Investigation of the post-exercise renal damage with reactive oxygen

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EARF (exercise-induced acute renal failure) :

- Acute renal failure induced by anaerobic exercise.
- Symptoms: severe loin pain, vomiting, and nausea
- 51% of EARF patients show renal hypouricemia.
- Wedge-shaped delayed contrast enhancement was found in renal CT scan.
  →arterial vasoconstriction?

The precise mechanism of EARF is still unclear.

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The hypothesis of pathogenesis of EARF

Exercise

Renin ↑

Ang II ⇧

hypertension

vascular endothelium damage

Inflammation

NADPH oxidase ⇧

ROS ⇧ → EARF

NO inactivation

uric acid (antioxidant)

Angiotensin II (Ang II)
- Vasoconstrictor that is elevated by exercise
- Radical oxygen species (ROS) generator

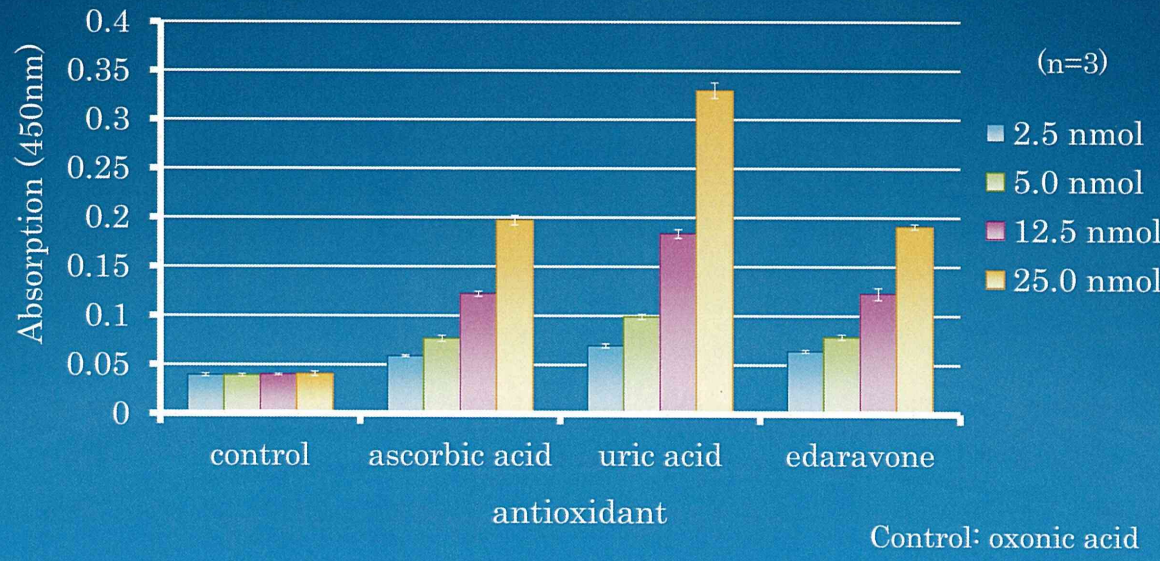
Hypouricemia patients have a risk for scavenging exercise-induced ROS.

The evaluation of antioxidant ability

- CUPRAC assay

antioxidants: $Cu^{2+} \rightarrow Cu^{+}$

Nc (neocuproine): $Cu^{+} \rightarrow Cu^{+}$-Nc ($A_{450nm}$)



Control: oxonic acid

Aim

Investigation of the hypothesis of EARF with experimental models

- Experiment 1:
  Evaluating renal cytotoxicity of ROS *in vitro*
- Experiment 2:
  In vivo estimating of vasoconstriction and renal oxidative stress by Ang II

