Experimental Ischemia/Reperfusion Renal Injury: Pathophysiology and Skills

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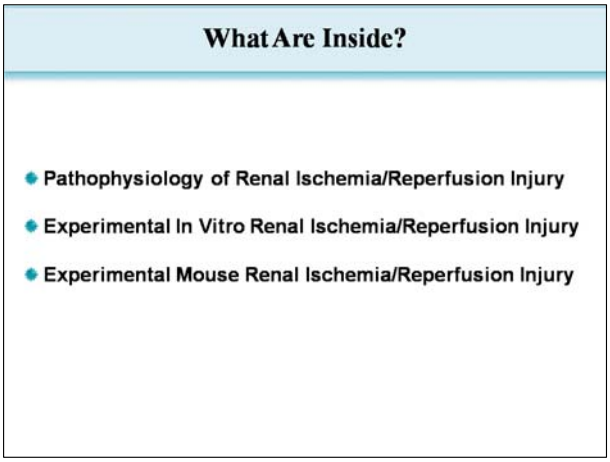
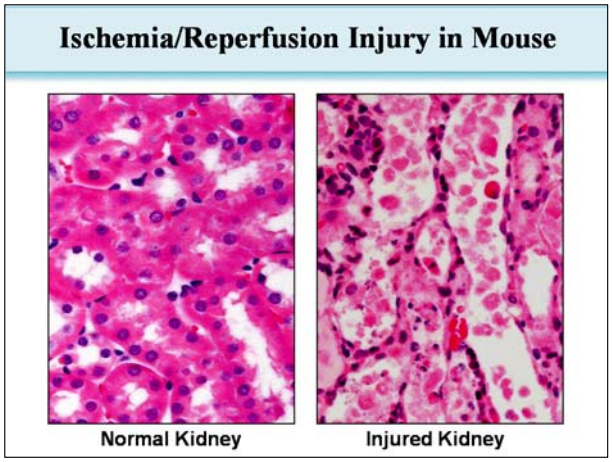
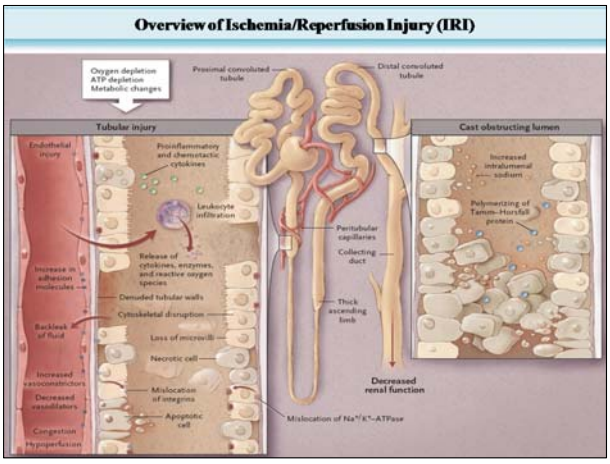
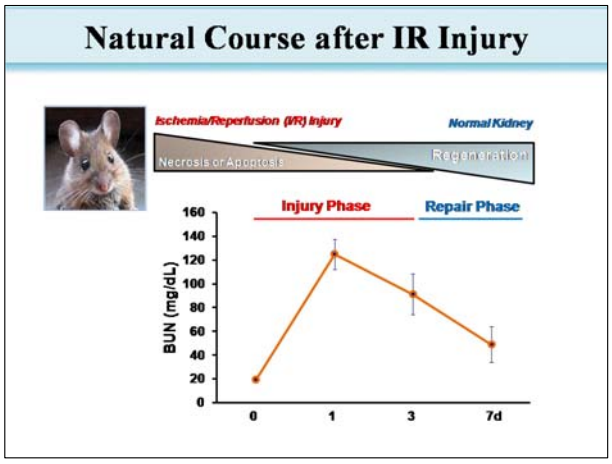


