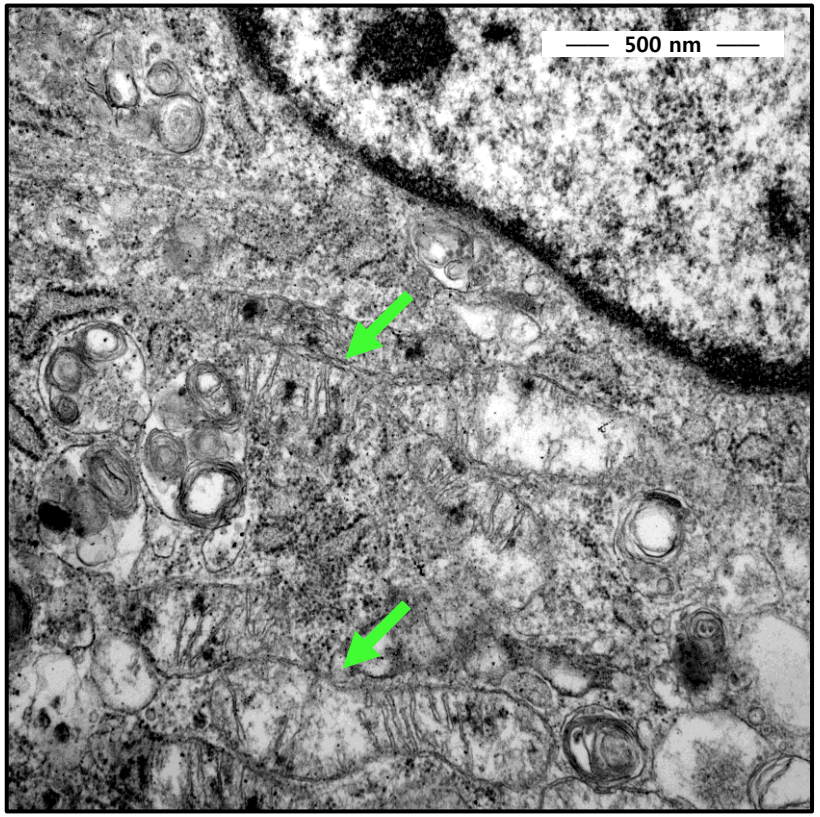
Vehicle + H-O/R



Intracytoplasmic vacuole

Swollen/ruptured mitochondria and degenerated crista

NecroX + H-O/R



Preserved normal mitochondria

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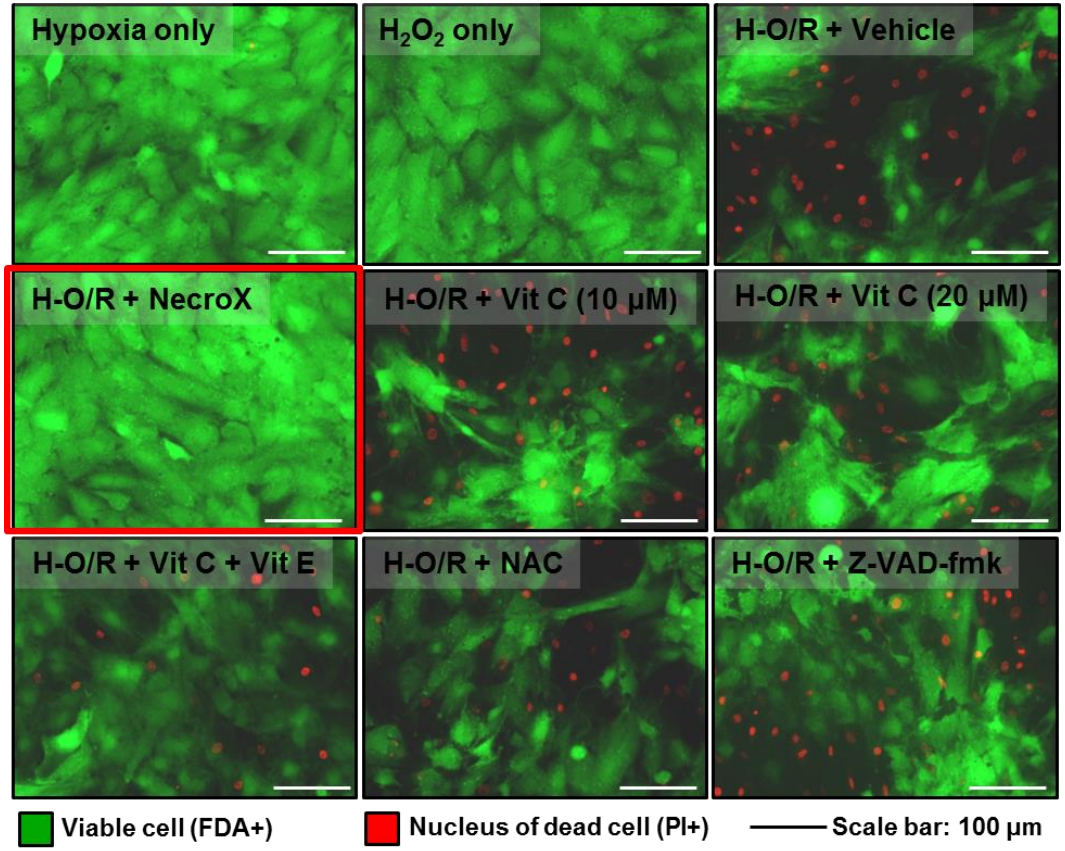
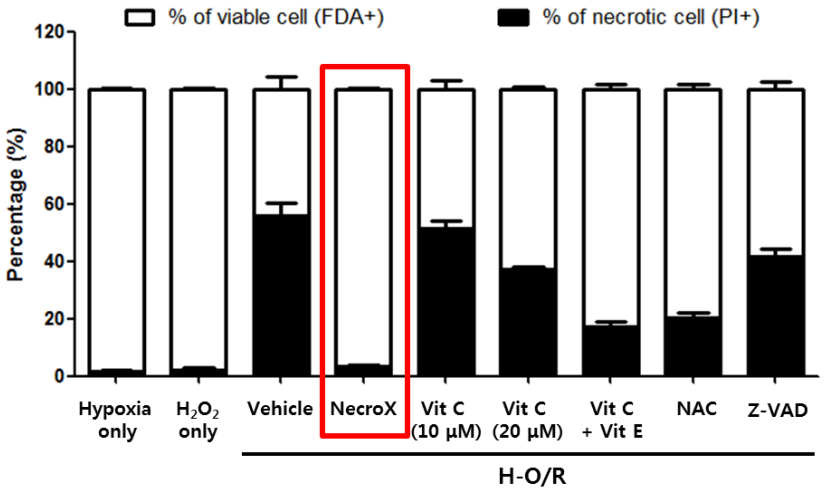
H-O/R denotes hypoxia-oxidative stress/reoxygenation.

PI/FDA staining & Counting the cell





NecroX protected H9C2 rat cardiomyoblasts from necrotic cell death after H-O/R stress.

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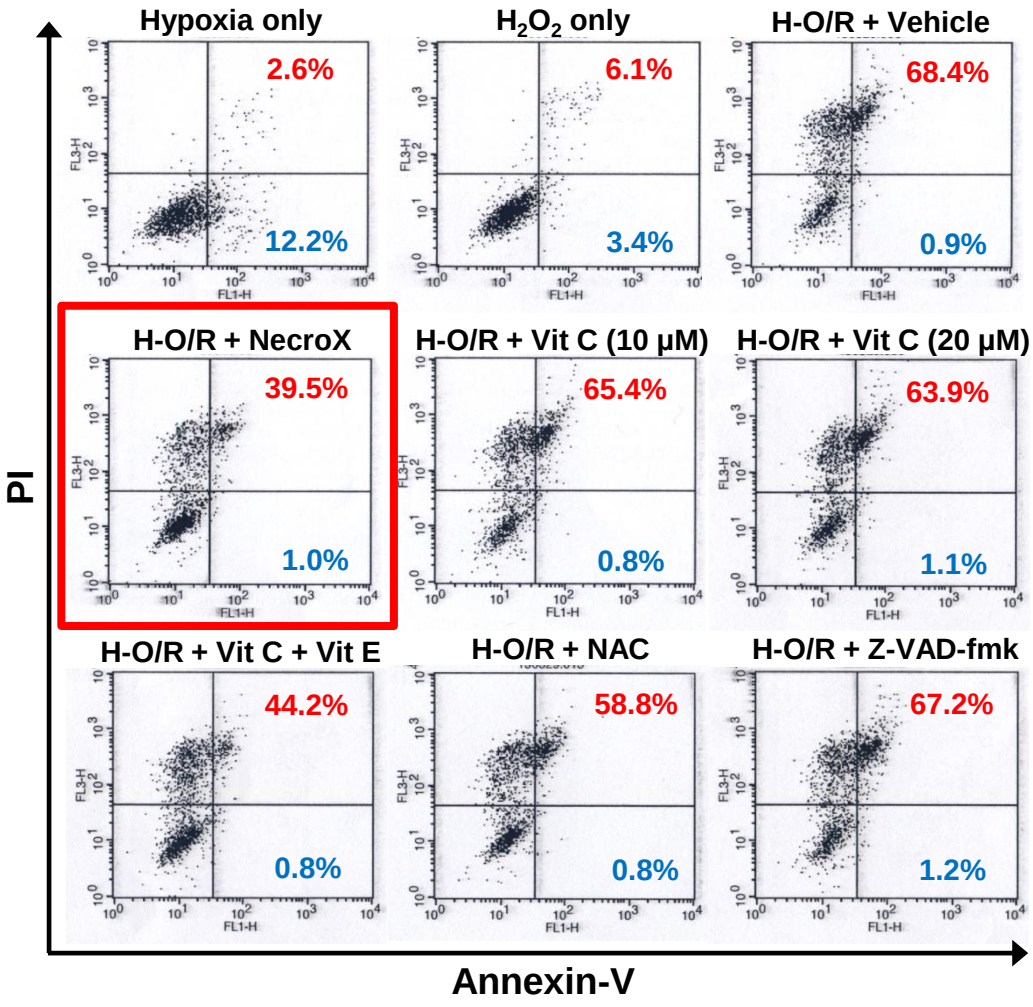
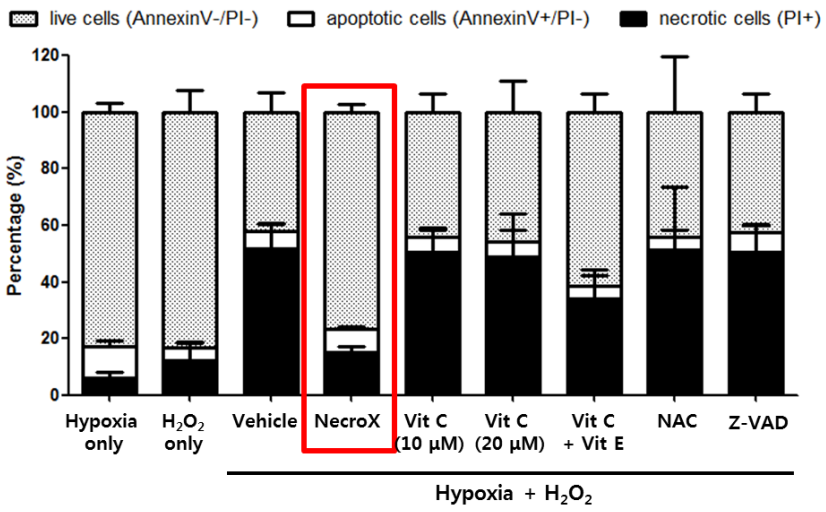
H-O/R : hypoxia-oxidative stress/reoxygenation.
NAC : N-acetylcysteine

Necrotic cells by FACS





NecroX protected H9C2 rat cardiomyoblasts from necrotic cell death after H-O/R stress.

