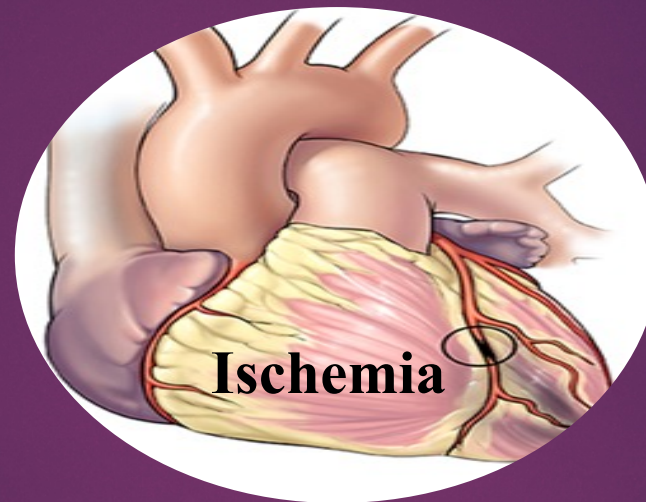


Cyclocreatine Phosphate: A Bioenergetic to Restore Cardiac Function in Animal Models of AMI, Cardiopulmonary Bypass, and Heart Transplantation



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Outline

- 1. Cyclocreatine (CCr) and Cyclocreatine Phosphate (CCrP) - *Background***
 - 1. Mechanism of Action**
 - 2. Bioenergetic Compounds**
- 2. Cardioprotection By CCr and CCrP - *Preclinical Studies***
 - 1. *Warm Ischemia:* Acute Myocardial Infarction**
 - 2. *Cold Ischemia:* Cardiopulmonary Bypass Surgery and Heart Transplantation**
- 3. *Preventive Therapy to Protect Hearts Against Ischemic Injury***
 - 1. Heart Transplantation**
 - 2. High-Risk Cardiopulmonary Bypass**
 - 3. High-Risk Interventional Cardiology**

1. Background:

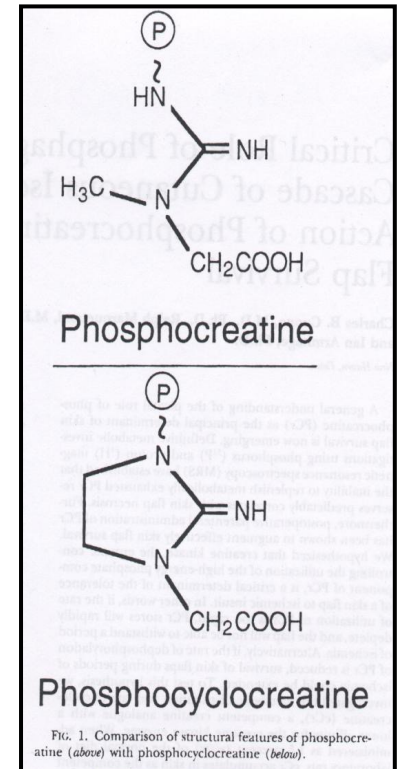
Cyclocreatine (CCr) & Cyclocreatine Phosphate (CCrP)

Creatine (Cr)

- Creatine is necessary for contractility
- Creatine Phosphate (CrP) is the source of P for ADP
- CrP stops working at low acidity in ischemic hearts

Cyclocreatine (CCr) & Cyclocreatine Phosphate (CCrP)

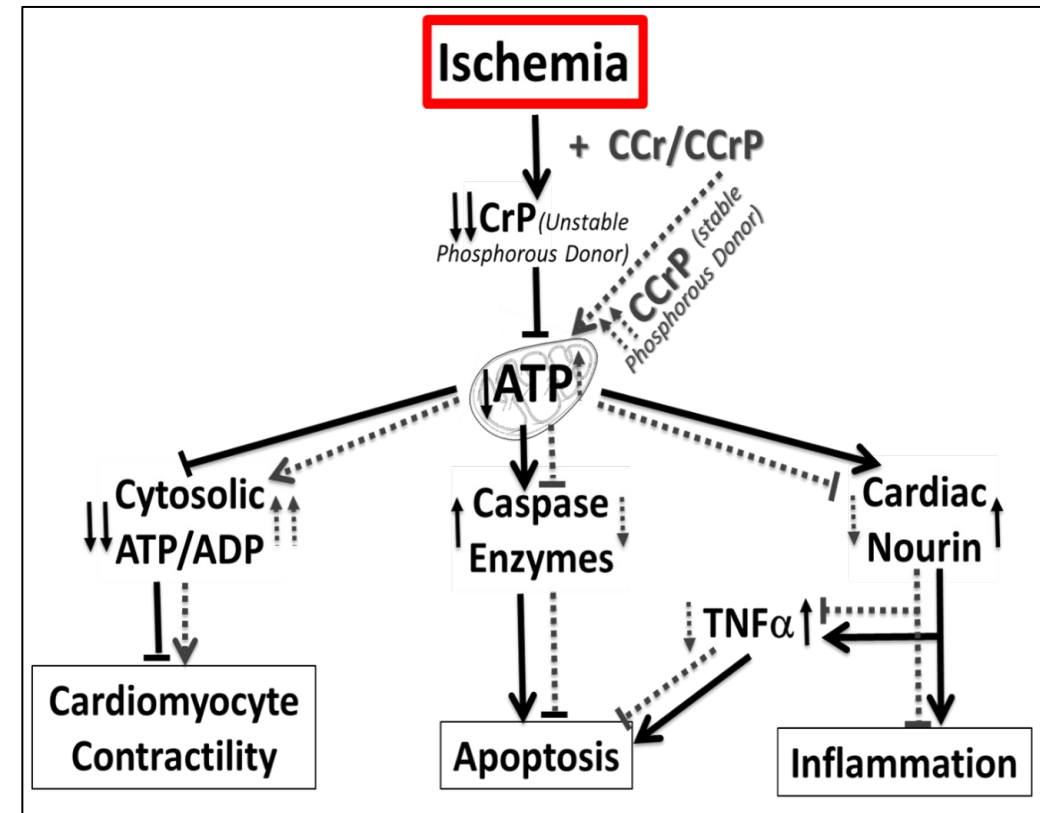
- CCr is a synthetic analogue of Creatine
- CCrP is more stable and superior than creatine phosphate in phosphorylating ADP to ATP during ischemia at low acidity
- CCrP continues to synthesize ATP during ischemia



What is Cyclocreatine?

A Novel “Bioenergetic” Compound!

- ▶ Ischemia causes irreversible myocardial injury through depletion of cellular ATP
- ▶ Cyclocreatine Phosphate (CCrP) is a “bioenergetic” compound maintains elevated cellular ATP during ischemia
- ▶ Preservation of cellular ATP by CCrP administration:
 - ▶ Protected against myocardial ischemic injury and:
 1. Reduced the inflammatory mediator, *Nourin*
 2. Reduced post-ischemic cardiac inflammation
 3. Reduced myocardial apoptosis and cell injury
 - ▶ Restored cardiac function immediately after reperfusion in animal models of:
 - Warm and cold ischemia



