Hsp72 is an early and sensitive biomarker to detect acute kidney injury

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Accepted November 08, 2010
• Background Acute Kidney Injury
• Background Heat Shock Response
• Manuscript
Acute Kidney Injury

Reduction of kidney function within 48 h:

- absolute increase in serum creatinine $\geq 0.3$ mg/dl
- percentage increase in serum creatinine $\geq 50\%$ (1.5 from baseline)
- reduction in urine output ($< 0.5$ ml/kg per h for minimum 6 hours)

Acute Kidney Injury

RIFLE criteria

- **Risk:** GFR decrease >25%, serum creatinine increased 1.5 times or urine production of <0.5 ml/kg/hr for 6 hours
- **Injury:** GFR decrease >50%, doubling of creatinine or urine production <0.5 ml/kg/hr for 12 hours
- **Failure:** GFR decrease >75%, tripling of creatinine or creatinine >4 mg/dl or urine output below 0.3 ml/kg/hr for 24 hours
- **Loss:** persistent AKI or complete loss of kidney function for more than 4 weeks
- **End-stage:** complete loss of kidney function for more than 3 months
Acute Kidney Injury

Therapie

• Intravenous fluid
• Norepinephrine/Dobutamine
• Diuretics (furosemide, fluid overload)
• Renal replacement therapy
Heat Shock Response

David Lanneau et al., Mini-Review: Apoptosis Versus Cell Differentiation
Role of Heat Shock Proteins HSP90, HSP70 and HSP27,
[Prion 1:1, 53-60; January/February/March 2007]; ©2007 Landes Bioscience
Heat Shock Response

1. Renal pyramid  
3. Renal artery  
5. Renal hilum  
7. Ureter  
9. Renal capsule  
11. Superior renal capsule  
13. Nephron  
15. Major calyx  
17. Renal column

2. Interlobular artery  
4. Renal vein  
6. Renal pelvis  
8. Minor calyx  
10. Inferior renal capsule  
12. Interlobar vein  
14. Minor calyx  
16. Renal papilla

Wikipedia, oct. 2012, Piotr Michał Jaworski
Heat Shock Response

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Schematic drawing of the anatomy of the kidney modified from by Uwe Gille 18:36, 12 August 2005 (UTC), wikipedia, oct. 2012

Wikipedia, oct. 2012, Piotr Michał Jaworski
Hsp72 is an early and sensitive biomarker to detect acute kidney injury

- HSP72 biomarker for AKI?
- as biomarker to monitor a renoprotective strategy?
72 Wistar rats
270 – 300 g

sham-operated
10 min
20 min
30 min
45 min
60 min

of bilateral renal ischemia

24 h reperfusion
30 Wistar rats
270 – 300 g
30 min ischemia

reperfusion

3h 6h 9h 12h 18h 24h 48h 72h 96h 120h
25 Wistar rats
270 – 300 g
30 min of ischemia
24 h reperfusion

no treatment
20 10 5 2.5
mg/kg bodyweight spironolactone p.o.
3 days before I/R
urine protein excretion
N-acetyl-β-D-glucosaminidase

- increased lysosomal activity in renal tubular cells
- a measure of altered function in the renal tubules
urine protein excretion
N-acetyl-β-D-glucosaminidase
Periodic acid-Schiff

- glycogen
- glycoproteins
- glycolipids
- cellulosis

urine protein excretion
N-acetyl-β-D-glucosaminidase

I/R
HSP72

Actin

Periodic acid-Schiff

• glycogen
• glycoproteins
• glycolipids
• cellulosis

HSP72 as a novel biomarker to detect AKI
urine protein excretion
N-acetyl-β-D-glucosaminidase

HSP72
NGAL
IL-18
Kim-1

Periodic acid-Schiff
- glycogen
- glycoproteins
- glycolipids
- cellulosis

HSP72 as a novel biomarker to detect AKI
5 healthy kidney donors

9 patients with septic AKI

patients with respiratory and organ failure

5 patients without AKI

5 patients with AKI
5 healthy kidney donors

9 patients with septic AKI

patients with respiratory and organ failure

HSP72

EMBO Molecular Medicine

Research Article

Hsp72 as a novel biomarker to detect AKI
72 Wistar rats
270 – 300 g

sham-operated 10 min 20min 30min 45min 60min of bilateral renal ischemia

24 h reperfusion
N-acetyl-β-D-glucosaminidase
- increased lysosomal activity in renal tubular cells
- a measure of altered function in the renal tubules
Different ischemia period

Result II

sham

10 min I

20 min I

30 min I

45 min I

60 min I

G

Cast Number per field

H

Tubular injured area %

Ischemia Period (min)
Different ischemia period

Result III

Hsp72/18S

RT-PCR

Ischemia Period (min)

Hsp72

Actin

Western Blot

O.D. Hsp72/actin

Ischemia Period (min)
Different ischemia period

Result IV

RT-PCR

ELISA

Western Blot

Hsp72 as a novel biomarker to detect AKI
Different ischemia period

Result V

ELISA
Different ischemia period

Result V

ELISA

highest correlation with tubular injury
30 Wistar rats
270 – 300 g
30 min ischemia

3h 6h 9h 12h 18h 24h 48h 72h 96h 120h
reperfusion
Different reperfusion period

Result I
Different reperfusion period

Result II

**ELISA**

- Hsp72 ng/mL

**Western Blot**

- O.D. Hsp72

**Reperfusion Period (h)**

**Tubular injured area %**
Different reperfusion period
Result III

ELISA

NGAL (ng/mL)

Hsp72 ng/mL

IL-18 (pg/mL)

Reperfusion Period (h)

Research Article
Hsp72 as a novel biomarker to detect AKI
Different reperfusion period

Result III

highest correlation with tubular injury
25 Wistar rats
270 – 300 g
30 min of ischemia
24 h reperfusion

no treatment
20
10
5
2.5
mg/kg bodyweight spironolactone p.o.
3 days before I/R
**Spironolactone**

Result I

- **Serum creatinine (mg/dl)**
  - I/R
  - I/R + Sp 20

- **Creatinine clearance ml/min**
  - I/R
  - I/R + Sp 20

- **Hsp72 ng/mL**
  - I/R
  - I/R + Sp 20

- **Western blot**
  - O.D. Hsp72

*Research Article*

Hsp72 as a novel biomarker to detect AKI
Spironolactone

Result II
5 healthy kidney donors

9 patients with septic AKI

patients with respiratory and organ failure

HSP72

HSP72

EMBO Molecular Medicine

Research Article
Hsp72 as a novel biomarker to detect AKI
Hsp72 as a novel biomarker to detect AKI
Patients with respiratory and organ failure
Conclusion

HSP72 as sensitive biomarker:

- stratify renal injury
- correlates with tubular injury
- correlates with tubular recovery
- tool to monitor effectiveness of a pharmacological intervention
- clinical setting
Thank you for your attention

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