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H-O/R : hypoxia-oxidative stress/reoxygenation.
NAC : N-acetylcysteine

in vivo study

in vivo protocol

Ischemia by Ligation of LAD

45min. of Ischemia | 14 days after I/RI injury

25min. | **20min.** | **3 Days** | **11 Days**

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

Reperfusion

EchoCG under anesthesia

EchoCG under anesthesia

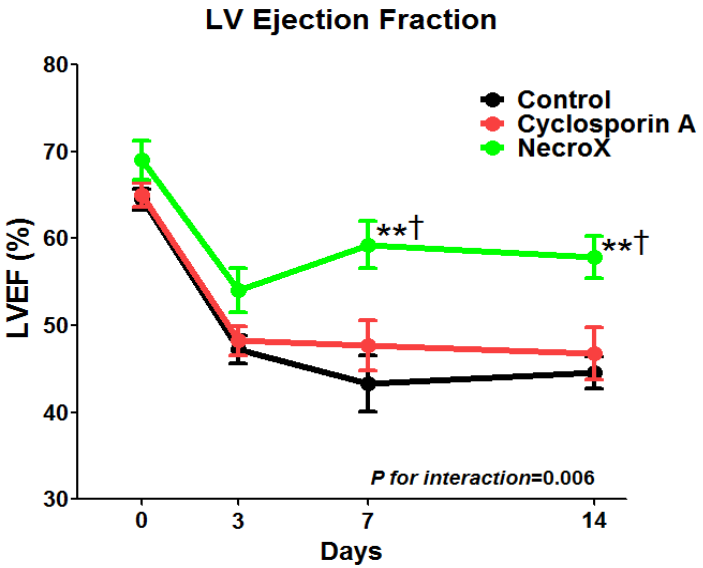
Harvest
After EchoCG & blood sampling

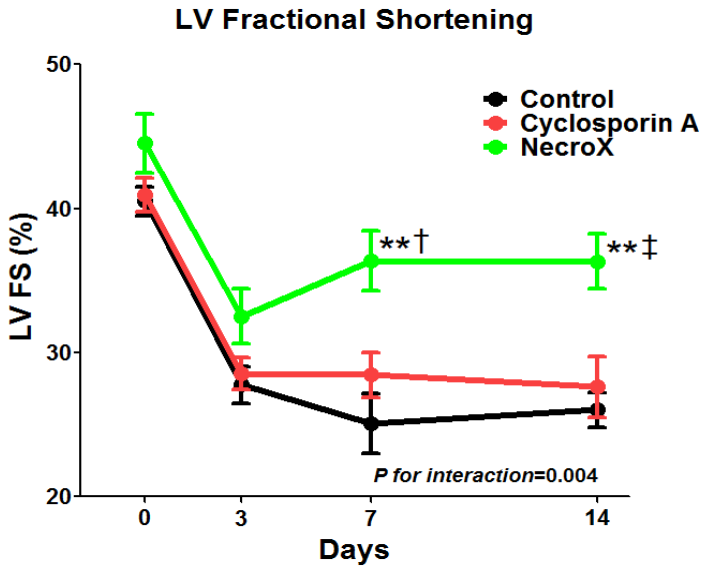
Formalin fixed tissue

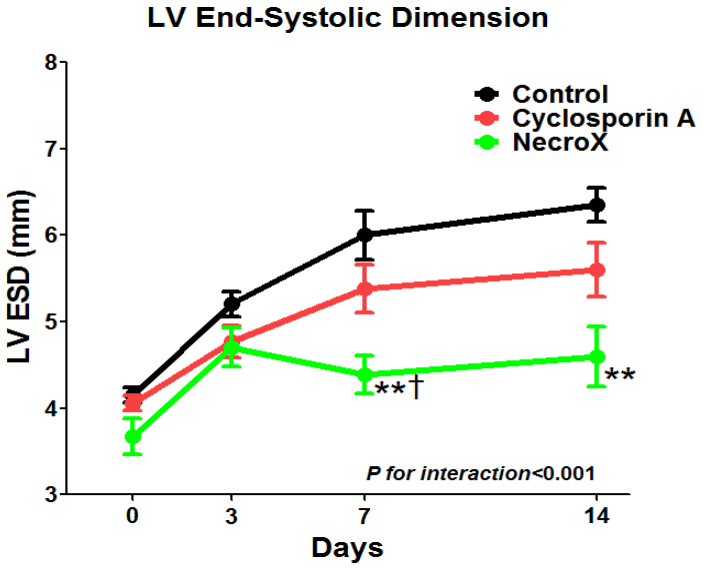
IV bolus infusion
Like as Preconditioning (N=13)

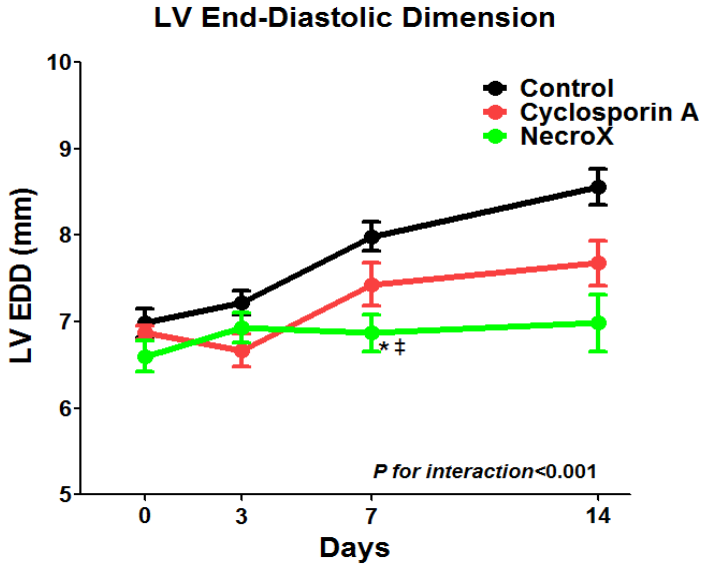
1. **Vehicle**
2. **Cyclosporin A** (CsA; 5 mg/kg)
   for positive control
3. **NecroX** (1 mg/kg)

NecroX preserved LVEF and inhibited LV remodeling









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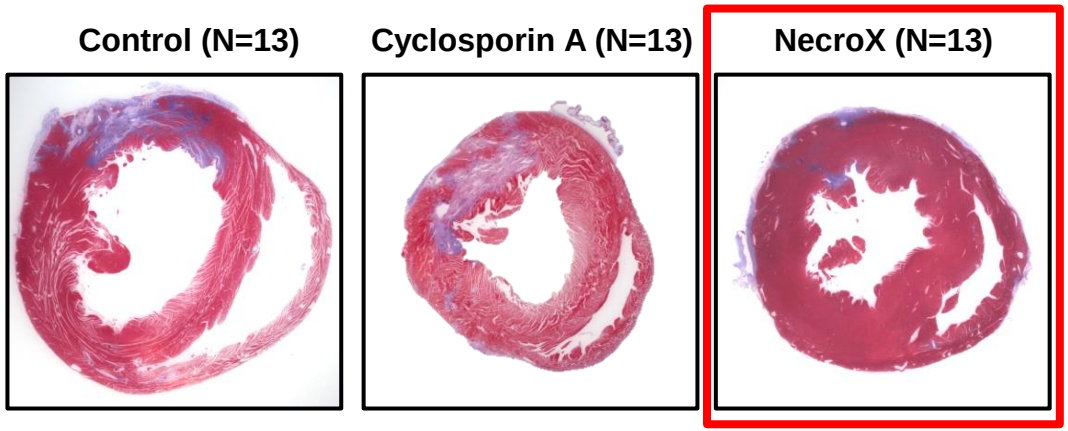
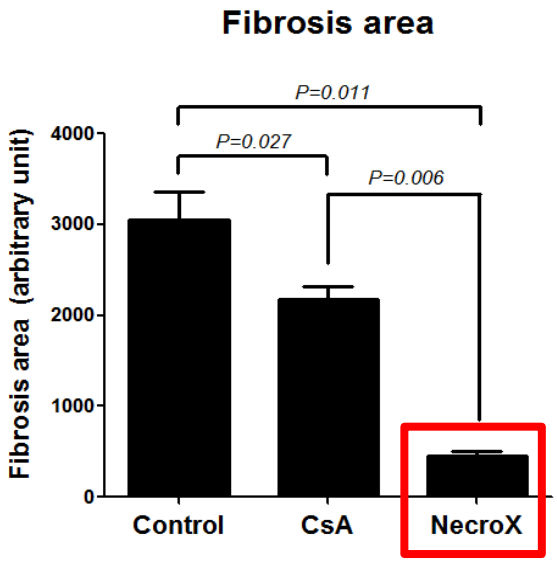
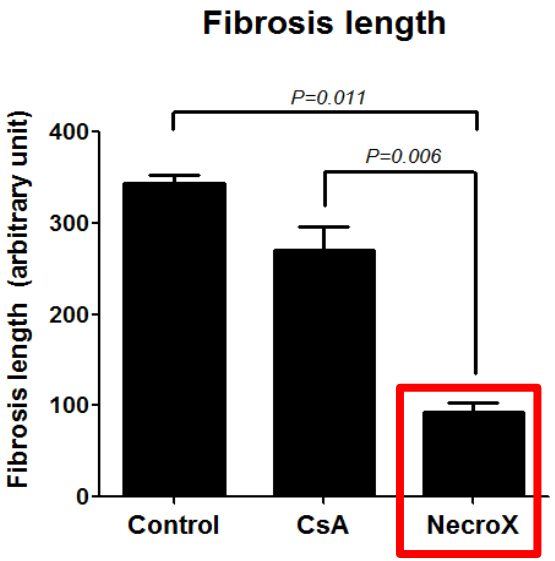
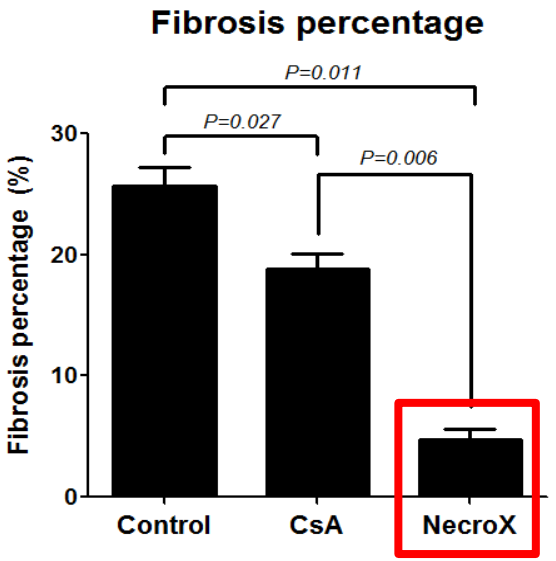
NecroX-treated rats: Reduction in Myocardial Fibrosis