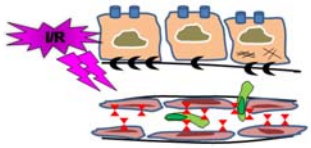






Mechanisms of IR Injury

- Vascular Factors
- Tubular Factors
- Inflammatory Cell Factors



Vascular Effect in IRI-1

Mechanisms of IR Injury

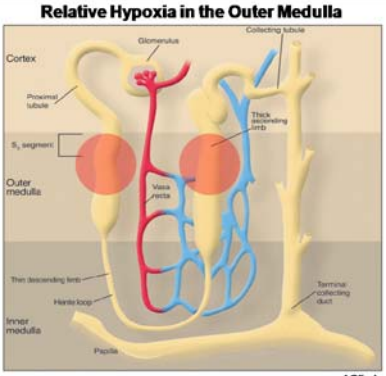
Unique microvasculature architecture of the kidney

- 0.5% of total body weight and 20% of CO
- oxygen tension: 50 mmHg in the cortex
  20 mmHg in the medulla
- highly sensitive to hypoxia
  high oxygen demand

N Engl J Med 1995; 332: 647-655

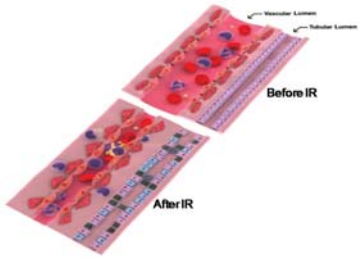
Vascular Effect in IRI-2

Relative Hypoxia in the Outer Medulla



J Clin Invest 2004; 114: 5-14

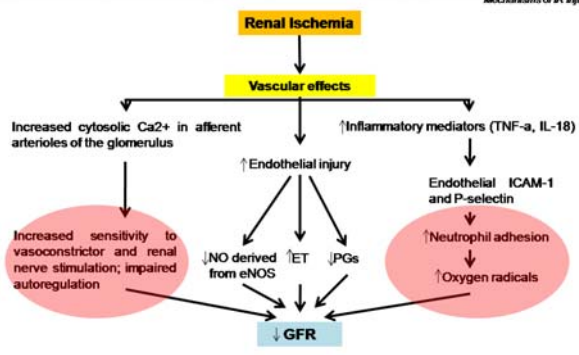
Vascular Effect in IRI-3



- No-reflow phenomenon
- Reflow paradox

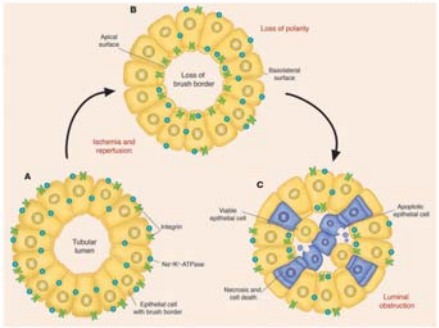
Vascular Effect in IRI-4

Mechanisms of IR Injury



Tubular Effect in IRI-1

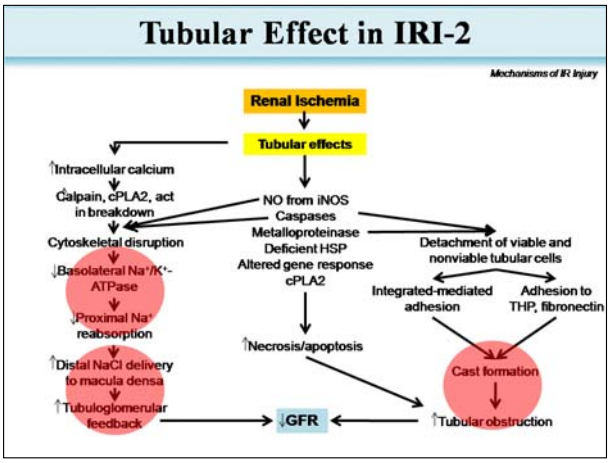
Mechanisms of IR Injury



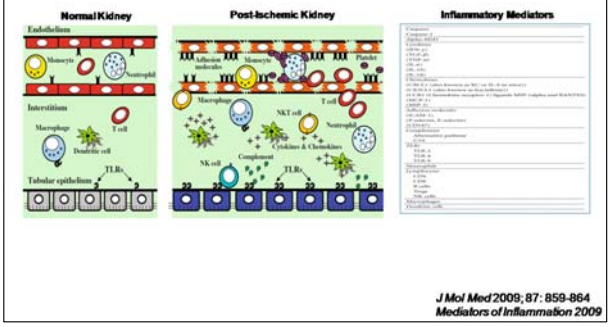
Morphological changes in the proximal tubule after IR