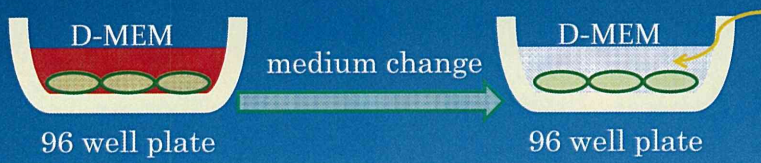
Experiment 1

<Method>

1. Incubating Cos7 in 96 well plate with D-MEM (37℃, 1 day)

Cos7: African green monkey kidney cell
D-MEM: Dulbecco's modified Eagle medium

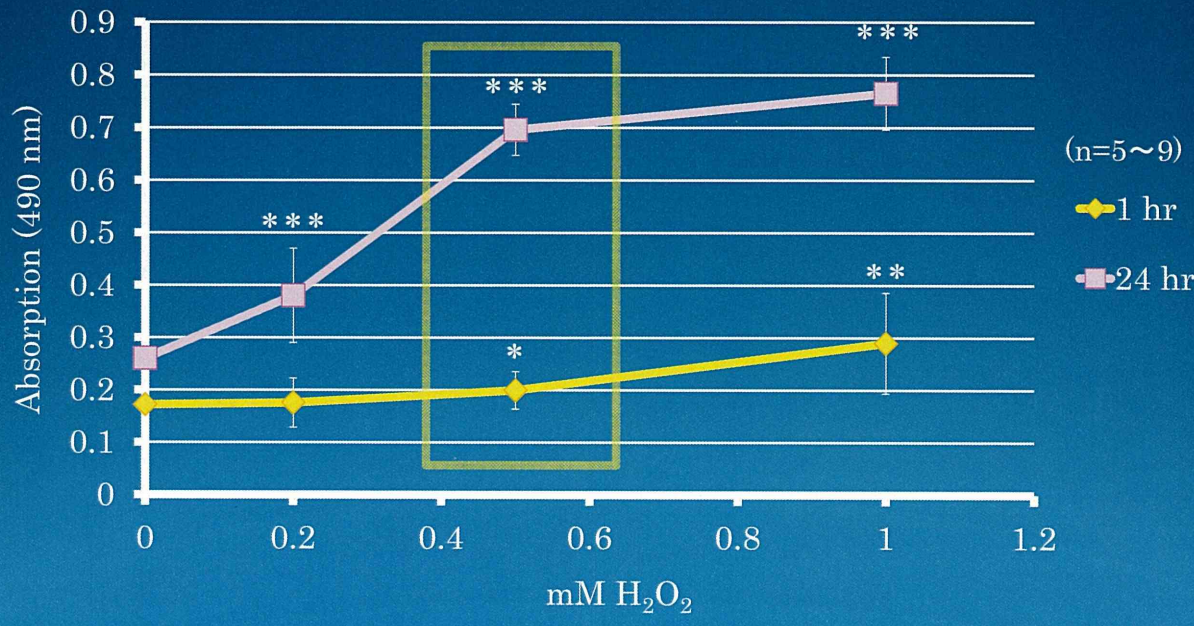
2. Exposing Cos7 to ROS



		concentration (mM)				
H_2O_2		0	0.5	0.5	0.5	0.5
antioxidant	ascorbic acid	0	0	0.8	0	0
	uric acid	0	0	0	0.8	0
	edaravone	0	0	0	0	0.8

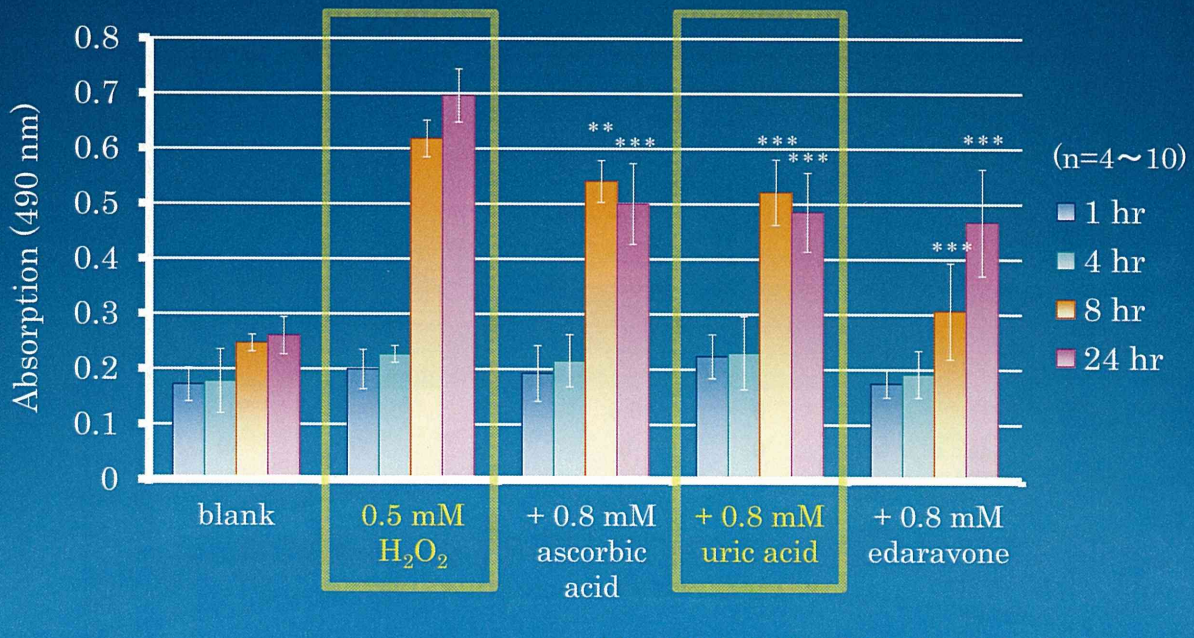
3. Incubating 1, 4, 8 or 24 hours (37℃)
4. Examining cytotoxicity of ROS by LDH assay (A_{490nm})