Day 14 harvested heart









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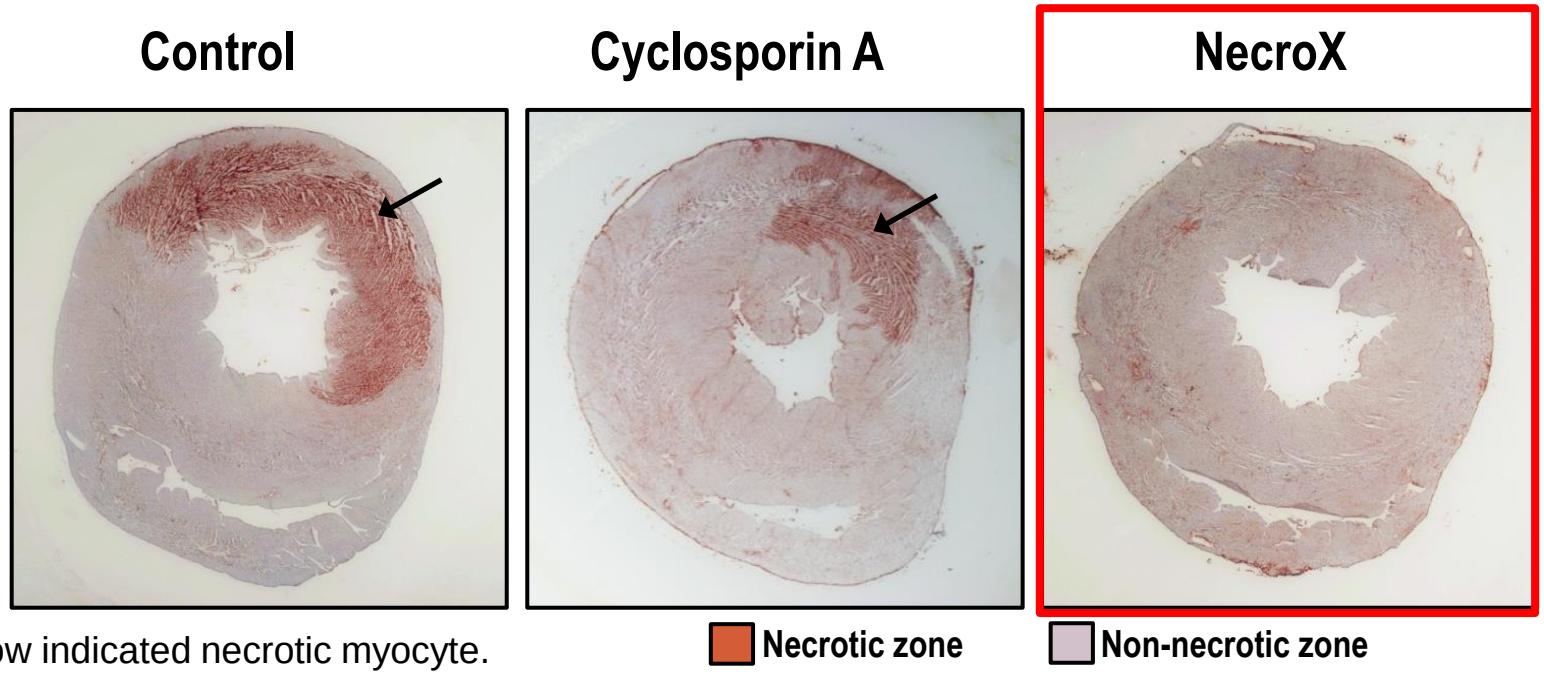
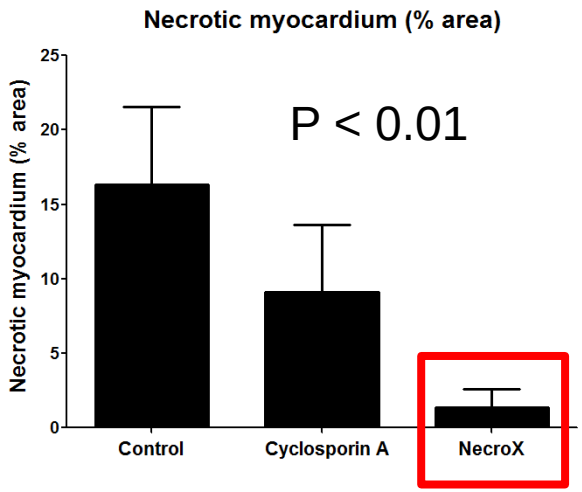
Necrotic myocardium quantification



Black arrow indicated necrotic myocyte.



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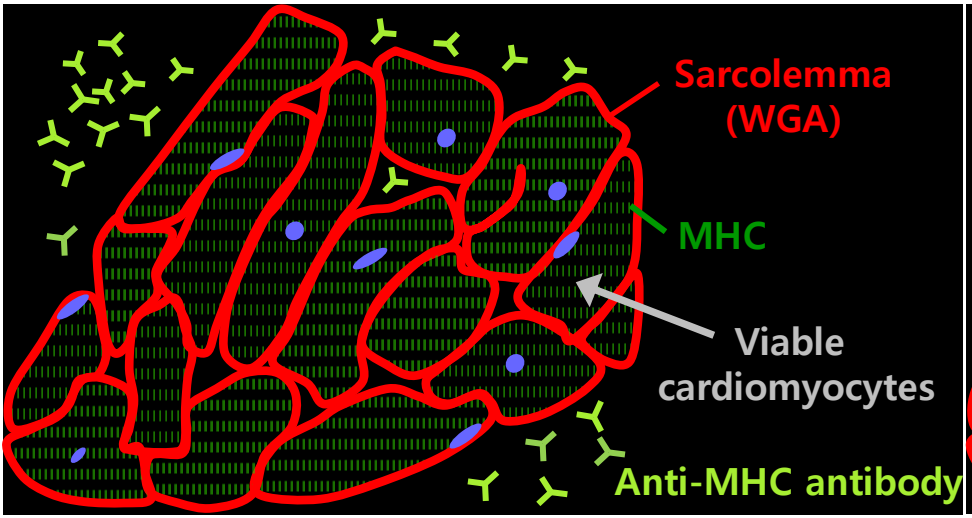
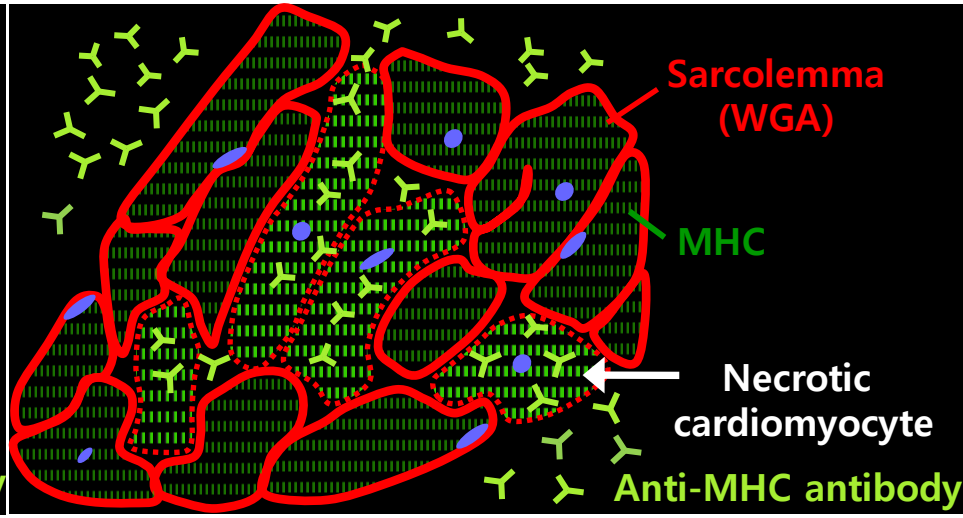
Necrotic myocardium specific quantification

In viable cardiomyocytes,
anti-MHC Ab cannot bind the cardiomyocytes.

Necrotic cardiomyocytes bind to anti-MHC antibody through broken sarcolemma.





Mechanism of anti-MHC antibody

WGA : Wheat germ agglutinin for sarcolemma stain
MHC : myosin heavy chain

Specific Quantification for Myocardial Necrosis

Ischemia by Ligation of LAD

20min. Before Ligation	45min. of Ischemia		12 hours
	25min.	20min.	

Anti-MHC antibody injection via internal jugular vein

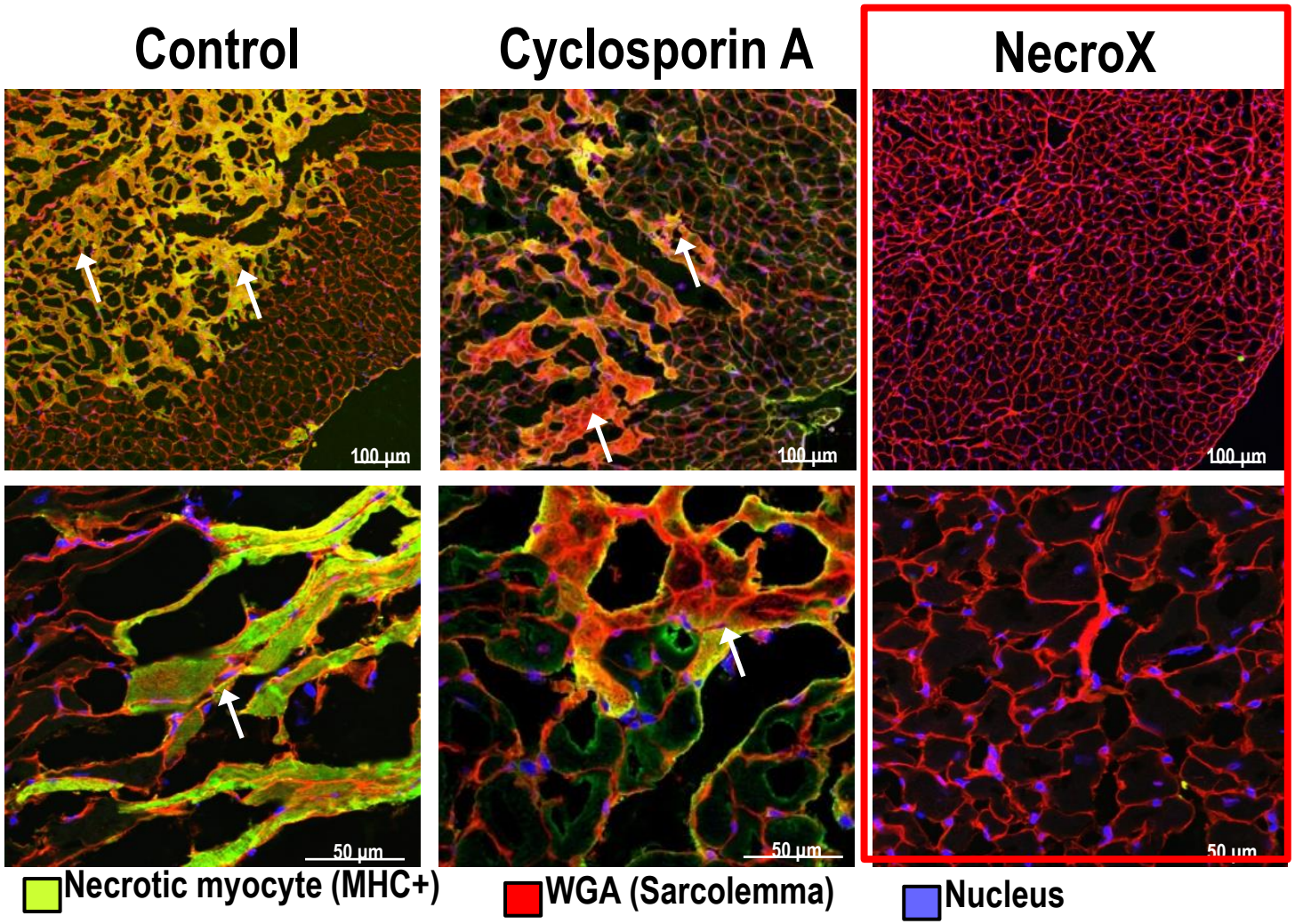
Reperfusion

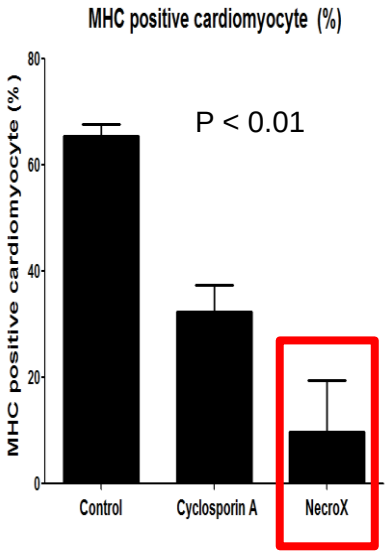
Harvest & OCT embedding

IV bolus infusion
Like as Preconditioning (N=4)
1. Vehicle
2. Cyclosporin A (CsA; 5 mg/kg)
3. NecroX-7 (1 mg/kg)

➔ **Why the anti-MHC Ab get injected before the ligation ?**

Necrotic myocardium specific quantification





White arrow indicated necrotic myocyte.