J Clin Invest 2004; 114: 5-14

Tubular Effect in IRI-2

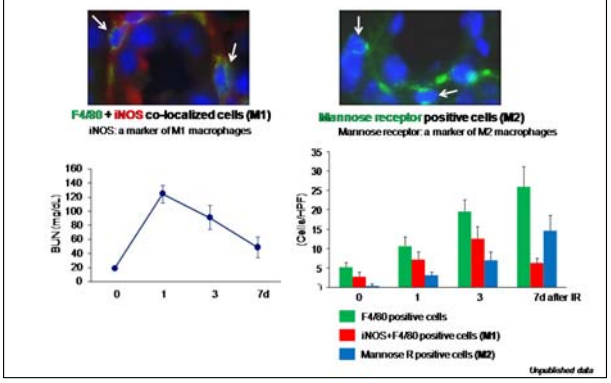


Inflammatory Cell Factors in IRI

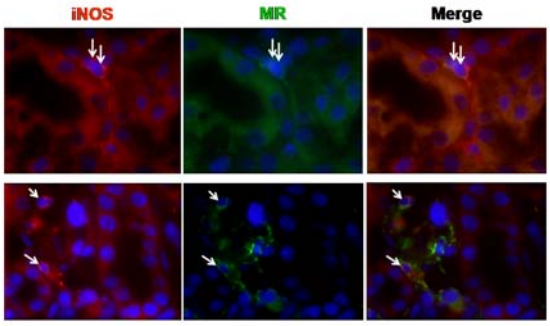


J Mol Med 2009; 87: 859-864
Mediators of Inflammation 2009

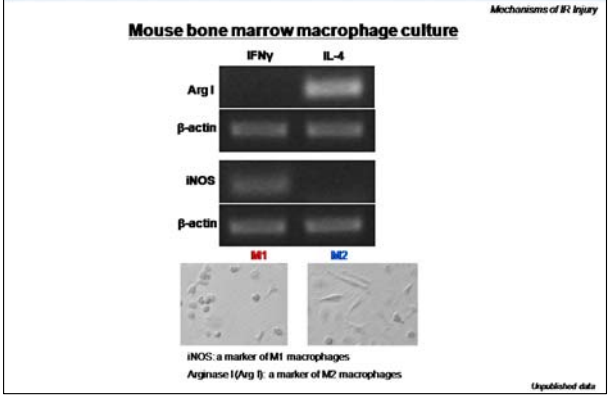
Existence of Different Phenotypes between Injury and Repair Phase after Ischemia/Reperfusion



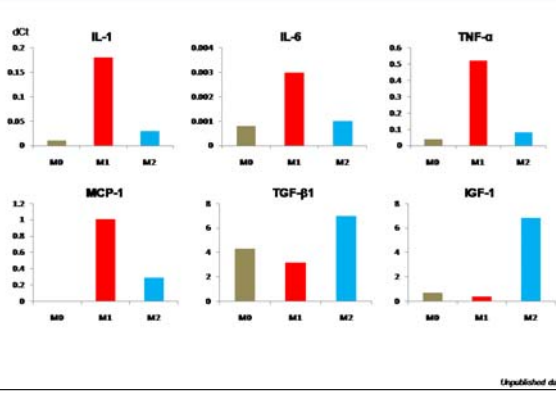
M1 Cells Switch into M2 Cells in Injured Kidney



