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Evaluation of the therapeutic effects of Argon inhalation in a renal transplantation model using MHC-inbred CLAWN miniature swine

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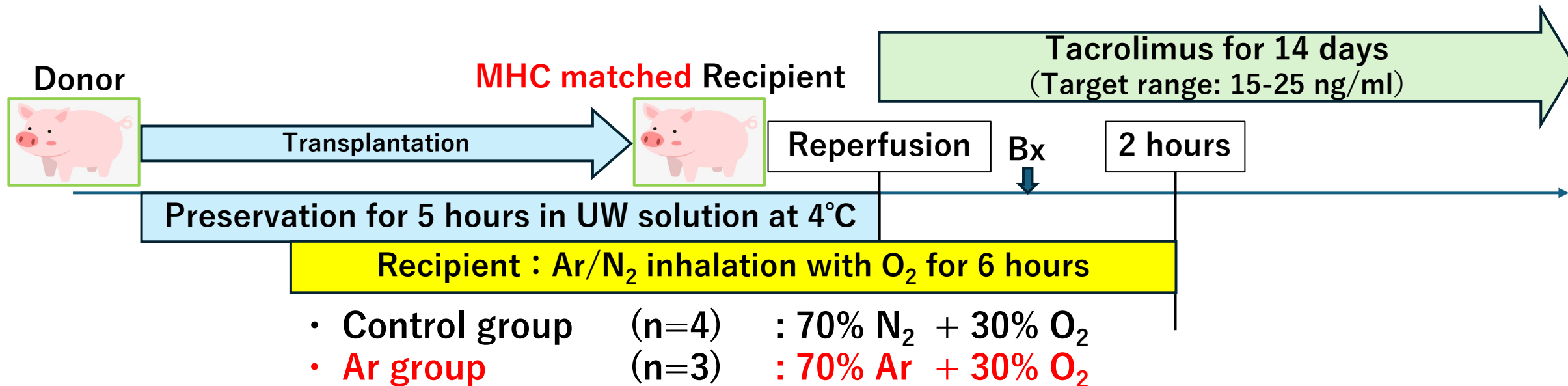
Background

- Advances in immunosuppressive drugs have significantly improved the prognosis of kidney transplantation (KTx); however, ischemia-reperfusion injury (IRI) remains a persistent problem in KTx. IRI affects not only short-term but also long-term graft survival. Therefore, the development of more effective preservation strategies to minimize IRI using perfusion devices or new drugs/methods is needed.

- The cytoprotective effects of chemically inert noble gases have been demonstrated, especially argon (Ar), which is non-anesthetic and the third most abundant gas in the atmosphere, suggesting higher safety levels and lower cost advantages.

- Previously, we reported the therapeutic effects of Ar inhalation in porcine lung IRI model induced by clamping of left pulmonary artery and veins via anti-apoptosis or anti-oxidative effect.
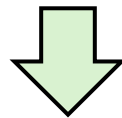
In this study, we investigated the therapeutic effects of Ar inhalation on renal IRI using MHC-matched KTx model using CLAWN miniature swine.

Methods

- Kidneys preserved in UW solution for 5 hours were transplanted into **MHC-matched CLAWN miniature swine**.

- The recipients were divided into two groups, Control and Ar, and inhaled for a total of 6 hours, starting at the beginning of surgery and continuing until 2 hours after reperfusion.

Donor

MHC matched Recipient

Tacrolimus for 14 days
（Target range: 15-25 ng/ml）

Transplantation

Reperfusion Bx 2 hours

Preservation for 5 hours in UW solution at 4℃

Recipient：Ar/N_2 inhalation with O_2 for 6 hours

- Control group　（n=4）　: 70% N_2 ＋30% O_2
- Ar group　　（n=3）　: 70% Ar ＋30% O_2

- Therapeutic efficacy of Ar was evaluated by assessing serum creatinine （sCr）, blood gas （base excess: BE, HCO_3^-）, cytokines of IL-6 and HMGB1, and graft biopsy at 1 hour post-reperfusion. The adverse effects were also monitored.