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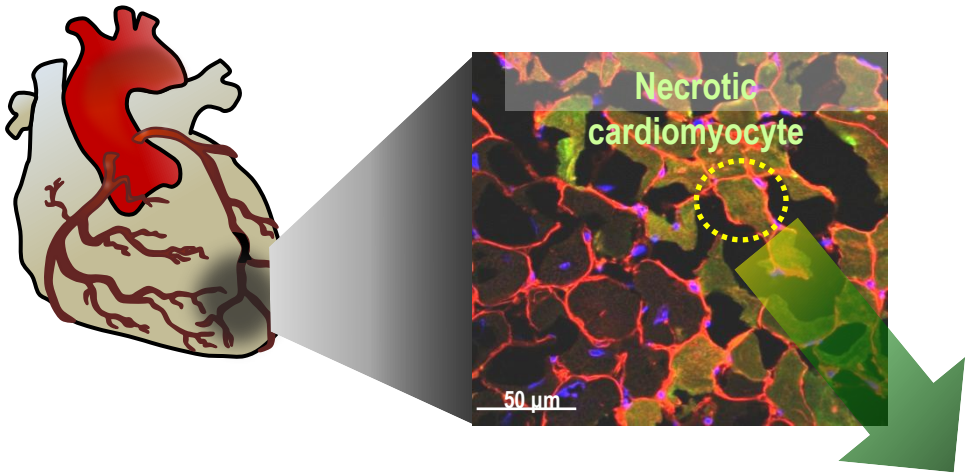
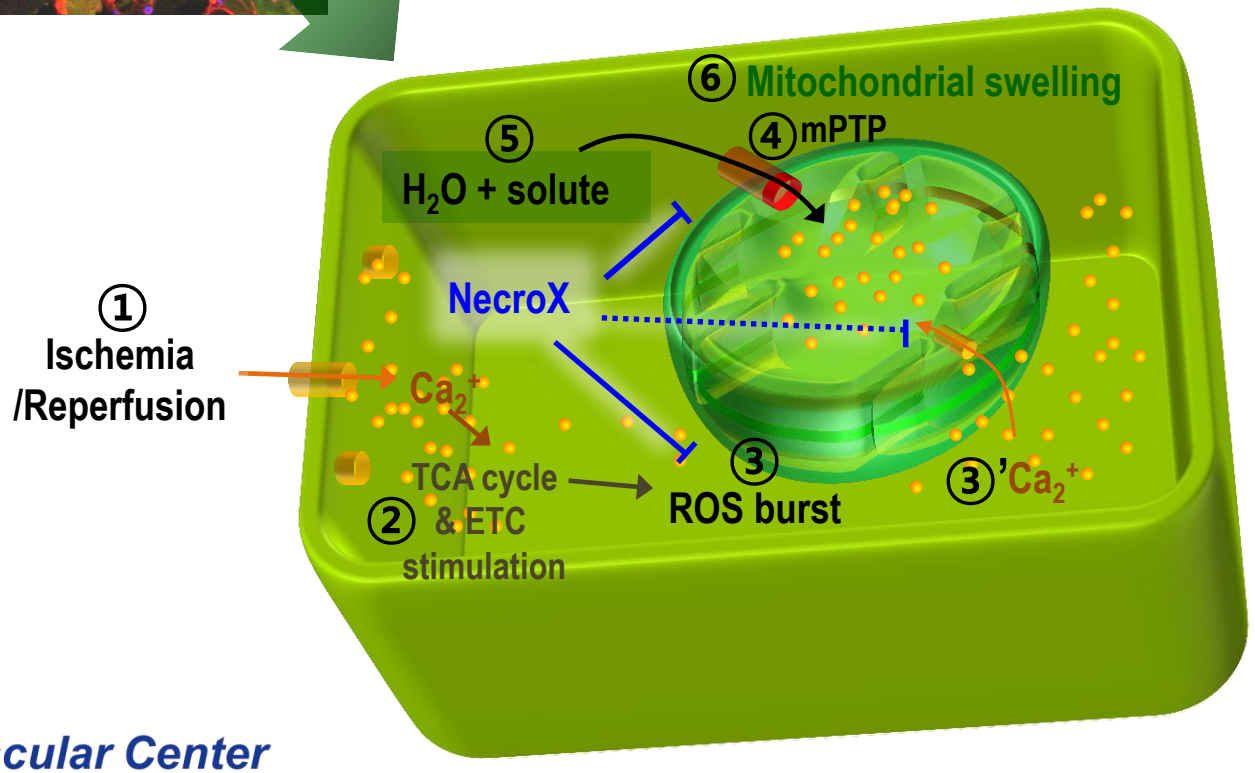
Mechanism of NecroX on I/R injury



Necrotic cardiomyocyte



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Clinical Trial: NEXsteMI RCT

Clinical trials…

Phase I trial : successfully finished !

Phase II multicenter RCT : on going

Phase I clinical trial

ClinicalTrials.gov
A service of the U.S. National Institutes of Health

Example: "Heart attack" AND "Los Angeles"
Search for studies: Search
Advanced Search | Help | Studies by Topic | Glossary

Find Studies | About Clinical Studies | Submit Studies | Resources | About This Site

Home > Find Studies > Search Results > Study Record Detail Text Size

Trial record **1 of 1** for: LC28-0126
Previous Study | Return to List | Next Study

Study to Investigate the Safety and Pharmacokinetic Characteristics of LC28-0126 in Healthy Male Subjects

This study is not yet open for participant recruitment.

Verified November 2012 by LG Life Sciences

Sponsor:
LG Life Sciences

Information provided by (Responsible Party):
LG Life Sciences

ClinicalTrials.gov Identifier:
NCT01737424

First received: November 27, 2012
Last updated: November 28, 2012
Last verified: November 2012
History of Changes

Full Text View | Tabular View | No Study Results Posted | Disclaimer | How to Read a Study Record

Purpose

To investigate the safety, tolerability and pharmacokinetic characteristics of **LC28-0126** in healthy male subjects

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Phase II NEXsteMI RCT

- **[NEXsteMI]**
- NECRO-X to reduced reperfusion injury
- after emergent PCI in patients with STEMI

- Multicenter,
  Randomized,
  Double-blinded,
  Placebo-controlled
- Efficacy & safety of single bolus IV infusion of Necro-X
  Just before emergent PCI for patients with STEMI
- Area under curve of cardiac enzyme
- Dose-finding

Phase II NEXsteMI RCT

기관 번호	실시 기관명	시험책임자
01	경상대학교병원	황 진 용 교수
02	경희대학교병원	김　원 교수
03	서울대학교병원	김 효 수 교수(CI)
04	연세대학교 세브란스병원	최 동 훈 교수
05	전남대학교병원	정 명 호 교수

Phase II NEXsteMI RCT

Number of Planned Patients = 60

4 Gr	Treatment group	No. of Pt.
Control	Placebo 1 vial	15
Tx 1	LC28-0126 1mg	15
Tx 2	LC28-0126 3mg	15
Tx 3	LC28-0126 10mg	15

Phase II NEXsteMI RCT

[1st screening criteria]

1) Age : 20 ~ 75

2) Onset of chest pain < 12 h

3) ST elevation > 0.1mV in two/more precordial leads

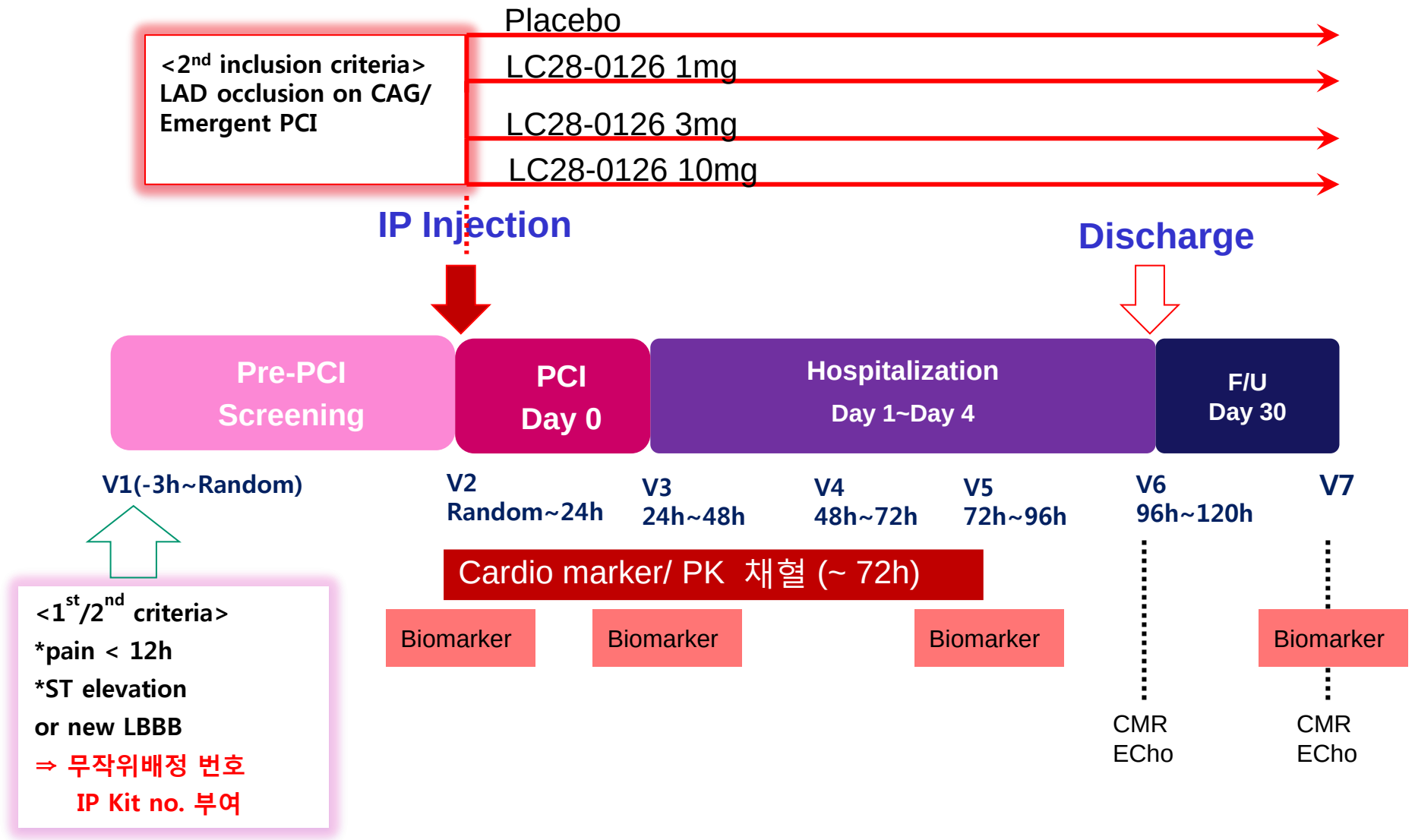
or new LBBB

[2nd inclusion criteria]

Occlusion at P.LAD or M.LAD (TIMI flow = 0 or 1)