Experiment 1 ＜Result 1＞
Cytotoxicity of ROS



*p < 0.05, **p < 0.01, ***p < 0.001 vs. 0 mM H_2O_2

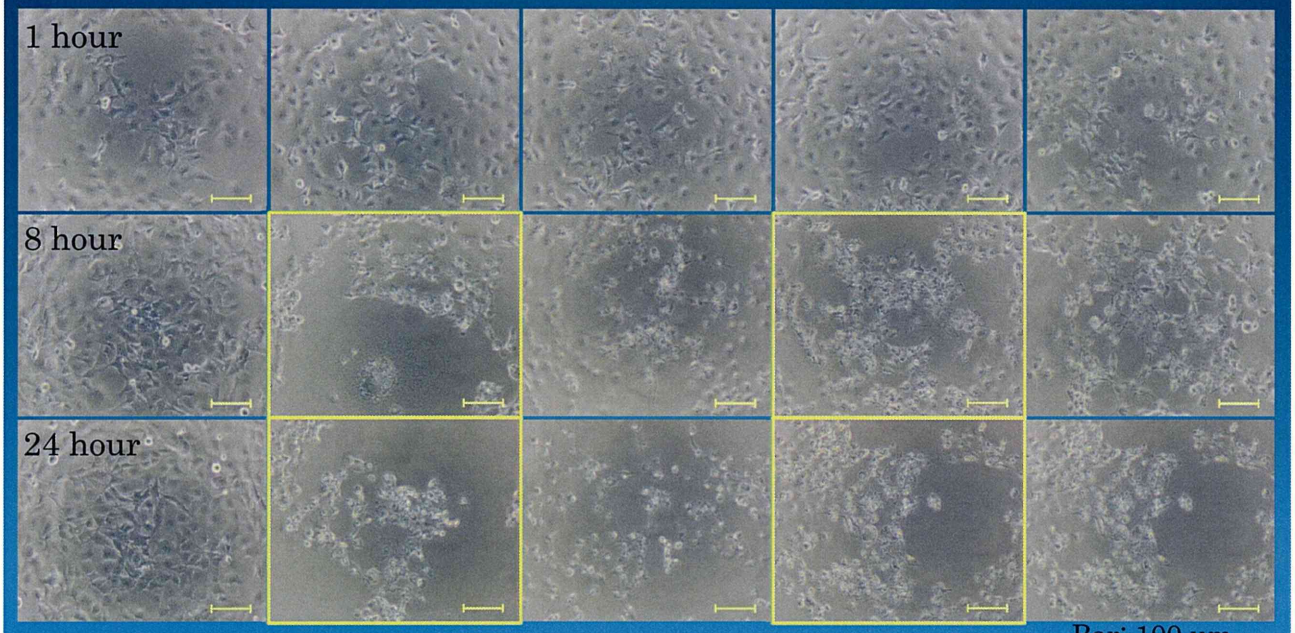
Experiment 1 ＜Result 2＞
Effect of antioxidants to ROS cytotoxicity



**p < 0.01, ***p < 0.001 vs. 0.5 mM H_2O_2

Experiment 1 ＜Result 3＞

Effect of antioxidants to ROS cytotoxicity



Bar: 100 μm

Experiment 1

＜Discussion＞

- Result 1

LDH assay — Addition of > 0.5 mM ROS caused renal cytotoxic effect.