2.1a CCr and Warm Ischemia - AMI Model

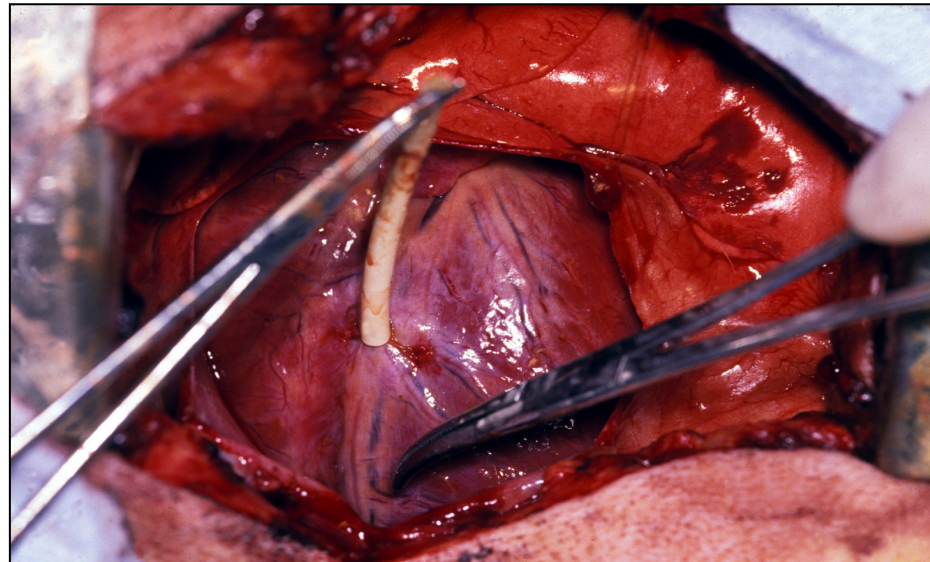
5

CCr Protects Canine Hearts Against Ischemic Injury and Restores Strong Cardiac Function During Early Reperfusion



Elgebaly SA, Poston R, Todd R, Helmy T, Almaghraby A, Elbayoumi T, Kreutzer DL.: Cyclocreatine Protects Against Ischemic Injury and Enhances Cardiac Recovery During Early Reperfusion. *Expert Review of Cardiovascular Therapy*, Volume 17(9), 683-697, 2019 (Review).

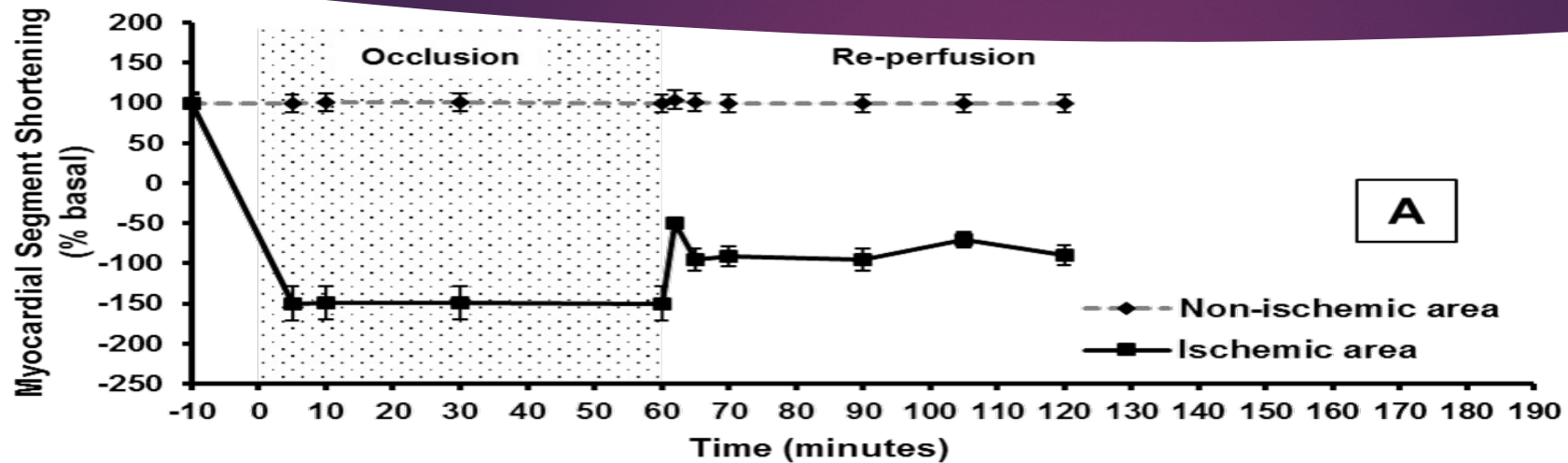
CCr and Warm Ischemia: Intact Canine Model of LAD Occlusion-Reperfusion



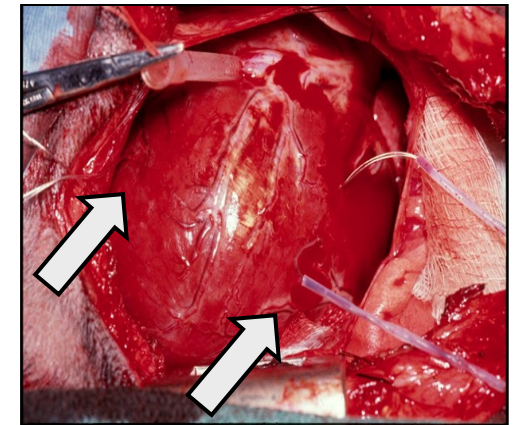
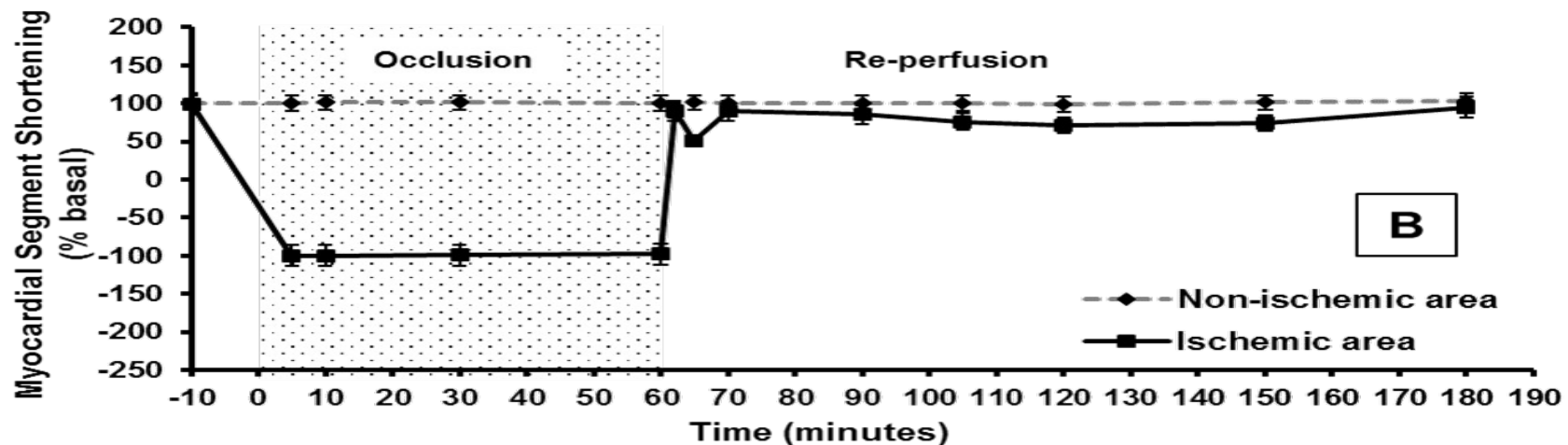
Cyclocreatine Injected IV once 1 hour before LAD occlusion
- 1 hour LAD Occlusion
- 2 hours Reperfusion

CCr and Warm Ischemia:

Rapid Restoration of Contractile Function in CCr [B] Hearts,
While Control Saline [A] Hearts Did Not Recover