Scheduled for emergent Primary PCI

Phase II NEXsteMI RCT



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Phase II NEXsteMI RCT

Primary Efficacy Endpoint

PCI 후 **72시간까지의 CK-MB의 AUC**

Secondary Efficacy Endpoint

1) PCI 후 **72시간까지의 Troponin I의 AUC**

2) PCI 후 **72시간까지의 CK 의 AUC**

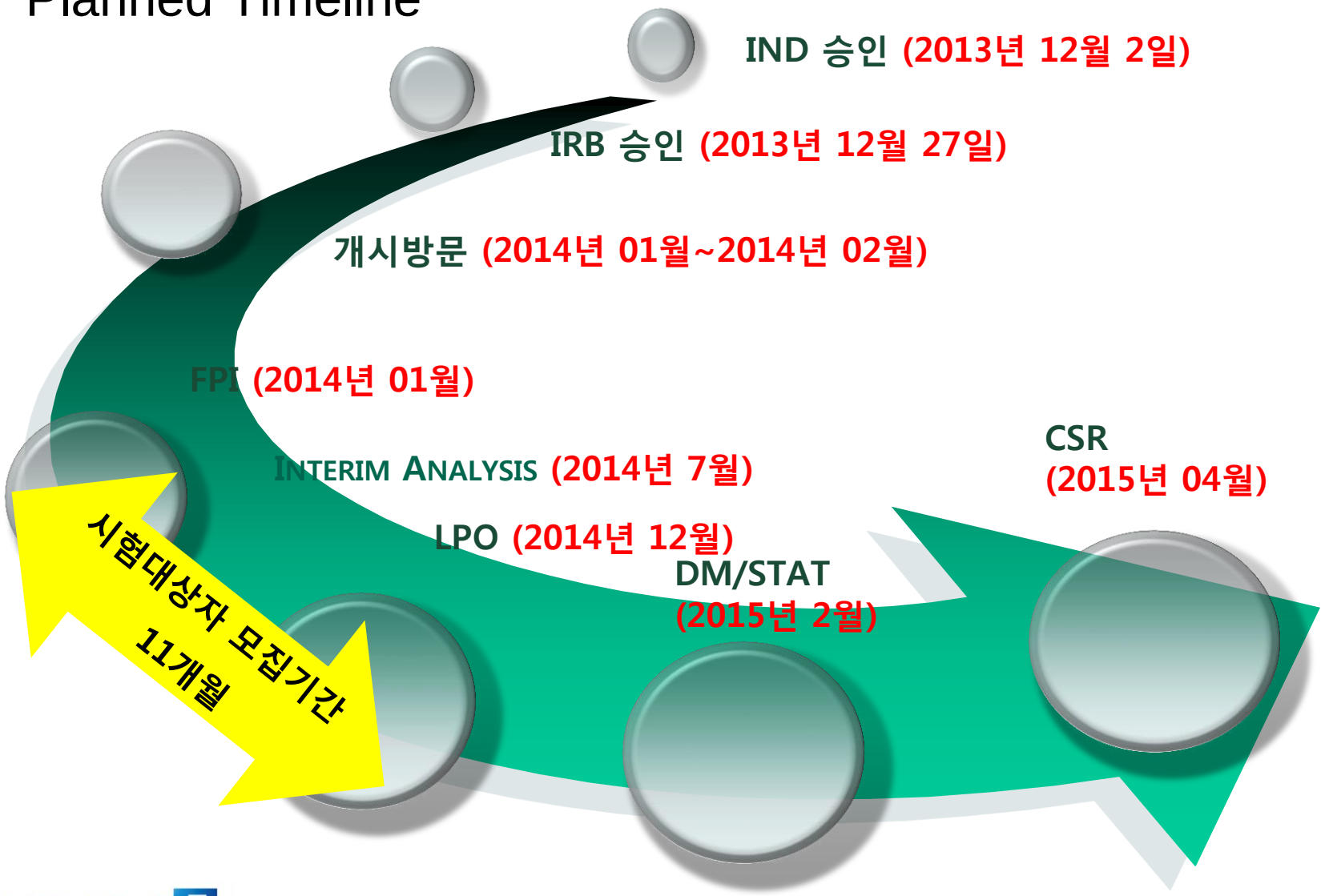
3) PCI 후 Day 4, 30에 **CMR**로 평가된 **Infarct size**

4) PCI 후 Day 4, 30에 **CMR**로 평가된 **LV function (LVEF, LVEDV, LVESV)**

5) PCI 후 Day 4, 30에 **심장초음파**로 평가된 **LV (LVEF,LVEDV, LVESV)**

Phase II clinical trial: NEXsteMI RCT

- Planned Timeline



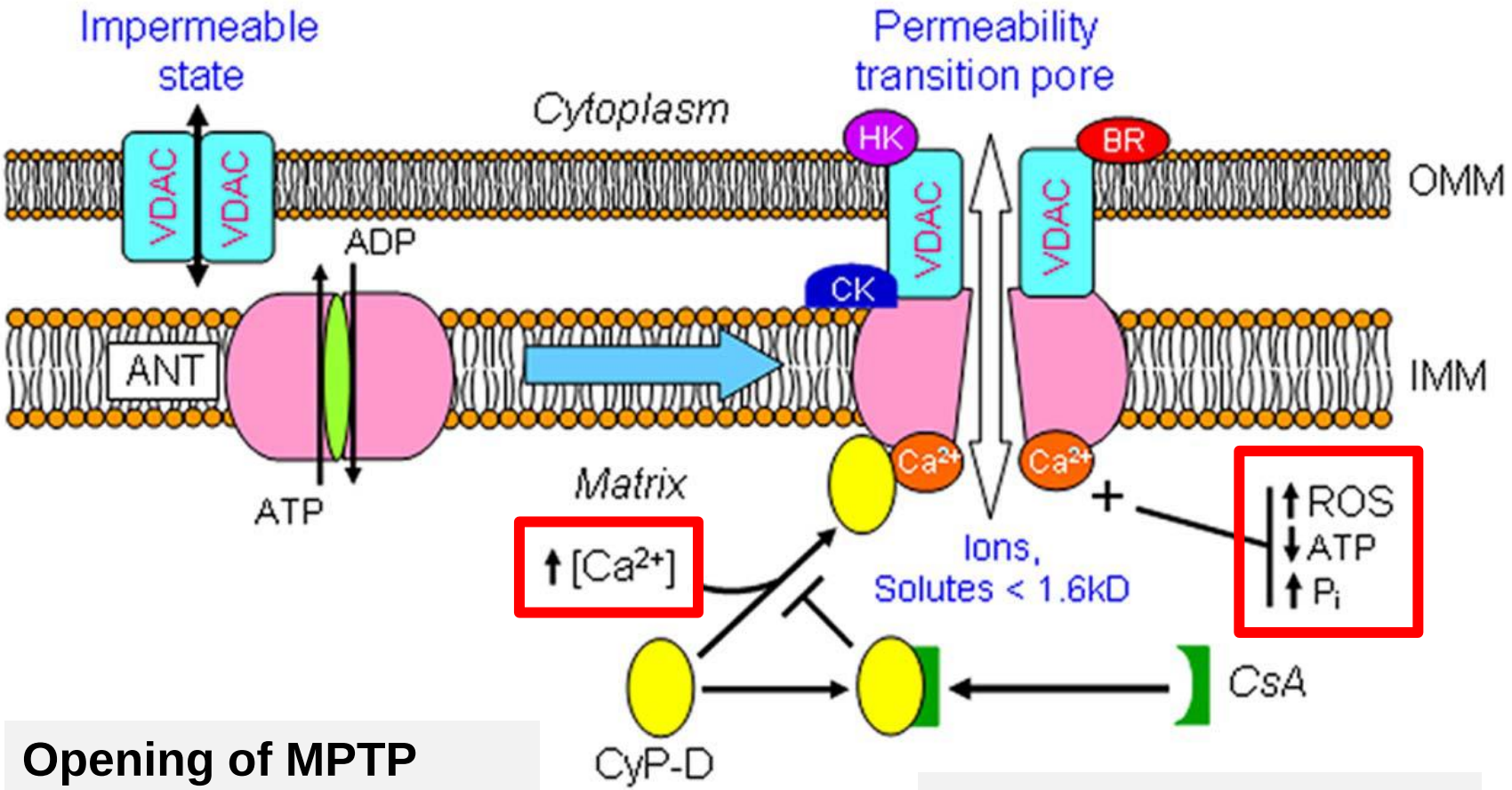
Novel Necrosis Inhibitor to prevent Myocardial Ischemia-Reperfusion Injury

Hyo-Soo Kim, MD/PhD/FAHA

Cardiovascular Center & Department of Internal Medicine,
Seoul National University Hospital

SNUH Cardiovascular Center

Structure and Mechanism of MPTP



Opening of MPTP

- Triggered by
  - ✓ **ROS overload**
  - ✓ **Ca^{2+} overload**
  - ✓ **pH normalization**

Opening of MPTP

- ✓ **Loss of ATP**
- ✓ **Mitochondrial swelling**
- ➔ **Necrotic cell death**

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Cell Physiol Biochem 2007;20:01-22

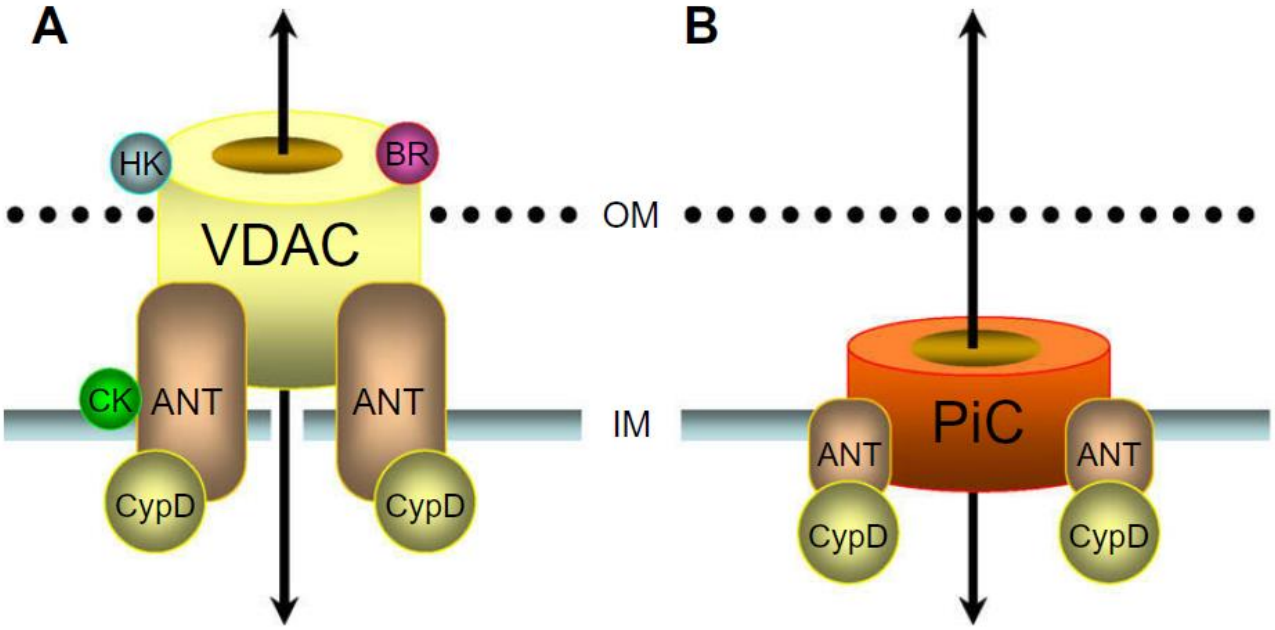
Structure and Mechanism of MPTP

Molecular components of MPTP

- Cyclophilin D (CypD)
- Adenine nucleotide translocase (ANT)
- Voltage-dependent anion channel (VDAC)
- Benzodiazepine receptor (BR)
- Creatine kinase (CK)
- Hexokinase (HK)

Opening of MPTP

- Triggered by
  - ✓ **ROS overload**
  - ✓ **Ca^{2+} overload**
  - ✓ **pH normalization**
- **Consequences**
  - ✓ **Loss of ATP**
  - ✓ **Mitochondrial swelling**
  - ➔ **Necrotic cell death**