- Result 2 and 3

LDH assay — Cytotoxicity of 0.8 mM antioxidant-added groups were decreased after 8 to 24 hours.

Phase-contrast microscopy — Cell viability seems to improve by incubation with 0.8 mM antioxidants for 8-24 hours.

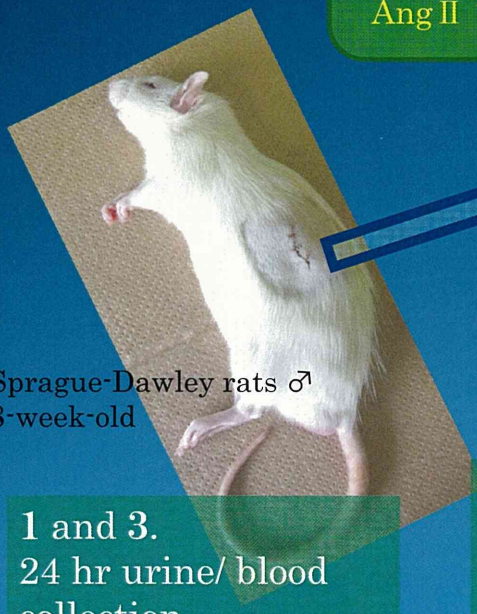
⇨ Addition of antioxidants prevented cytotoxicity of ROS.

Experiment 2
<Method>

Exercise

ROS ⇧ → EARF

Ang II ⇧



Sprague-Dawley rats ♂
8-week-old

Kidney

artery

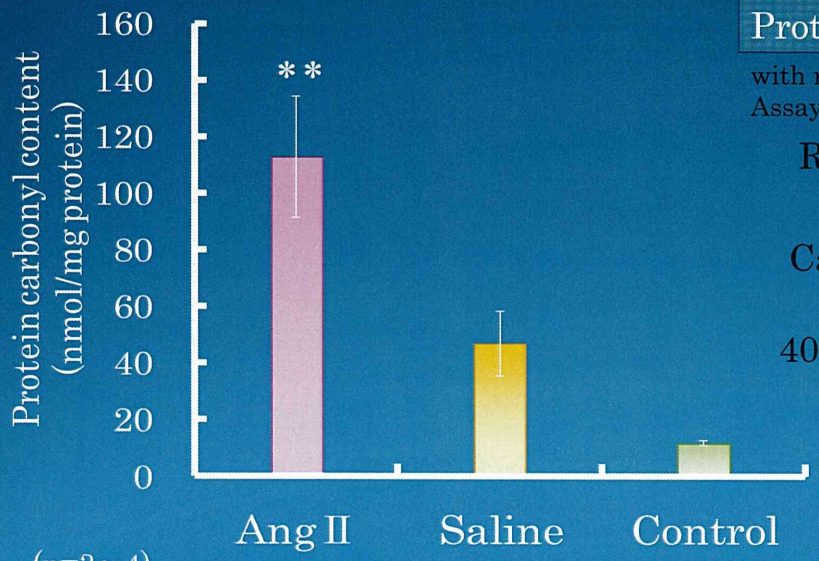
4. Kidney excision

2. Ang II infusion
(1 mg/kg weight/10min)

1 and 3.
24 hr urine/ blood collection

5. Evaluation of renal damage
- Protein carbonyl assay
- Hematoxylin-eosin (HE) staining
- Creatinine clearance (Ccr)

Experiment 2 <Result 1>
Renal oxidative stress induced by Ang II infusion



(n=3～4)

**p < 0.01 vs. saline

Protein carbonyl assay

with reference to Protein Carbonyl Assay kit (Cayman)