Results ① Porcine IRI-KTx model in Control group

Two out of 4 recipients in Control group showed delayed recovery of sCr after KTx following 5 hours of cold preservation (lower figure). The metabolic acidosis was observed until around POD2 (right figure).
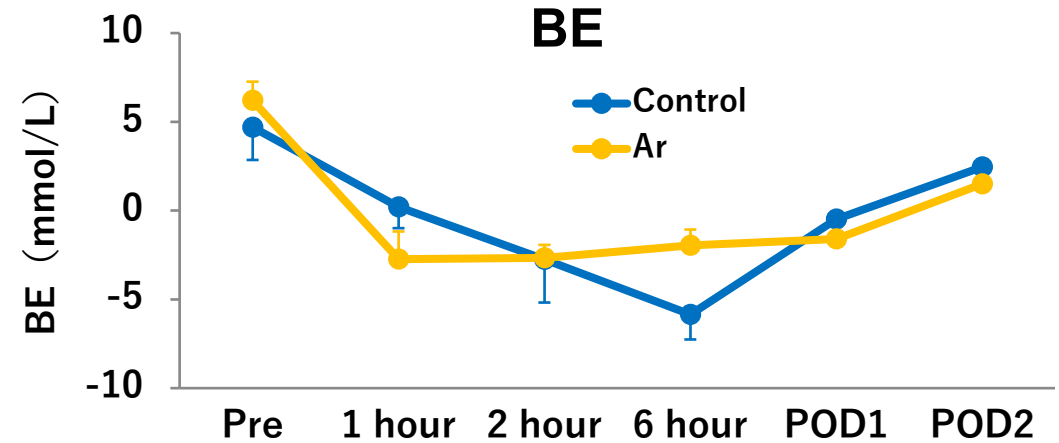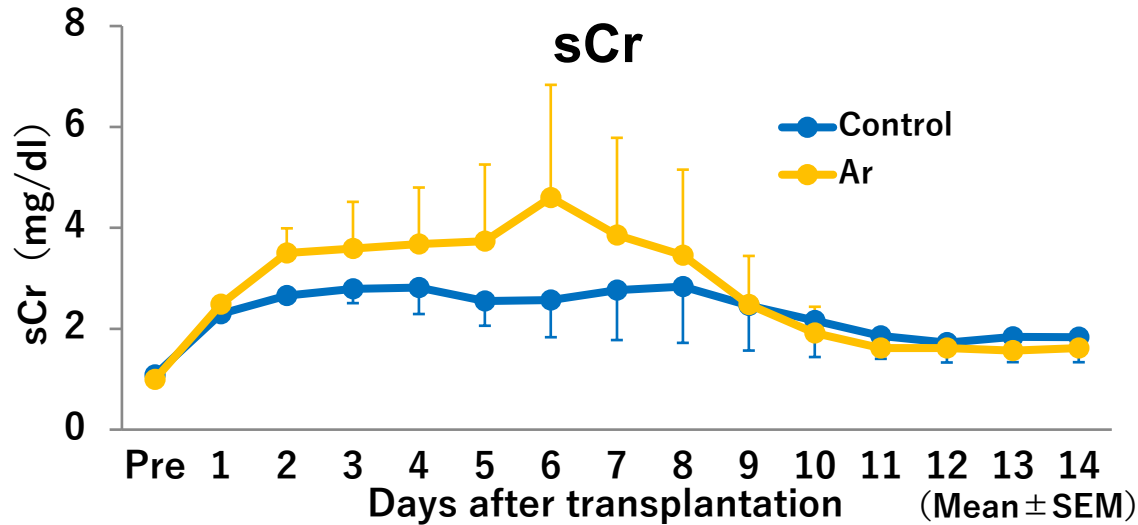
sCr: Control group

Blood gas analysis

BE (base excess)