- Restoration of ATP in CCr hearts
- Depletion of ATP in Saline hearts



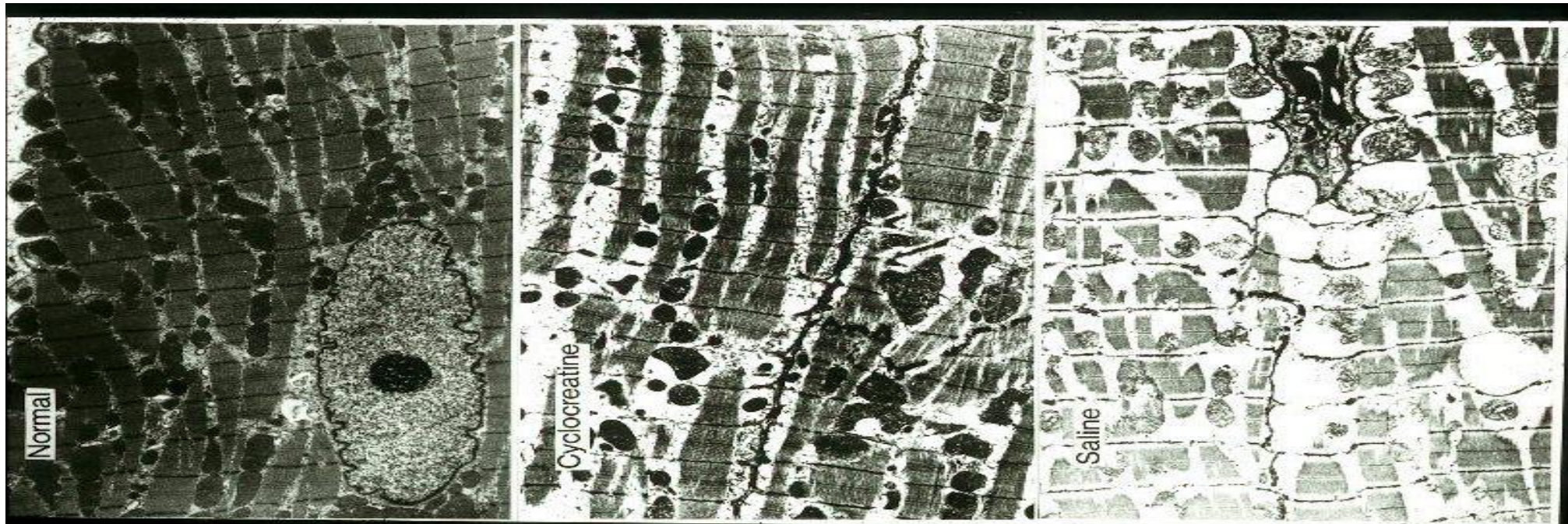
A: Saline
B: CCr

CCr and Warm Ischemia:
Less Myocardial Cell Injury in LAD/CCr Hearts
Compared to LAD/Saline Control

Normal Heart

LAD + Cyclocreatine

LAD + Saline



2.1b CCrP *and Warm Ischemia* – Global Arrest

9

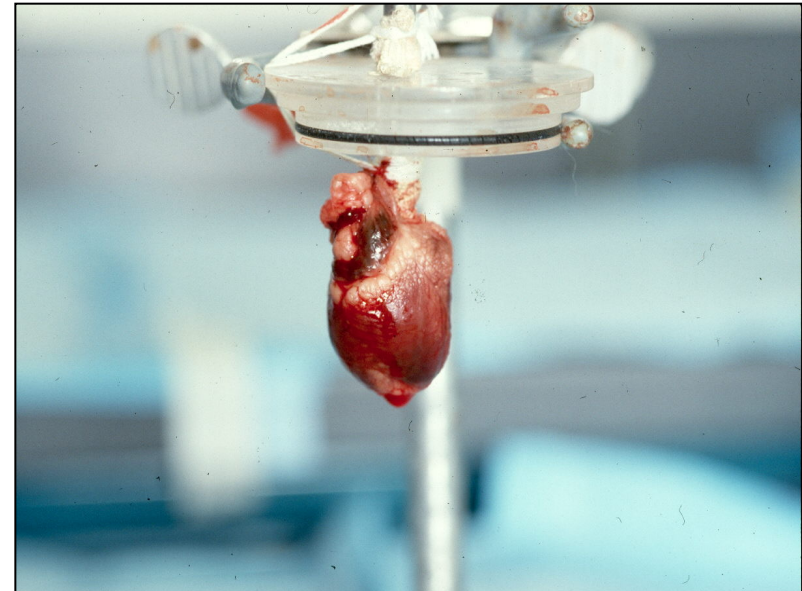
Rat Heart Model of Global Warm Cardiac Arrest



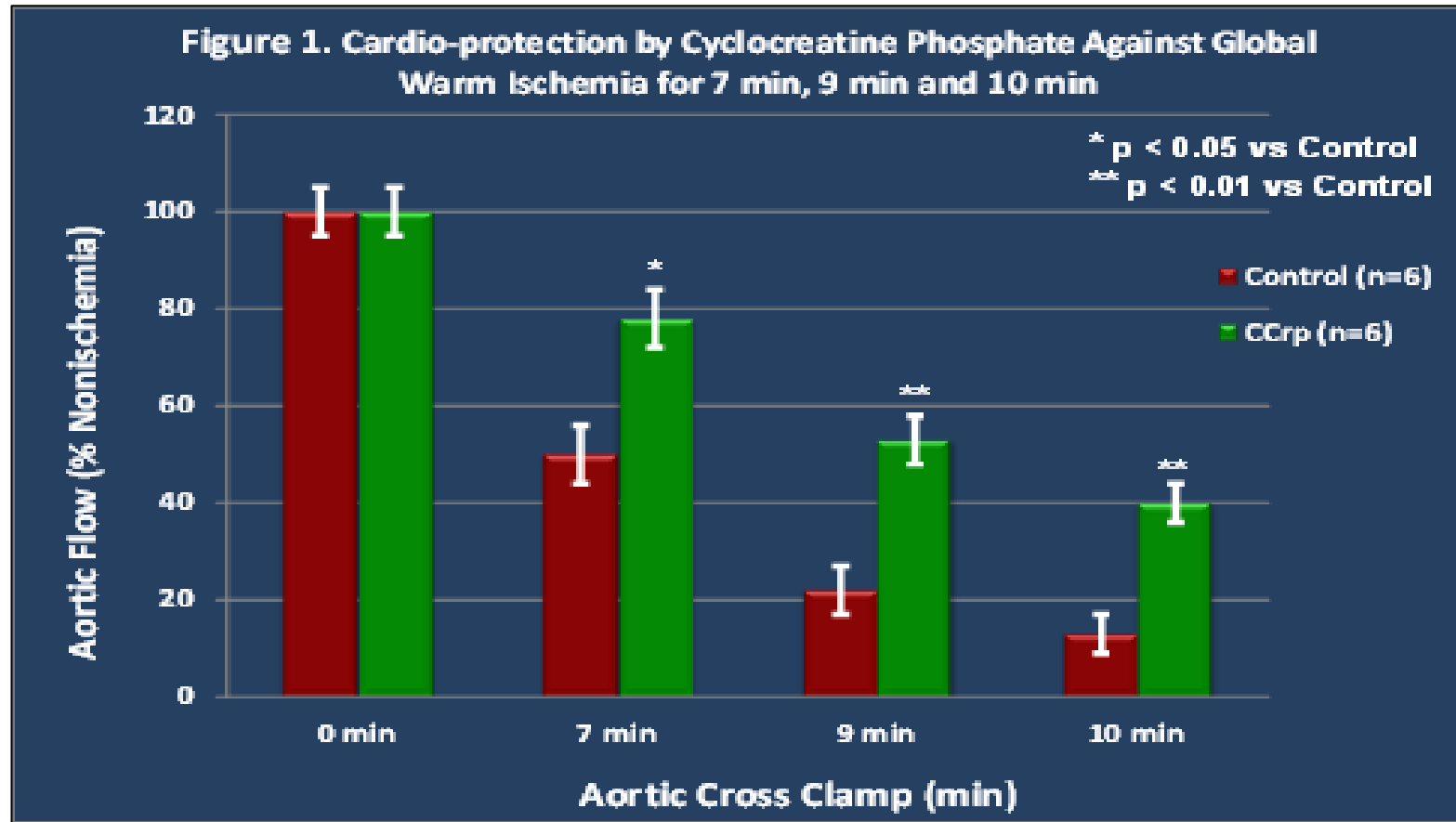
Elgebaly SA, Poston R, Todd R, Helmy T, Almaghraby A, Elbayoumi T, Kreutzer DL.: Cyclocreatine Protects Against Ischemic Injury and Enhances Cardiac Recovery During Early Reperfusion. *Expert Review of Cardiovascular Therapy*, Volume 17(9), 683-697, 2019 (Review).