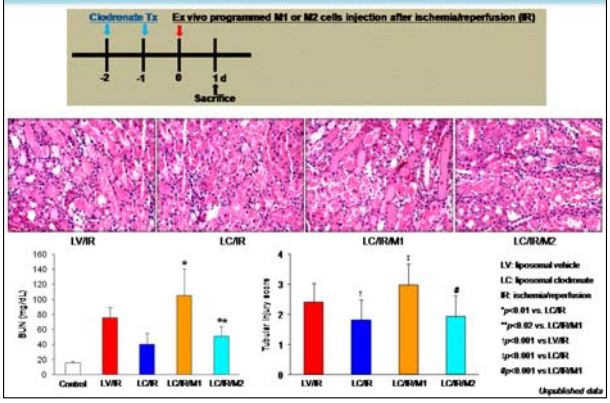
Macrophage Activation and Programming



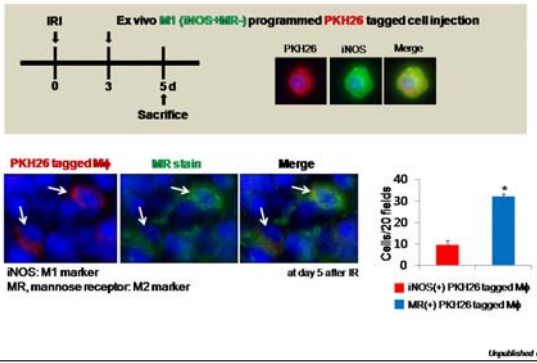
Cytokines in Induced Macrophages



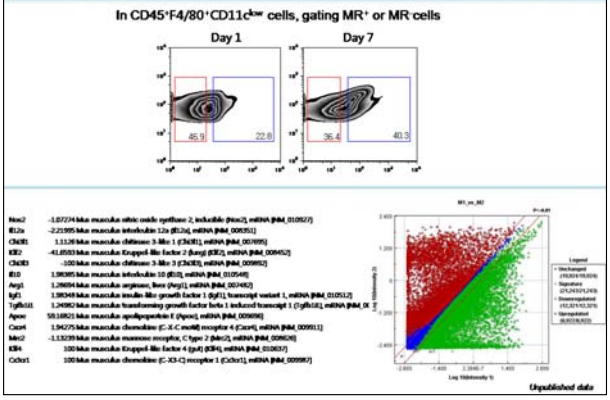
Effect of Different Phenotypic Macrophages in Renal Injury and Repair



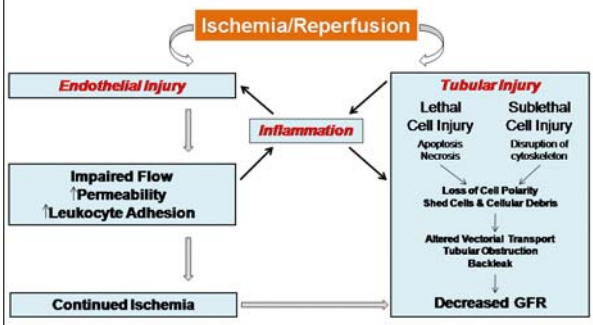
Transition from M1 into M2 cell at Day 5 after IR



Transcriptional Profiling of Mouse Monocyte-to-Macrophage Differentiation and Polarization after Ischemia/Reperfusion Injury



Summary-1

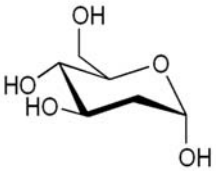


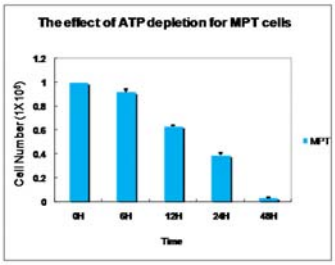
Experimental Ischemia/Reperfusion Model

In Vitro IRI Model

Mild ATP depletion (Chemical Hypoxia)

- MPT cells: mouse proximal tubular cell lines
- Culture in D-MEM supplemented with 2%FBS, 10 mM 2-Deoxy-D-glucose, no glucose at 37℃.