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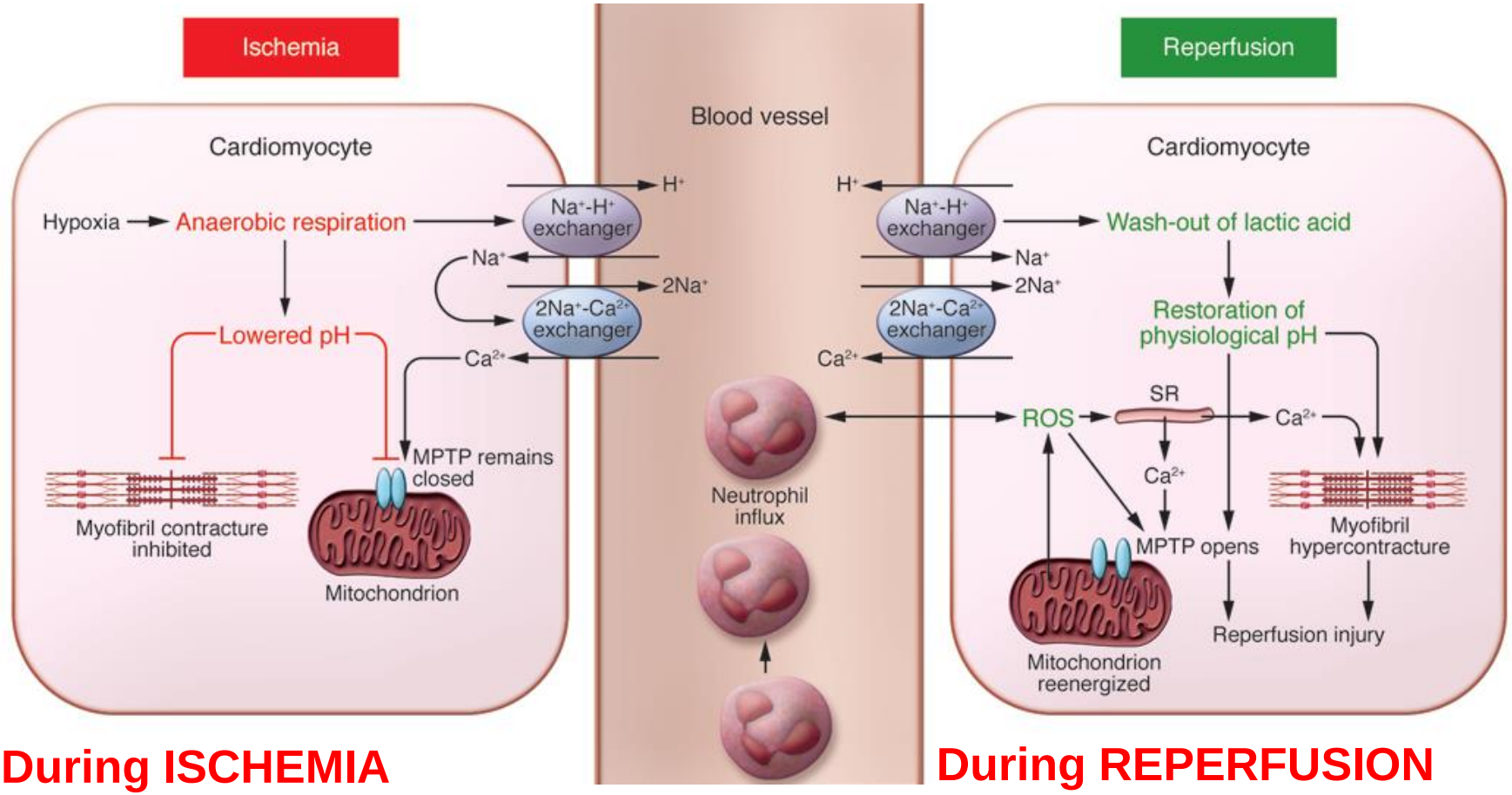
FEBS Lett. 2010;584(10):2161-6.

MPTP in Myocardial Ischemia-Reperfusion Injury



During ISCHEMIA

- Inhibited Ox-Phos
  ➔ ATP depletion & Acidic pH
- Minimal Mitochondrial Ca^{2+} uptake
- MPTP: closed

During REPERFUSION

- Mitochondrial ROS generation
- Mitochondrial Ca^{2+} overload
- Normalization of pH
- MPTP opening ➔ Cell Death

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J Clin Invest. 2013;123(1):92–100.

Myocardial Ischemia-Reperfusion Injury : Neglected Therapeutic Target

Prevention of Myocardial Reperfusion Injury

- Cardioprotection Trials

- **Aims: To reduce the amount of necrosis after myocardial ischemia-reperfusion.**
- **No cardioprotective interventions have been included in guidelines or clinical practice.**

	Intervention	Target	Patients (n)	Outcome
Ion flux or metabolism				
EMIP-FR	Trimetazidine	Glucose metabolism	19725	No difference in mortality at 35 days
MAGIC	Magnesium	Membrane stabilisation	6213	No difference in mortality at 30 days
		Metabolism	120	Percentage reduction in myocardial infarct size (as % of myocardium at risk), by MRI at 4 days
		Metabolism	20201	No difference in mortality at 30 days
		Sodium accumulation	430 (stage 1); 959 (stage 2)	No difference in myocardial infarct size, by enzyme
	infusion for 6 h	GLP1 receptor	107	Increase in myocardial salvage index at 90 days, by MRI
	infusion	ATP-sensitive potassium channel	545	No difference in myocardial infarct size, by enzyme or 6 month left-ventricular ejection fraction
	4 h infusion	Complement	5745	No difference in mortality at 30 days
		Inflammation	232	No difference in myocardial infarct size, by MRI at 5 days or 4 months
	h	Protein kinase C	1083	No difference in myocardial infarct size
		Natriuretic peptide receptor	569	15% reduction in myocardial infarct size, by enzyme and 2·0% absolute increase in left-ventricular ejection fraction
HEBE-III	Epoetin alfa	Protective kinases	529	No difference in left-ventricular ejection fraction at 6 weeks or size, by enzyme or troponin T
				-ventricular ejection fraction or myocardial infarct MRI
				ocardial infarct size at 6 days or 3 months, by MRI
				ocardial infarct size at 5–14 days, by SPECT

However, several are currently under investigation - Cyclosporin, TRO40303, NO, Metformin, etc

Notable forthcoming or ongoing studies				
CIRCUS	Ciclosporin	..	972	..
CYCLE	Ciclosporin	..	444	..
EMBRACE	Bendavia	..	200	..
MitoCare	TRO40303	..	180	..
DETO$_2$X-AMI	Oxygen	..	6600	..
PRESERVATION 1	IK-5001	..	306	..
MVO	Vasodilators	..	297	..
GIPS-III	Metformin	..	380	..
NOMI	Nitric oxide	..	230	..

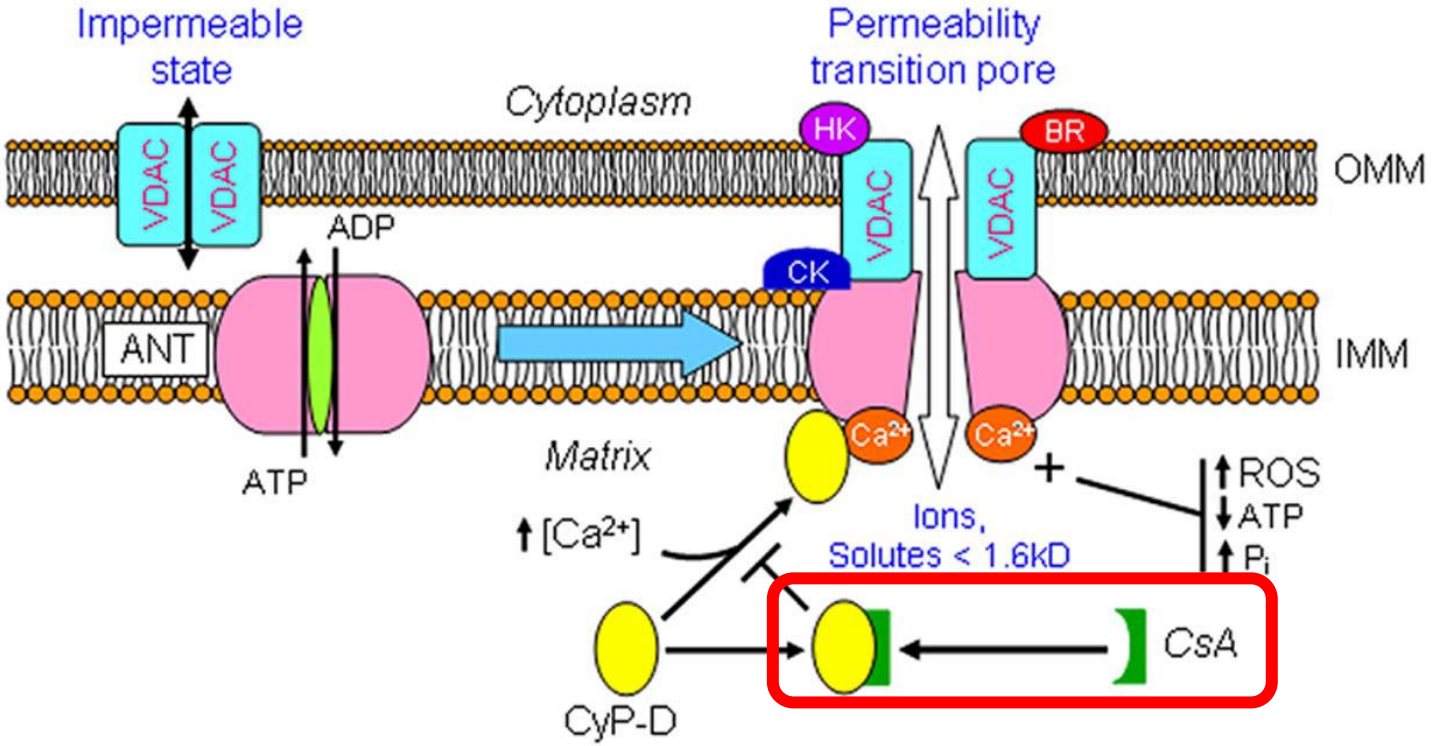
References or NCT identifiers of trials are provided in the appendix (pp 1–2). PCI=percutaneous coronary intervention. STEMI=ST-segment elevation myocardial infarction. TIMI=thrombolysis in myocardial infarction.

Table 3: Cardioprotection trials of more than 100 STEMI patients

Lancet 2013; 382: 644–57

Prevention of Myocardial Reperfusion Injury

- **Cyclosporin A (CsA; inhibitor of MPTP opening)**
- **Mechanism of Action**
  - CsA binds to & inhibits cyclophilins
  - CsA/CypD complex inhibits MPTP opening



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Cell Physiol Biochem 2007;20:01-22

NECRO-X DATA IN OUR LAB

- **Hypothesis / Aim of study**
  - To reduce the infarct size of the heart after ischemia-reperfusion injury by using the necrosis inhibitor, NecroX-7
  - The necrosis inhibitor will reduce the necrotic cell death and the infarct size in the *in vitro* and *in vivo* models of myocardial ischemia-reperfusion injury

In vitro evaluation

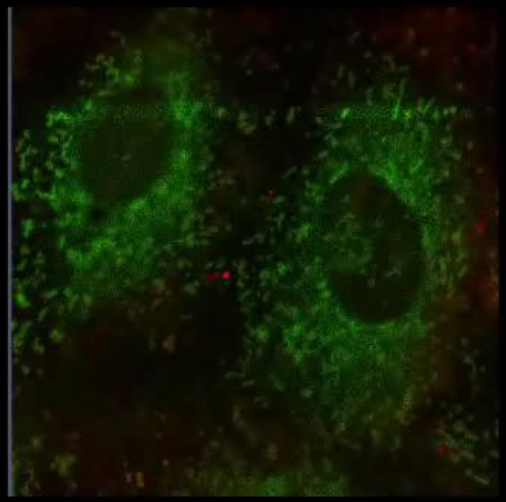
1. Mitochondria swelling measurement (Turbidity)
2. Mitochondria swelling using Confocal Images
3. TEM for Mitochondria swelling and Nucleus
4. PI / FDA staining and cell count
5. FACS analysis using PI / Annexin-V
6. Mitochondria membrane potential measurement using JC-1
7. Western blot for necrotic/apoptotic signaling pathways
8. Ca^{2+} influx in mitochondria under hypoxia-oxidative stress/reoxygenation stress
9. ROS scavenging activity measurement (DHR 123, DCF-DA)
10. NADPH oxidase activity measurement

Measurement of mitochondrial Ca^{2+} influx

Under hypoxia-oxidative stress/reoxygenation condition

Vehicle (3~5 minutes)

[Vehicle-treated H9C2 cells]

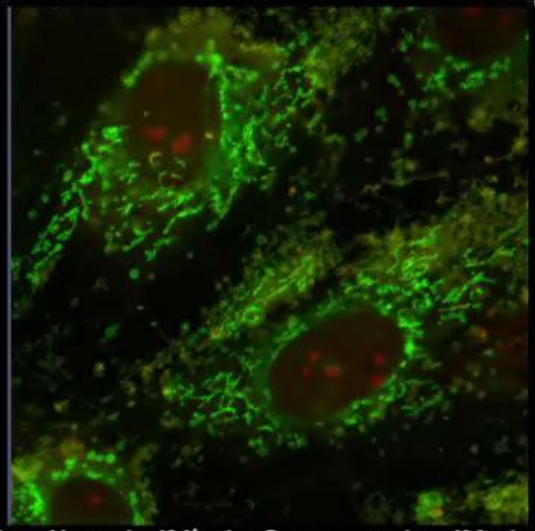


* Condition: Hypoxia (24hrs) - Reoxygenation (90mins)
* Red (Rhod-2 fluorescence): free Ca2+ level
* Green (Mitotracker): mitochondrial shape

H9C2 cells pretreated with vehicle (DMSO) exhibiting rapid increase in Ca2+ influx and mitochondrial swelling.