CCrP and Warm Global Ischemia: Rat Model of Cardiac Arrest for 7, 9, and 10 Minutes

- CCrP injected IV once 1 hr. before heart arrest (n=21)
- Saline rats (n=21)
- Aortic cross clamping (warm global cardiac arrest) for:
 - 7 minutes
 - 9 minutes
 - 10 minutes
- Contractility on Langendorff apparatus for 30 minutes:
 - Aortic Flow



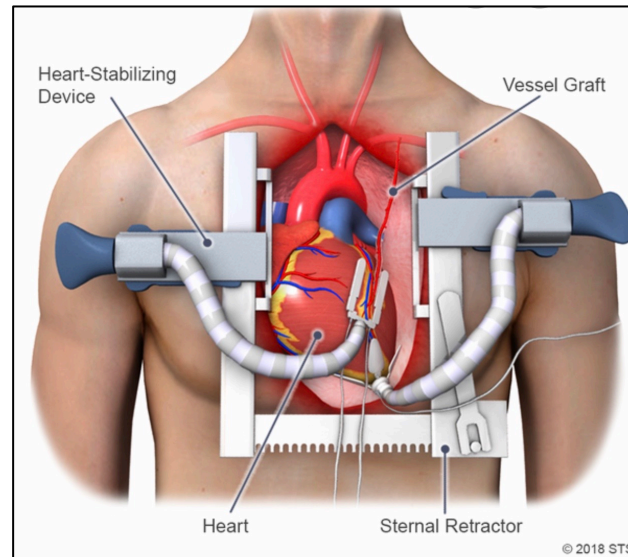
CCrP and Warm Global Ischemia: Significantly Stronger Recovery of Cardiac Function in CCr Rat Hearts Compared to Saline Controls



2.2 CCr and Cold Ischemia - Bypass Model

12

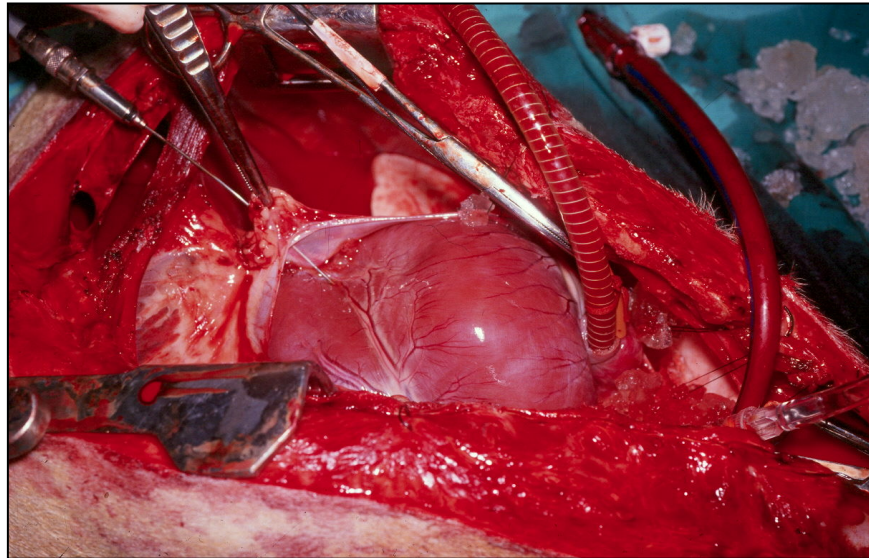
CCr Protects Canine Hearts Against Ischemic Injury Resulting in Strong Cardiac Function During Early Reperfusion



Elgebaly SA, Poston R, Todd R, Helmy T, Almaghraby A, Elbayoumi T, Kreutzer DL.: Cyclocreatine Protects Against Ischemic Injury and Enhances Cardiac Recovery During Early Reperfusion. *Expert Review of Cardiovascular Therapy*, Volume 17(9), 683-697, 2019 (Review).

CCr and Cold Ischemia:

Dogs Underwent Cold Cardioplegic Arrest for 1 and 3 Hours Followed By Reperfusion for 4 Hours

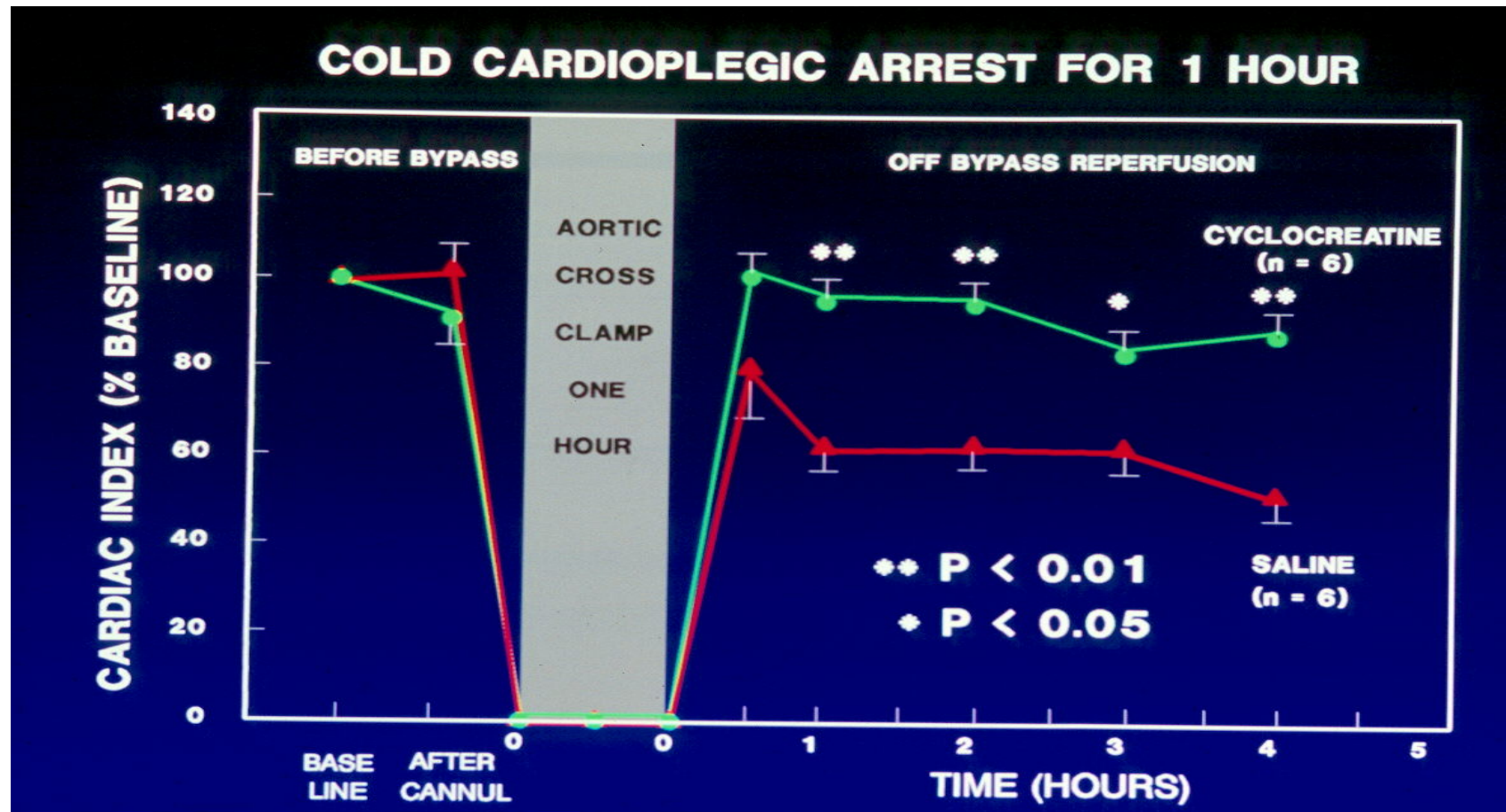


Cyclocreatine Injected IV once 1 hour before Cold Cardioplegic Arrest

- 1 hour Arrest Followed by 4 hours Reperfusion
- 3 hours Arrest Followed by 4 hours Reperfusion