Murine Ischemia/Reperfusion Model

Murine Ischemia/Reperfusion Model

Operating Environment

- Clean, Comfortable, Quiet, and Separate Space

Murine Ischemia/Reperfusion Model

Stuff Preparation

- **Stuffs for Ischemia/Reperfusion Surgery**

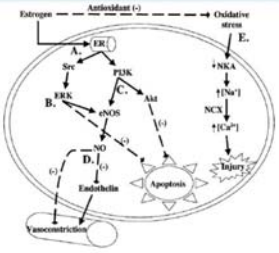
Sweetheart for mouse
Atraumatic microaneurysm clamps
Autoclaved gauze (small and large size)
Autoclaved cotton tip
Sterile DPBS or saline
70% Ethanol
Timer
1 cc syringe for anesthesia and fluid injection (2x)
Petri dish (2x)
Anesthesia (ketamine and xylazine)
Painkiller
Tape (for fixing mouse on the surgery table)
Betadine
Thermostatically regulated heating mat
Overhead lamps
Rectal temperature probe and monitor
Surgery instruments (scissors, forceps, silk, staple etc)

Murine Ischemia/Reperfusion Model

Selection of Mice

- **Genetic Background:**
  Strain difference in response to I/R
  (C57/BL6 mice > BALB/c mice > NIH Swiss mice)
- **Gender**
  Male mice > Female mice
- **Age**
  8 ~ 10 weeks old

Cellular and Molecular Mechanisms of Sex Differences in Renal Ischemia/Reperfusion Injury



Effects of estrogen and testosterone on mechanisms involved in I/R injury

Mechanism	Estrogen	Testosterone
Mitogen activated protein kinases (MAPK)	Increases ERK activation, decreases JNK activation	Increases JNK and p38 activation
Endothelial nitric oxide synthase (eNOS)	Increases eNOS activity	Decreases eNOS activity
Endothelin	Decreases production	
ATP sensitive potassium channels (K_{ATP})	Activates K_{ATP} channels	Activates K_{ATP} channels
Akt	Increases the sustained activation of Akt	Decreases the sustained activation of Akt

ERK indicates extracellular regulated kinase, JNK is c-Jun N-terminal kinase and p38 is p38 mitogen activated protein kinase.

Cardiovascular Research 2005; 67: 594-603

Murine Ischemia/Reperfusion Model

Anesthesia

- **Anesthetic agent**
  - 1 mL Ketamine (100 mg/mL)
  - 0.1 mL Xylazine (100 mg/mL)
  - 8.9 mL Normal saline or PBS solution
  - 200㎕ SC or IM per mouse

Murine Ischemia/Reperfusion Model

Animal Preparation

- Surgical clip

- Surgical scrub

Murine Ischemia/Reperfusion Model

Temperature Effect

Hypothermia

- Slowing metabolic processes
- Reducing ATP depletion
- Cold preservation of kidney transplant; protection from I/R injury

Hyperthermia

- Cytokine release
- More severe kidney failure
- Increased mortality as is seen in heat stress

Murine Ischemia/Reperfusion Model

Temperature Control

- Ambient room temperature: 21~27℃
- Rectal temperature: 37℃
- Throughout surgery, Ischemic interval, During the early stages of recovery
- Heated plate/Heated pad/Overhead lamp

Murine Ischemia/Reperfusion Model

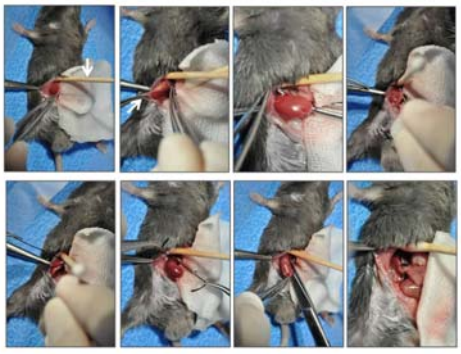
Incision

- Type of Incision

Midline laparotomy	Flank incision
Relatively simple Rapid exposure of both renal pedicles Greater fluid loss More marked systemic inflammatory response	좁은 시야 확보 Less fluid loss