Necrosis Inhibitor (over 30minutes)

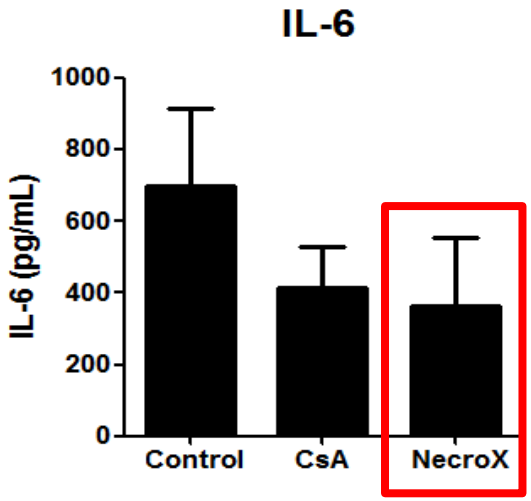
[NecX-treated H9C2 cells]

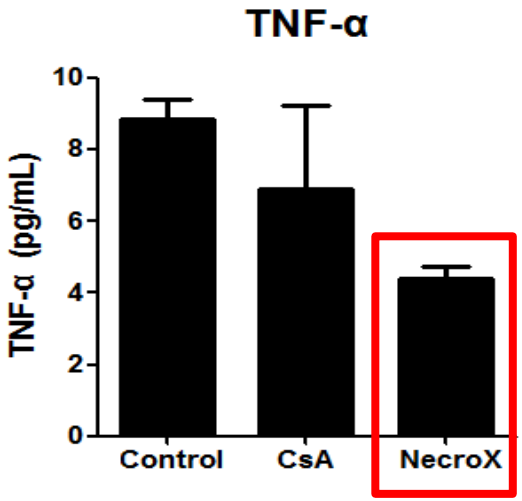


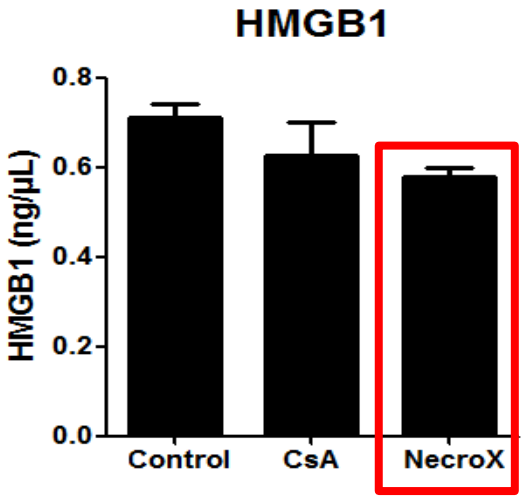
* Condition: Hypoxia (24hrs) - Reoxygenation (90mins)
* Red (Rhod-2 fluorescence): free Ca2+ level
* Green (Mitotracker): mitochondrial shape

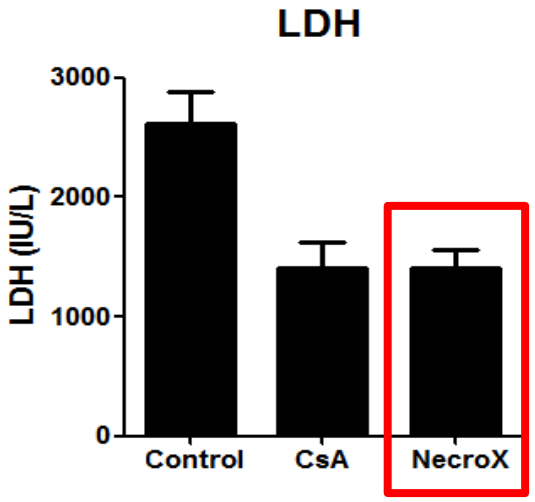
H9C2 cells pretreated with necrosis inhibitor (NecX) showing minimal increase in Ca2+ influx and preserved mitochondrial shapes.

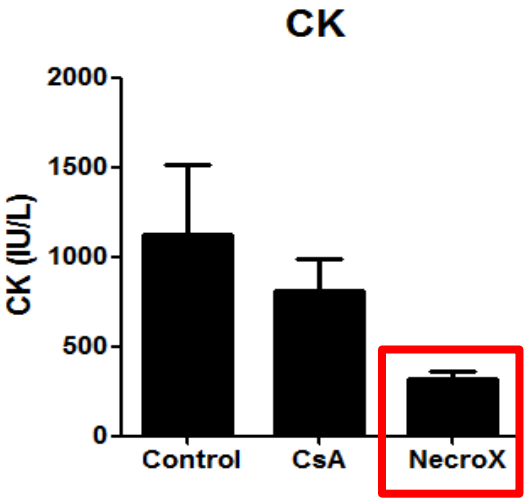
Measurement of inflammatory cytokine & cardiac enzymes at 12 hours after I/R injury

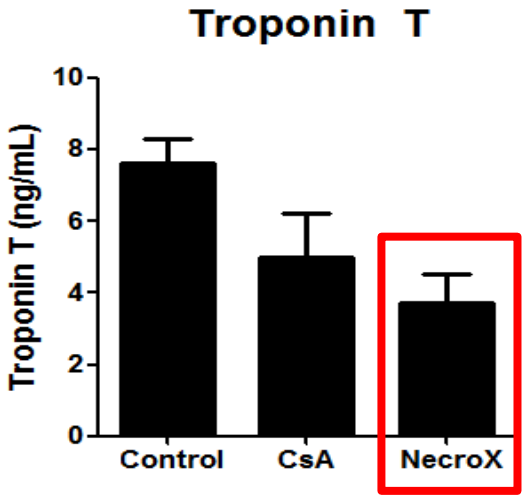












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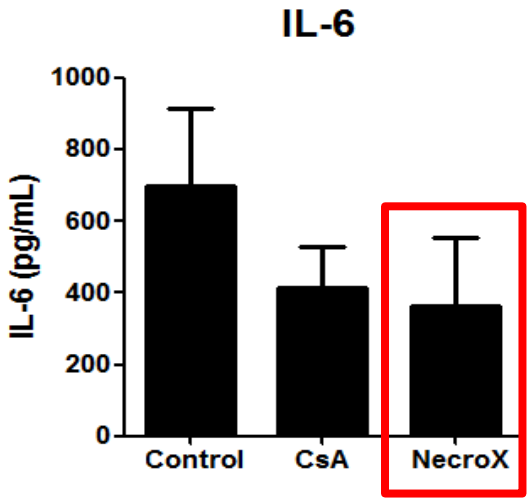
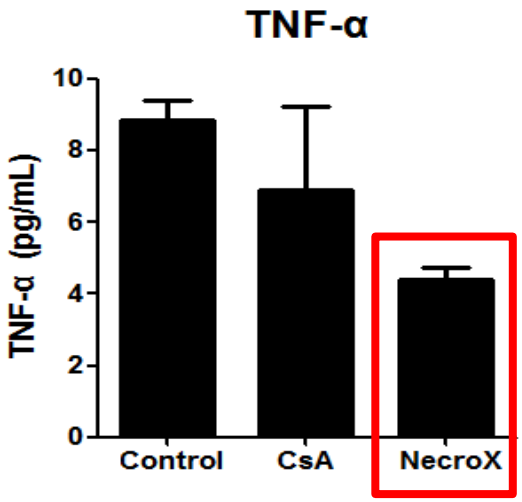
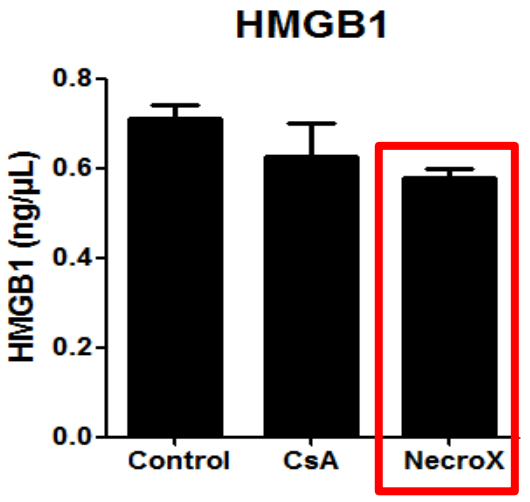
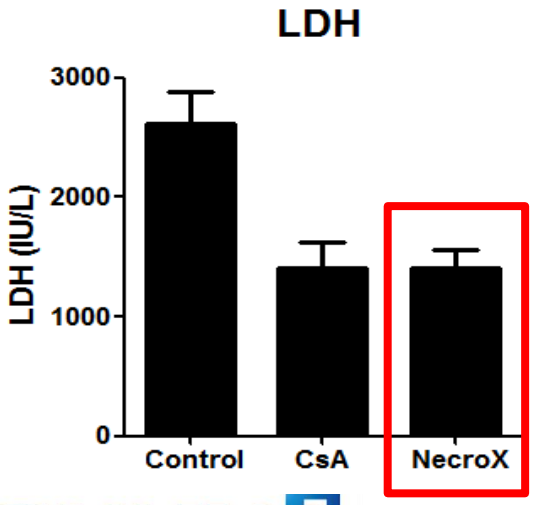
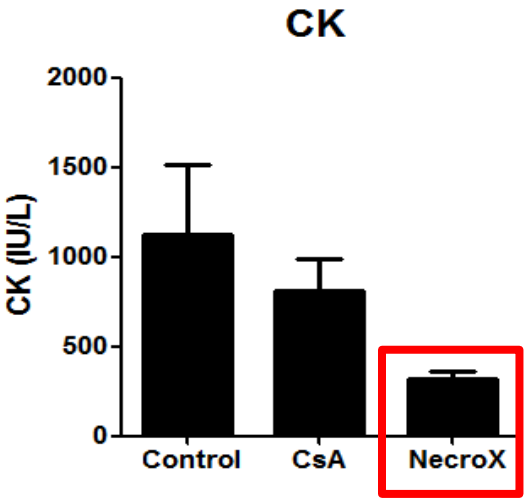
Inflammatory cytokines & Cardiac enzymes were significantly lower in the NecroX-treated rats.