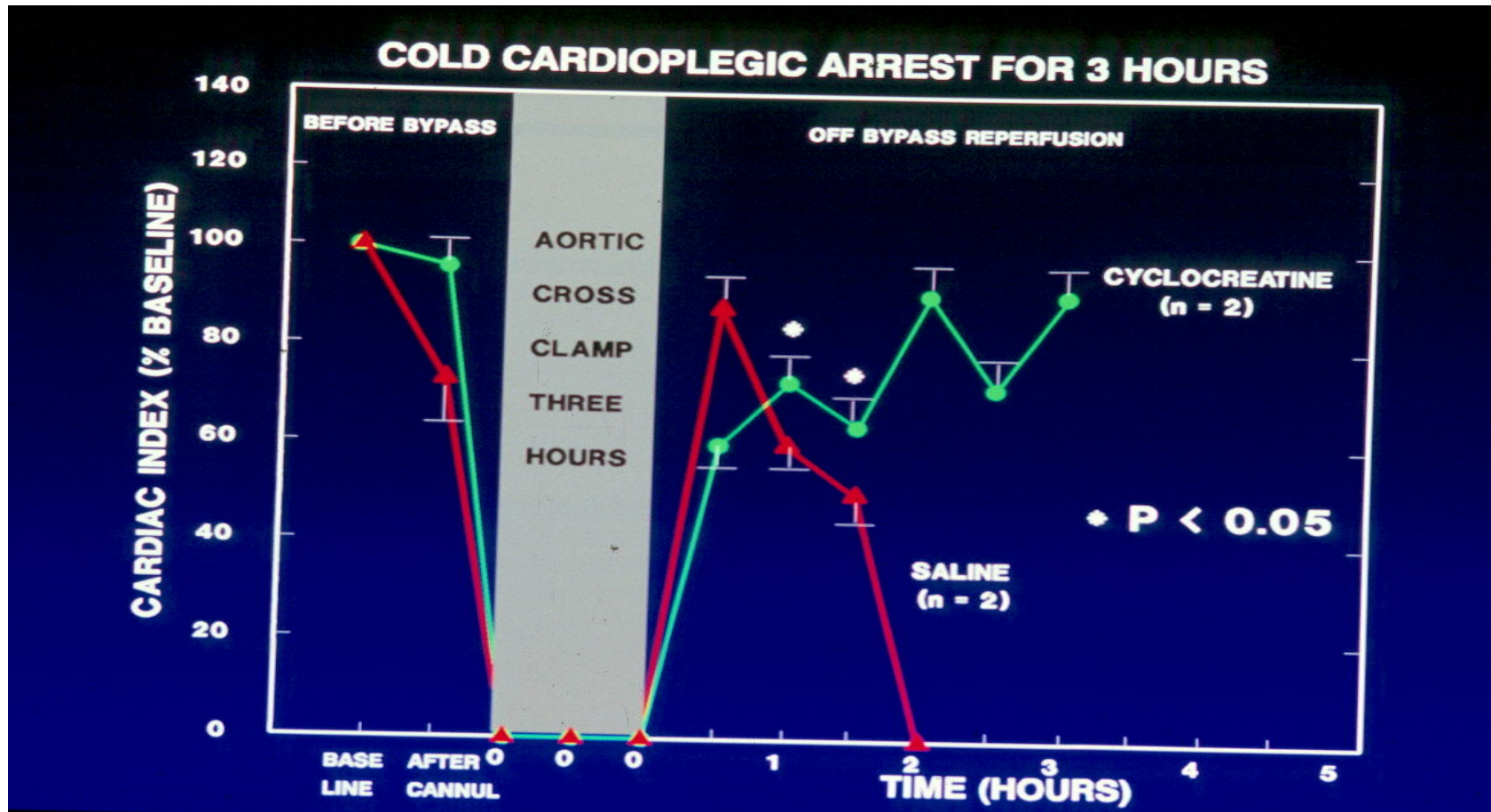
CCr and Cold Ischemia:

Significantly Stronger Cardiac Recovered in CCr-Treated Hearts after 1 Hour of Cold Cardioplegic Arrest Compared to Saline Hearts



CCr and Cold Ischemia:

CCr Hearts Recovered After 3 hours of Cold Cardioplegic Arrest and Continued Contracting for an Additional 4 hours During reperfusion, While All Control Hearts Ceased Contractility and died



2.3 CCr & CCrP and Heart Transplantation

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Restoration of Strong Cardiac Function In vivo After Surgical Heart Transplantation and Ex vitro After Prolonged Storage



Elgebaly SA, Poston R, Todd R, Helmy T, Almaghraby A, Elbayoumi T, Kreutzer DL.: Cyclocreatine Protects Against Ischemic Injury and Enhances Cardiac Recovery During Early Reperfusion. *Expert Review of Cardiovascular Therapy*, Volume 17(9), 683-697, 2019 (Review).

2.3a CCrP and Heart Transplantation for 3 Days

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In vivo Rat Syngeneic Abdominal Heterotopic Heart Transplantation After Prolonged Cold Storage (22 hours)

- Lewis *Donor & Recipient rats were used* to avoid immunologic rejection
- **Donor CCrP:** (n=6) rats infused once IV over 10 minutes
Recipient rats did not receive CCrP
- **Donor Saline:** (n=6) rats infused once IV over 10 minutes
- **Incubation Time:** 22 Hours (Cold Storage)
- **Measurements:** Contractility
Heart beating scores
ECHO Analysis
- **Graft Survival:** Day 0 (Surgical Transplantation)
Day 3 (Three Days After Surgery)



CCrP and Cold Ischemia:

CCrP Immediate Recovery at Day 0 of Surgical Transplantation After 22 Hours of Prolonged Cold Storage

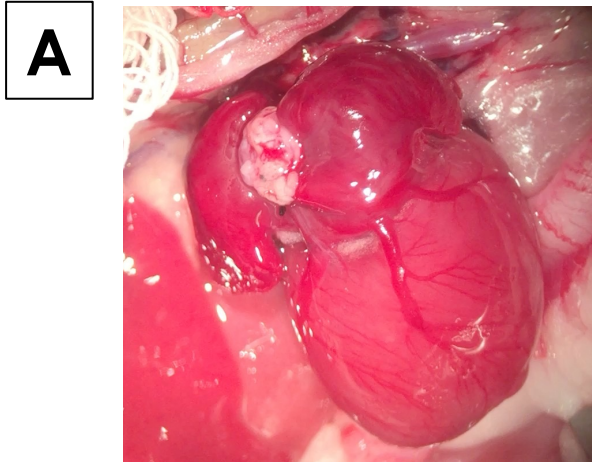


Photo A: Day Zero of Control Saline

- 1- *“Delayed heart function”* in first 2 minutes.
- 2- Heart beating scores ranged from: 1+ and 2+
- 3- *ECHO* analysis confirmed weak and ischemic injury