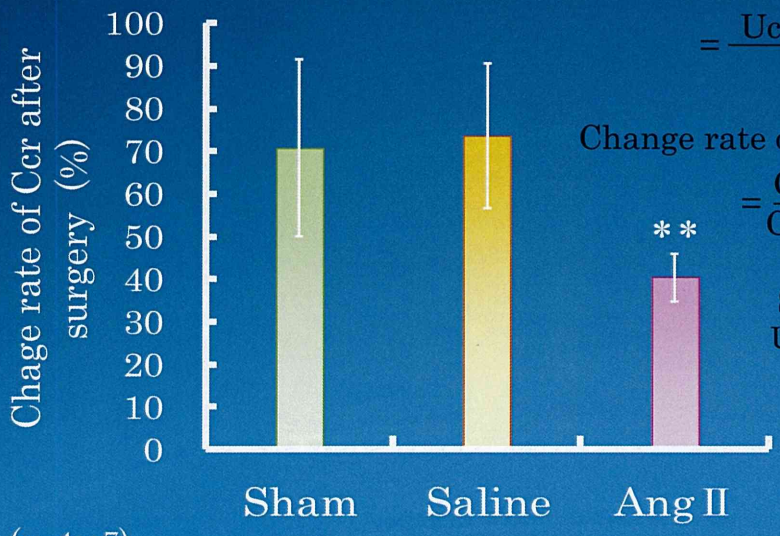
Renal Protein
↓ ROS
Carbonyl protein
↓ *DNPH
405 nm absorption

*DNPH :
2,4-Dinitrophenylhydrazine that form stable chromophore with carbonyl protein.

Saline : Saline infusion
Control : without operation

Experiment 2 <Result 2>

Effect of Ang Ⅱ infusion on Ccr



$$\text{Ccr (mL/hr)} = \frac{\text{Ucr (mg/dL)} \times \text{V (mL/hr)}}{\text{Scr (mg/dL)} \times 24}$$

$$\text{Change rate of Ccr after surgery (\%)} = \frac{\text{Ccr after surgery}}{\text{Ccr before surgery}} \times 100$$

Scr : serum creatinine
Ucr : urinary creatinine
V : urine volume

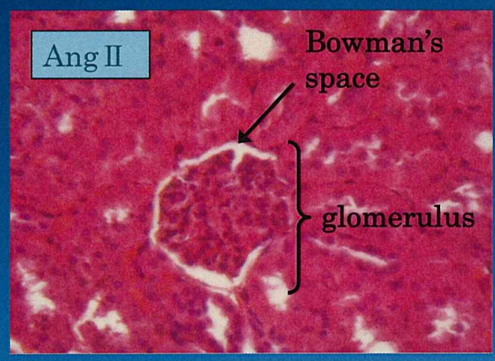
Sham operation : without infusion

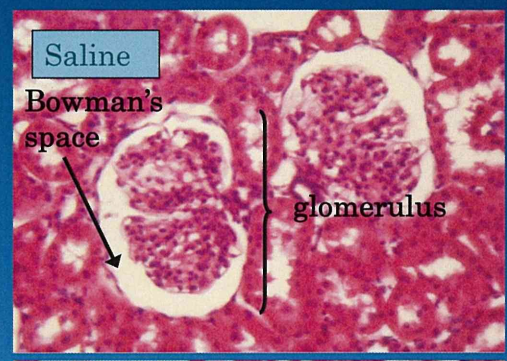
(n=4～7)

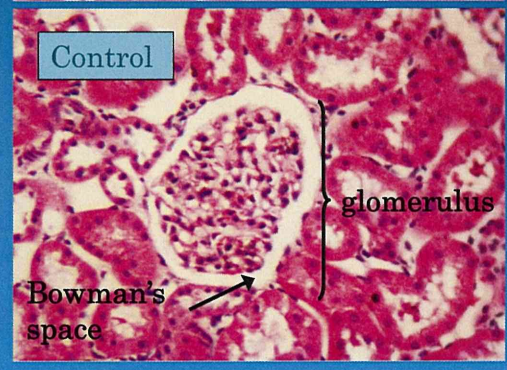
**p < 0.01 vs. saline

Experiment 2 <Result 3>

HE staining of Ang Ⅱ - infused kidney







➡ Ang II infusion caused
- stricture of Bowman's spaces.
- size-reduction of constriction of glomerulus.

Experiment 2

<Discussion>

- Result 1

  Protein carbonyl assay revealed that Ang II -infusion induced renal oxidative stress.

- Result 2

  Ang II -caused decrease of Ccr indicated renal damage.

- Result 3

  HE staining result of Ang II -administrated renal tissue suggested glomerular capillary contraction.

Conclusion

- EARF animal model was constructed by Ang II infusion.

⇒ Ang II -induced oxidative stress could cause EARF with vasoconstriction.

- Uric acid prevented renal cell damage by ROS scavenging.

⇒ Hypouricemia can be a risk factor of EARF.

Next

Examination with serum uric acid-controlled EARF models.

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