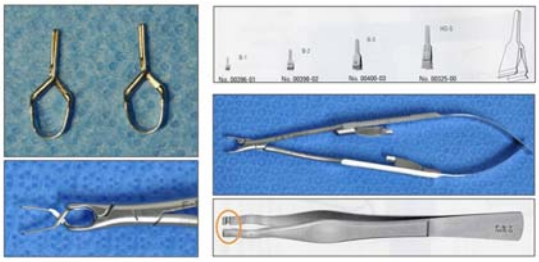
Murine Ischemia/Reperfusion Model

Removal of Right Kidney



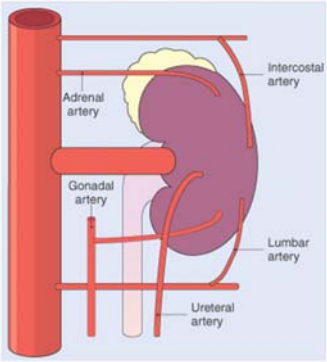
Murine Ischemia/Reperfusion Model

Clamp and Forcep



Murine Ischemia/Reperfusion Model

Renal Collateral Blood Supply



Murine Ischemia/Reperfusion Model

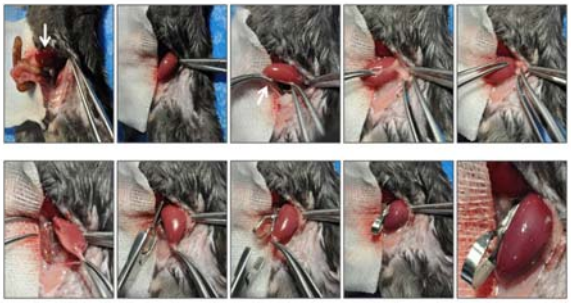
Clamping-1

- Type of Clamping


	One kidney removal and the other clamping	Both clamping
Short term (24-48hrs)	Outcomes: No difference	
Long term	Nephron reduction becomes more relevant	

Murine Ischemia/Reperfusion Model

Clamping-2



Murine Ischemia/Reperfusion Model

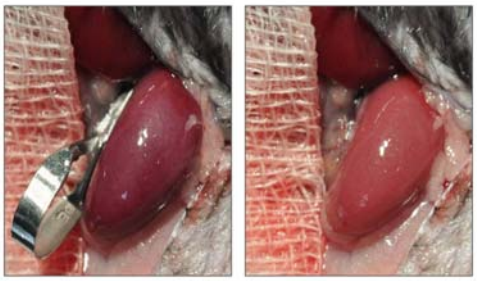
Duration of Ischemia

- Ischemic time: 22-35 min
- Variability according to strains

C57/BL6 mice > BALB/c mice > NIH Swiss mice

Murine Ischemia/Reperfusion Model

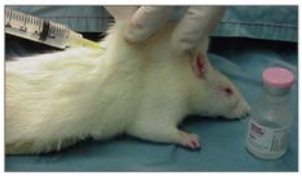
Removal of Clamp



Murine Ischemia/Reperfusion Model

Fluid Management

- Volume and Route of Fluid Administration
  - Normal saline
  - 500 μL during surgery via intraperitoneal route
  - 500 μL immediately after surgery

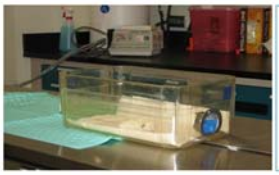


Post-operative fluids
- Increase drug metabolism
- Aid hydration
- Speed recovery

Murine Ischemia/Reperfusion Model

Postoperative Care

- In a quiet warm environment with free access to food and water



Analgesia (pain control)
- 1 mL Buprenorphine (0.3 mg/mL)
- 9 mL Normal saline or PBS solution
- 200 μl SC or IM per mouse bid for 3 days

- Postoperative recovery

- Within sight; monitor for sternal recumbancy
- Bedding free; to eliminate risk of aspiration
- Warmed; to speed recovery

Murine Ischemia/Reperfusion Model

Post-Operational Care Record

Rodent Post-Op Care Record

PI: Sik Lee Protocol:
Contact Info: Sik Lee Phone:
Procedure: Renal Artery Clamping

Date:____/_____/__2010

Time:_____

Fluids: ☐ 500 µl NS sq post op

☐ Ketamine/xylazine IP (10mcg/ g)

☐ Analgesia: Buprenex (75mcg/kg)

Complication comments: (pre, intro, post-op):

Animal ID	Weight	Appearance	Initials	Comments
		D0:		
		D1:		
		D2:		
		D3:		
		D4:		
		D5:		
		D6:		
		D7:		

Summary-2

The Less touch and Faster, The Better !

The more practice, The Better !