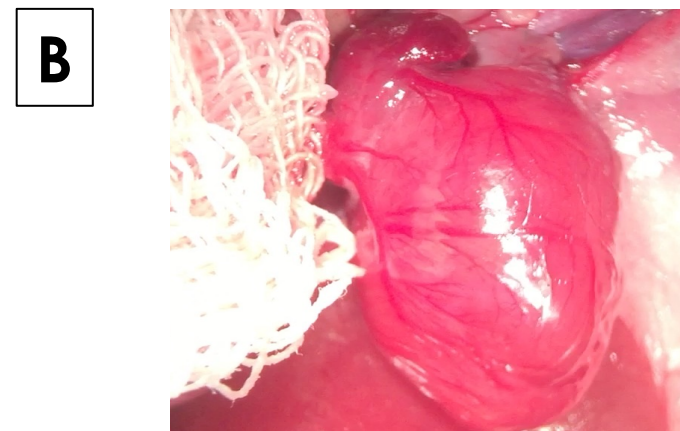


Photo B: Day Zero of CCrP Treated

- 1- No *“delayed heart function”*, but immediate recovery of contractile function
- 2- Heart beating scores ranged from: 3+ and 4+
- 3- *ECHO* analysis confirmed strong cardiac recovery

CCrP and Cold Ischemia: Persistent Advantage of CCrP Hearts in LV Recovery after Surgical Transplantation for 3 Days

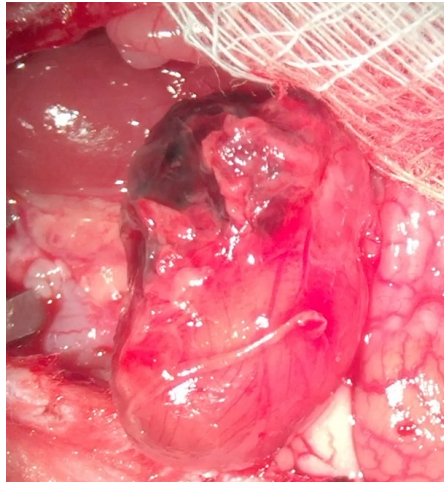
C

Photo C: Day Three of Control Saline

- 1- Heart beating scores ranged from: 1+ and 2+**
- 2- ECHO analysis confirmed weak and ischemic injury after 3 days with some thrombosis**
- 3- Low graft survival**

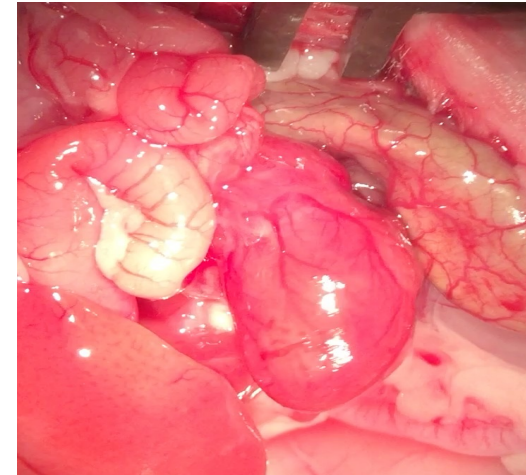

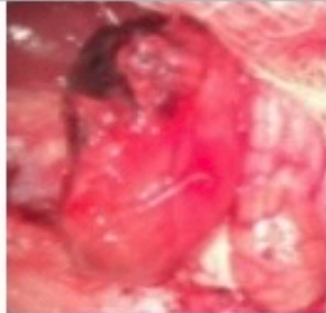
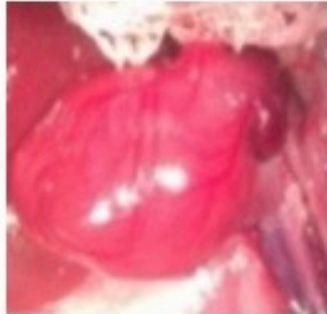
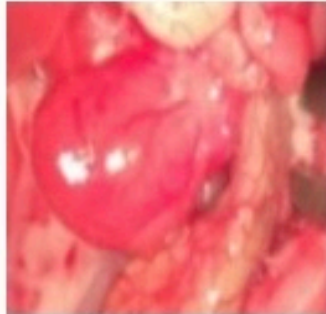
D

Photo D: Day Three of CCrP Treated

- 1- Heart beating scores ranged from: 3+ and 4+**
- 2- ECHO analysis confirmed healthy hearts with strong cardiac recovery after three days**
- 3- Increased Graft Survival**

CCrP and Cold Ischemia:

CCrP Prevents Ischemic Injury, Extends Transport Time, and Increases Graft Survival Compared to Control Hearts

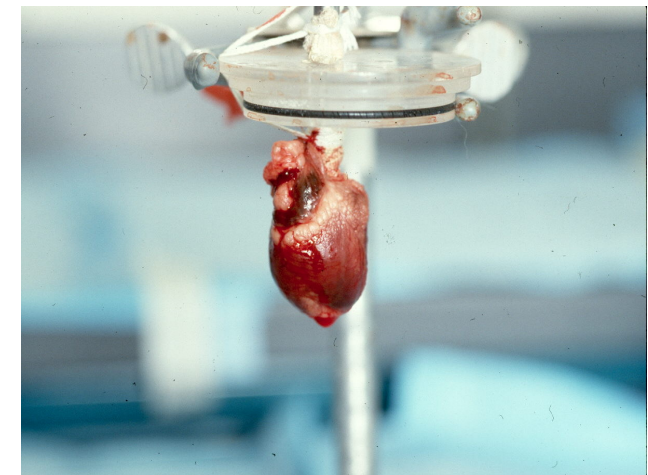
Treatment	Rat #	Incubation Time (hours)	Dose (g/kg)	ECHO Analysis [Wall Thickness and LV Mass]	Heart Beating Score	Potential Graft Survival	Graft (Day 0)	Graft (Day 3)
Saline	1	22	--	Loss	1+	Poor		
	2	24	--	Partial loss	1+ - 2+	Poor		
	3	24	--	Loss	1+ - 2+	Poor		
	4	22	--	Loss	1+	Very poor		
	5	22	--	Loss	1+	Poor		
CCrP	6	22	1.5	Preservation	3+	Excellent		
	7	22	1.2	Preservation	4+	Excellent		
	8	24	0.5	Partial Preservation	2+	Average		
	9	24	0.5	Preservation	3+	Very good		
	10	22	0.8	Preservation	4+	Excellent		
	11	22	0.8	Partial Preservation	2+	Very good		