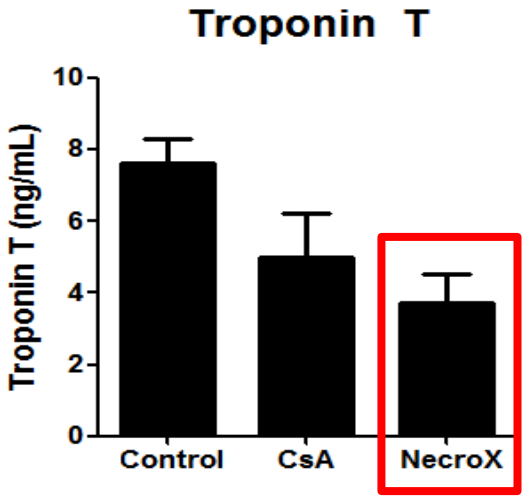












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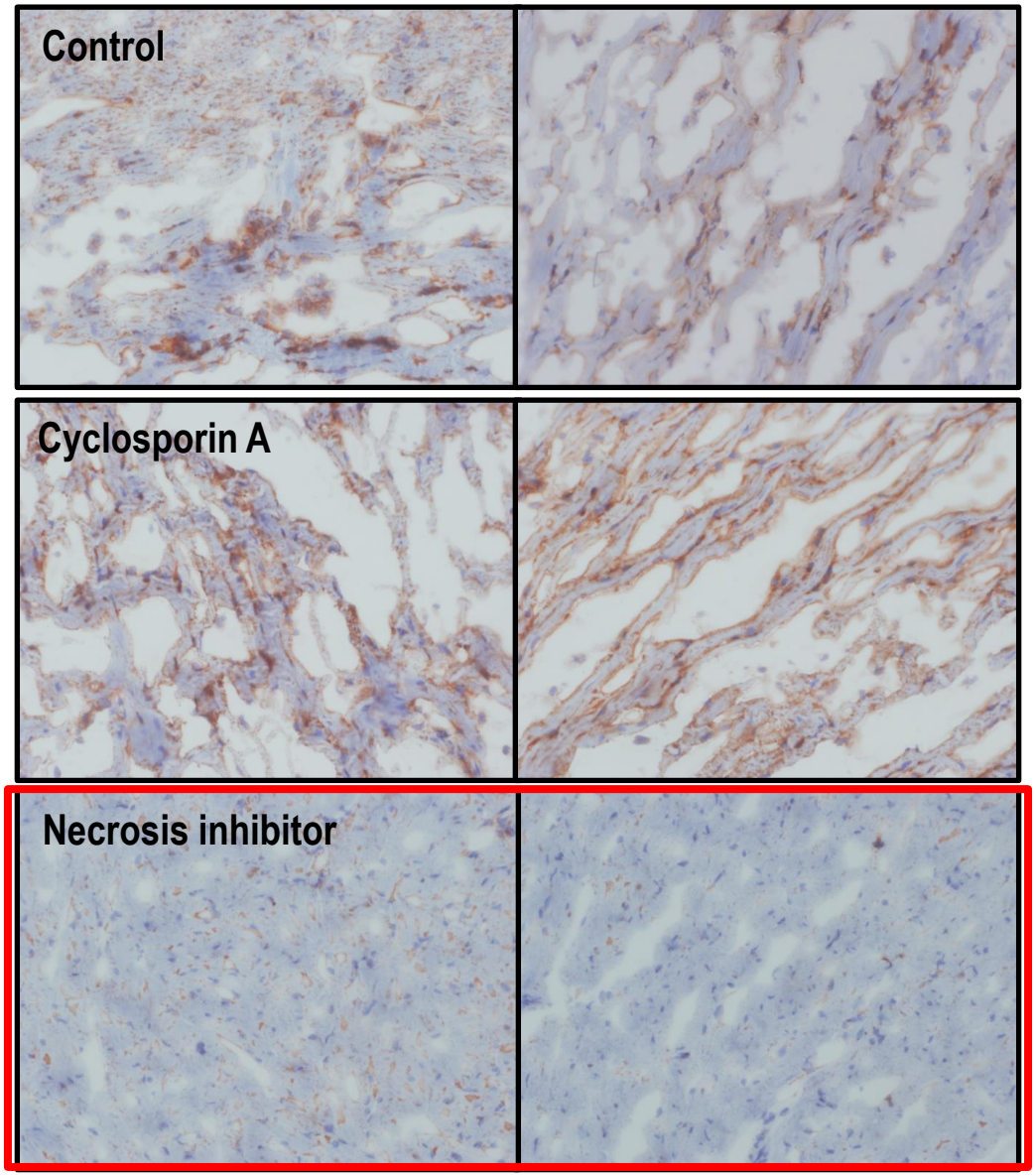
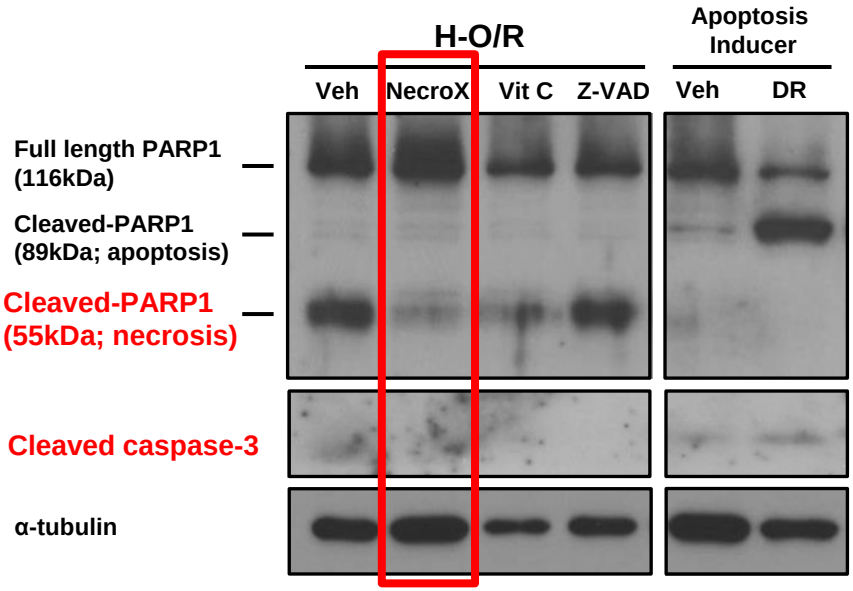
IHC for HMGB1 at 12hr after I/RI

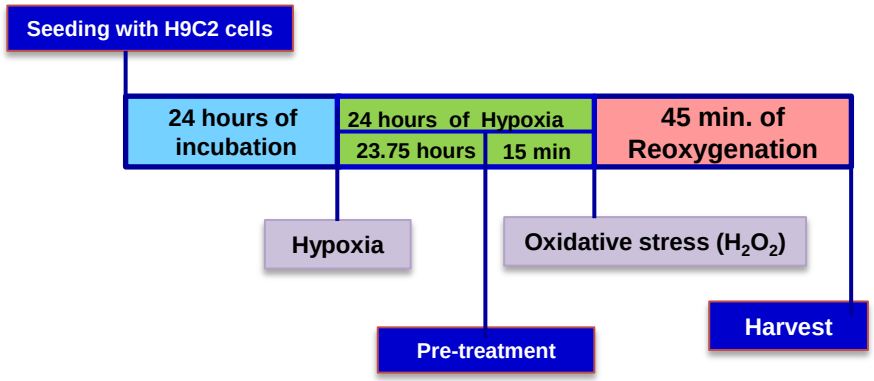


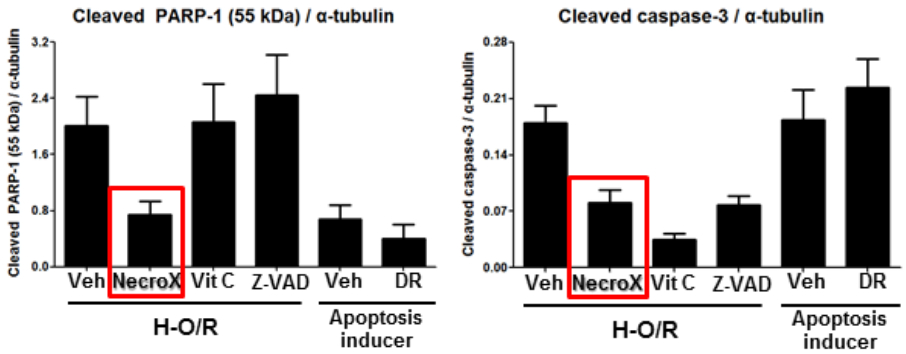
Brownish stained HMGB1

Why 12 hours after MI/R injury?
: Necrosis is early process in MI/R

NecroX blocked the necrotic signaling pathways



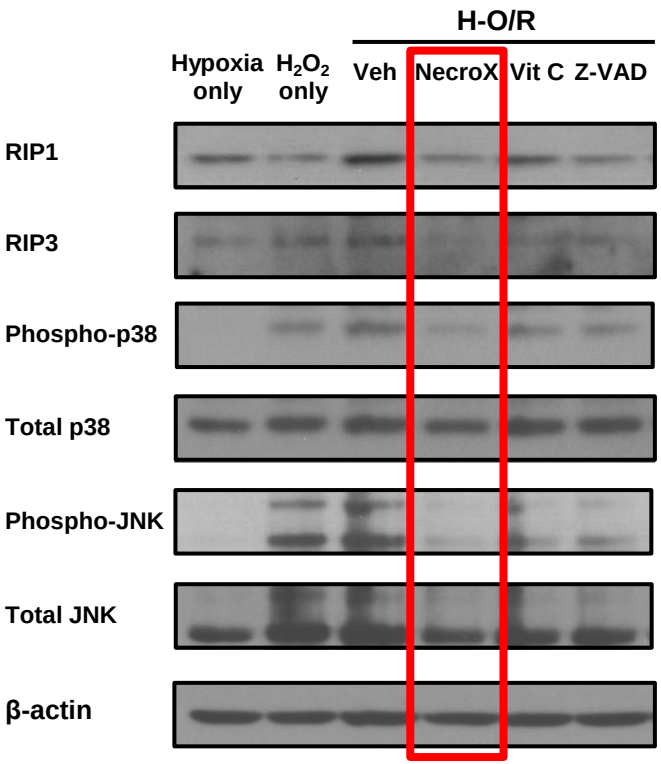


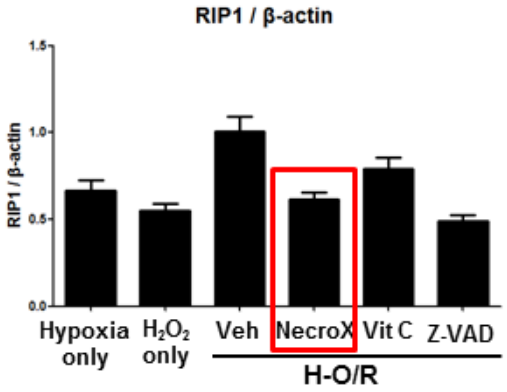


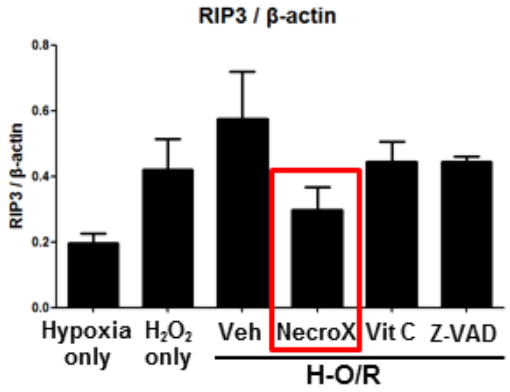
- **Cleaved-PARP1 (55kDa), the necrotic fragment was significantly expressed in the Vehicle-treated H9C2 cells under H-O/R stress.**
- **The necrotic fragment was reduced by NecroX.**
- **The main mechanism of cell death after H-O/R stress was not apoptosis but necrosis that was prevented by NecroX.**

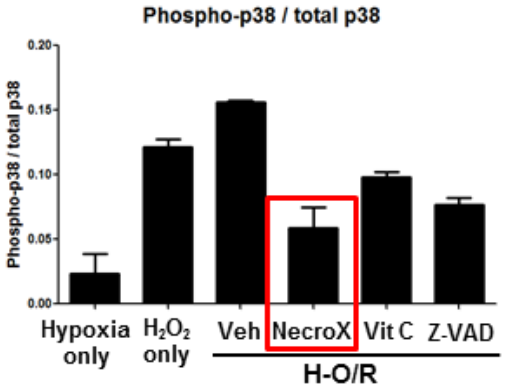
Veh: Vehicle
DR: Doxorubicin (apoptosis inducer)
H-O/R: Hypoxia-Oxidative stress/Reoxygenation

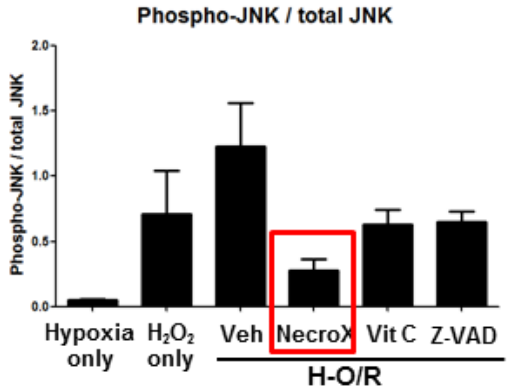
NecroX blocked the necrotic signaling pathways











- **Necrotic signal pathways were effectively blocked by the treatment with NecroX.**

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Veh: Vehicle
H-O/R: Hypoxia-Oxidative stress/Reoxygenation