2.3b CCrP and Heart Preservation for 6 Hours

21

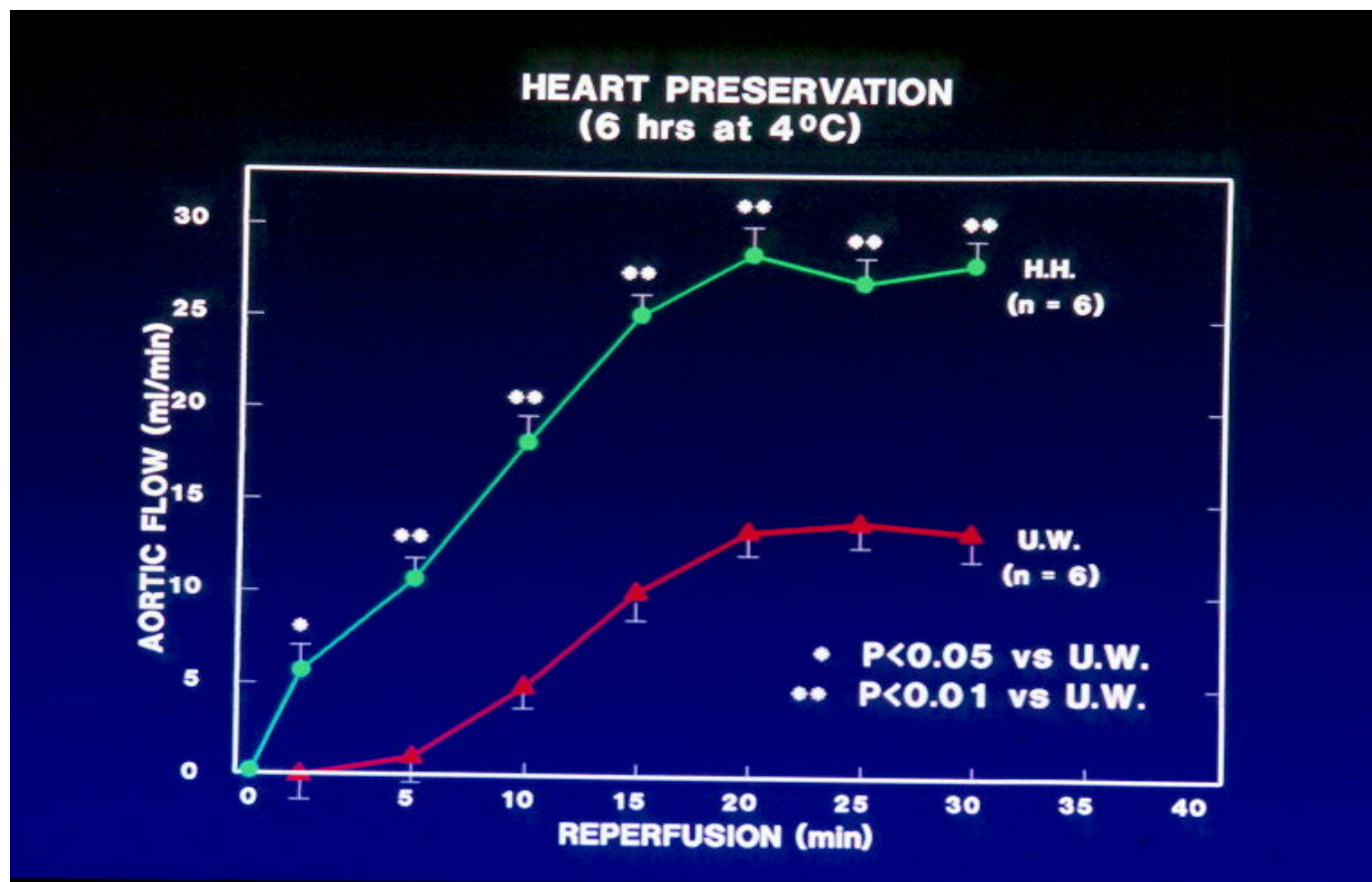
Standard Cold Storage for 6 Hours Followed by In vitro Cardiac Function Analysis

- CCrP injected IV once 1 hour before heart removal
- CCrP rats (n=6)
- Saline rats (n=6)
- Prolonged Preservation Ex vivo:
 - Hearts incubated in UW solution for 6 hours in cold storage
 - UW + CCrP (HH) for 6 hours in cold storage
- Heart weight was measured at the end of 6 hours
- Contractility on Langendorff apparatus for 30 minutes:
 - Aortic Flow

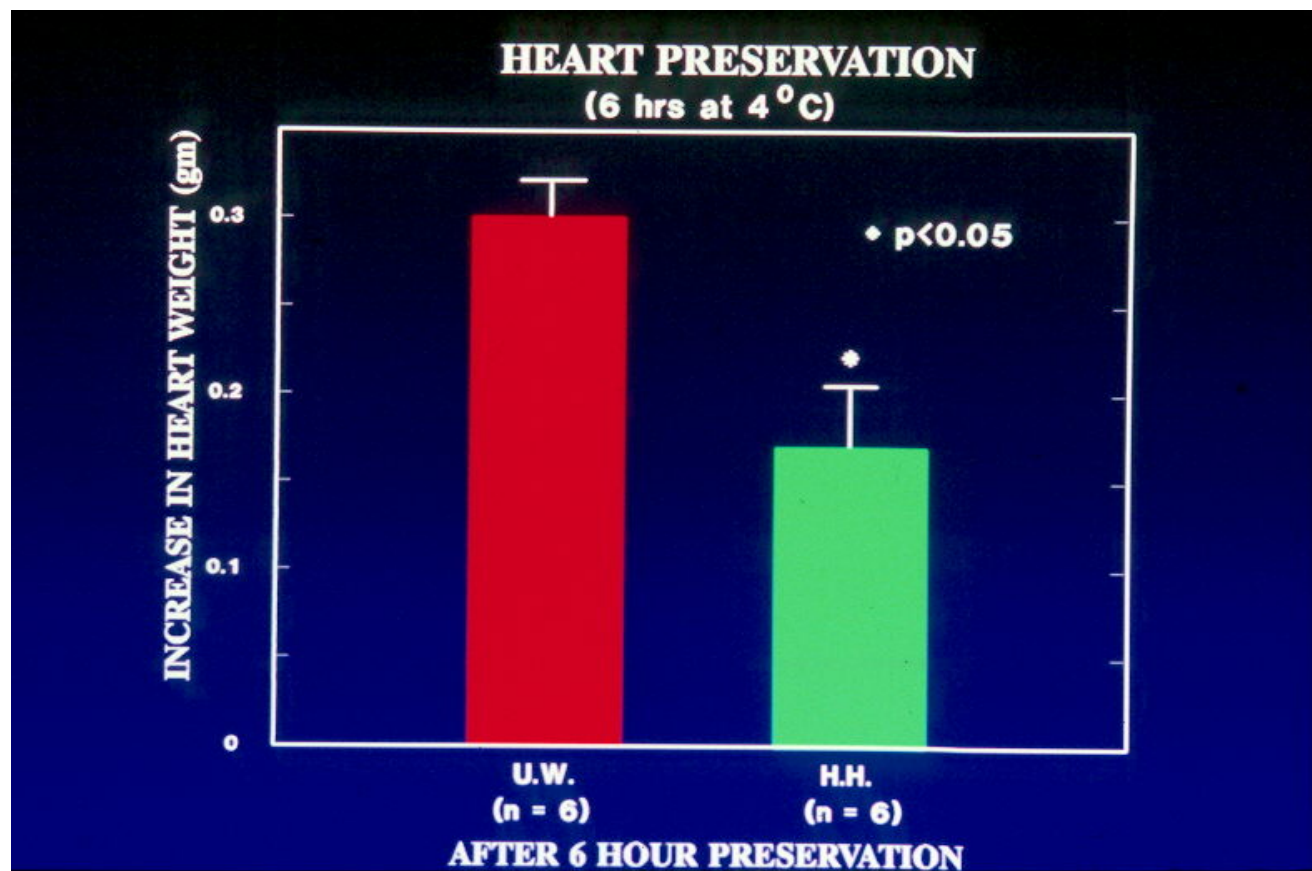


CCrP and Cold Ischemia:

Greater Cardiac Recovery of CCrP Rat Hearts (HH) After Standard Cold Storage for 6 Hours



CCrP and Cold Ischemia: Less Myocardial Edema in CCrP Hearts (HH) After Standard Cold Storage for 6 hours

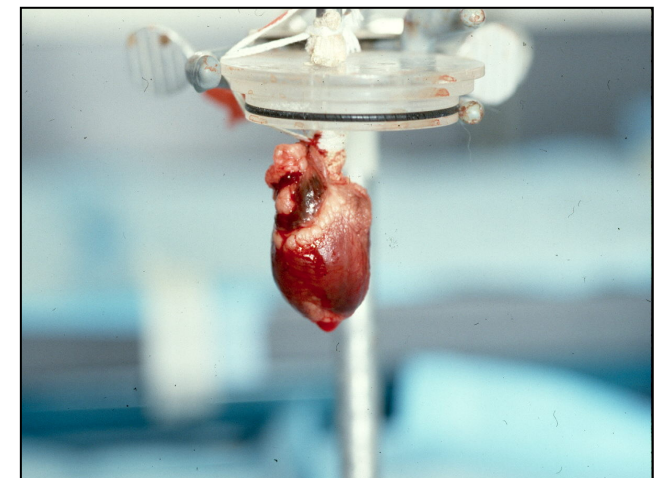


2.3c CCrP and Ex vivo Non-heartbeating Donor

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Ex vivo Dog Non-heartbeating Donor Model Heart Preservation For Transplantation

- Cyclocreatine injected IV once 60 minutes before cardiac arrest
- Cyclocreatine Dog (n=1)
- Saline Dogs (n=4)
- Aortic cross clamping for 1 hour (warm ischemia)
- Prolonged cold preservation for 4 hours (cold ischemia)
- Perfusion for 60 minutes on Langendorff apparatus
- Measured ATP, acidity, cell injury marker, and edema
- Measured cardiac apoptosis
- Contractility on Langendorff apparatus for one hour



CCrP and Warm Ischemia / Cold Storage: Myocardial Acidity in CCrP & Saline Hearts

- **Hearts Stop Beating:**
 - Cyclocreatine - 9 minutes
 - Controls - only 2 minutes

- **Myocardial pH Measured After 1 hr. arrest & 4 hrs. of Perfusion**
 - Baseline level - pH of 7.11
 - Cyclocreatine - pH of 7.04 ± 0.1
 - Controls - pH of 6.00 ± 0.25 and never returned back

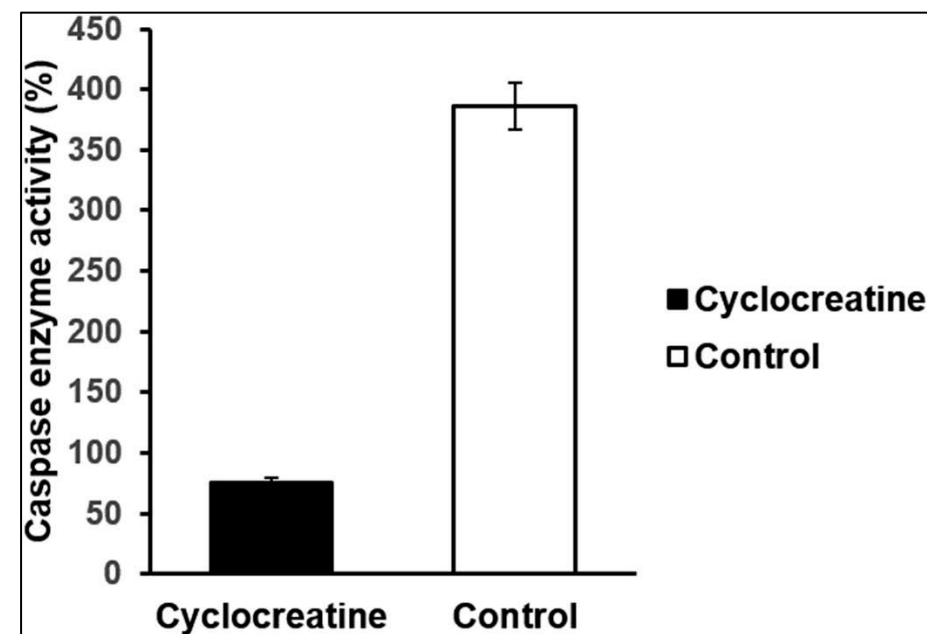
- **Lactic Acidosis Measured by spectroscopic imaging on MRI**
 - Reduced in Cyclocreatine heart compared to control hearts

CCrP and Warm Ischemia / Cold Storage:

Myocardial ATP, Contractile Function and Apoptosis in CCrP and Saline Hearts

- **ATP** - Three-fold increase in Cyclocreatine heart
- **Intracellular Edema**
 - Reduced in Cyclocreatine heart as measured by diffusion weighted imaging on MRI
- **Cell Injury Marker Malondialdehyde**
 - Reduced level in Cyclocreatine heart
- **Contractile Function**
 - Cyclocreatine - strong contractility for 1 hr. period
 - Control - declined after 15-20 min.
- **Apoptosis** – significant protection by Cyclocreatine (Figure)

Cardiac Apoptosis



FDA Orphan Drug Designation for CCrP for Prevention of Ischemic Injury in Heart Transplantation

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1 - 25 of 1

Designations: 1

#	Generic Name	Orphan Designation	Designation Date	Designation Status
1	cyclocreatine phosphate	Prevention of ischemic injury to enhance cardiac graft recovery and survival in heart transplantation.	01/17/2018	Designated

1 - 25 of 1

3. Clinical Applications

Preventive Therapy to Protect Hearts Against Ischemic Injury

▶ ***Predictable Myocardial Ischemia***

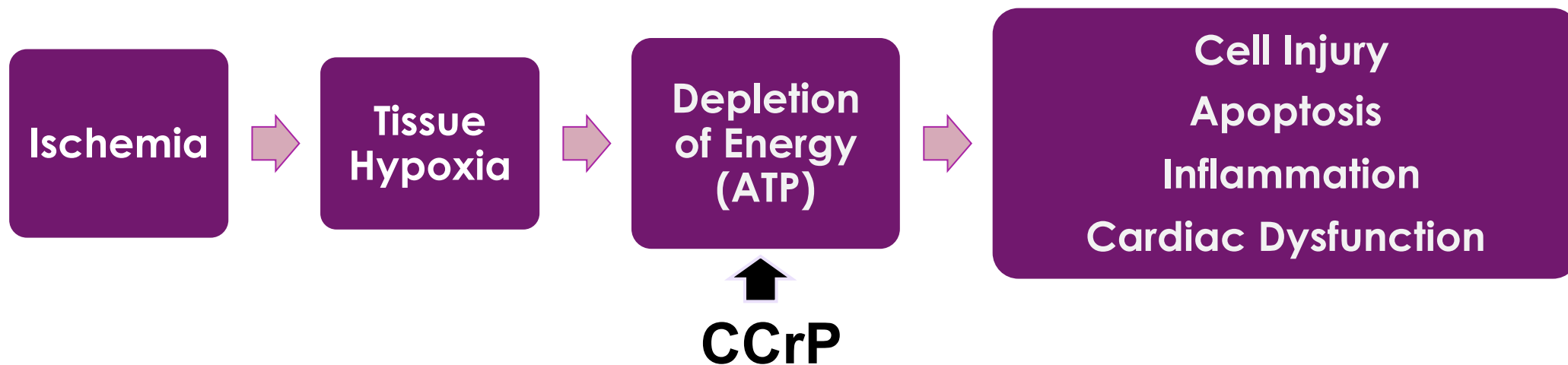
1. Heart Transplantation
2. High-Risk Cardiopulmonary Bypass
3. High-Risk Interventional Cardiology

▶ ***Rationale***

- ▶ During these procedures, myocardial ischemia occurs, which can cause low cardiac output syndrome (LCOS) at the end of procedures.
- ▶ Efforts to improve heart protection against ischemia during surgery, using CCrP, may improve cardiac function after surgery and reduce the mortality associated with LCOS.

Conclusions

CCrP Prevents “Acute” Myocardial Ischemic Injury and Restores Cardiac Function



- ▶ **CCrP is a paradigm shift in the treatment of myocardial ischemia/hypoxia.**
- ▶ **CCrP is a bioenergetic that prevents ischemic injury and downstream endpoints of ischemia, including: cell injury, apoptosis, inflammation and cardiac dysfunction.**
- ▶ **CCrP is a safe drug with no toxic effect on cardiac, liver and kidney function.**

Thank You.



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