HCO_3^-
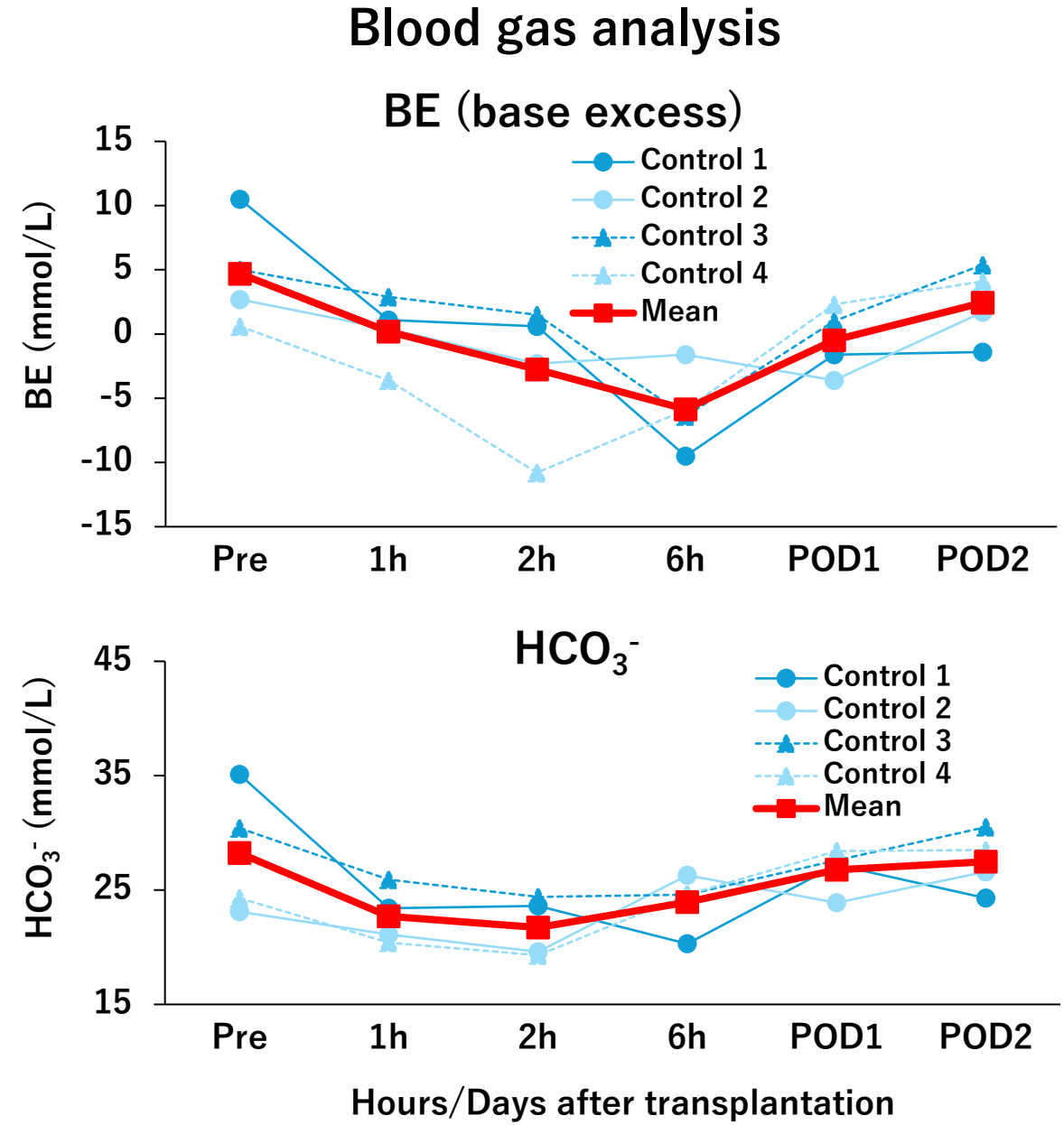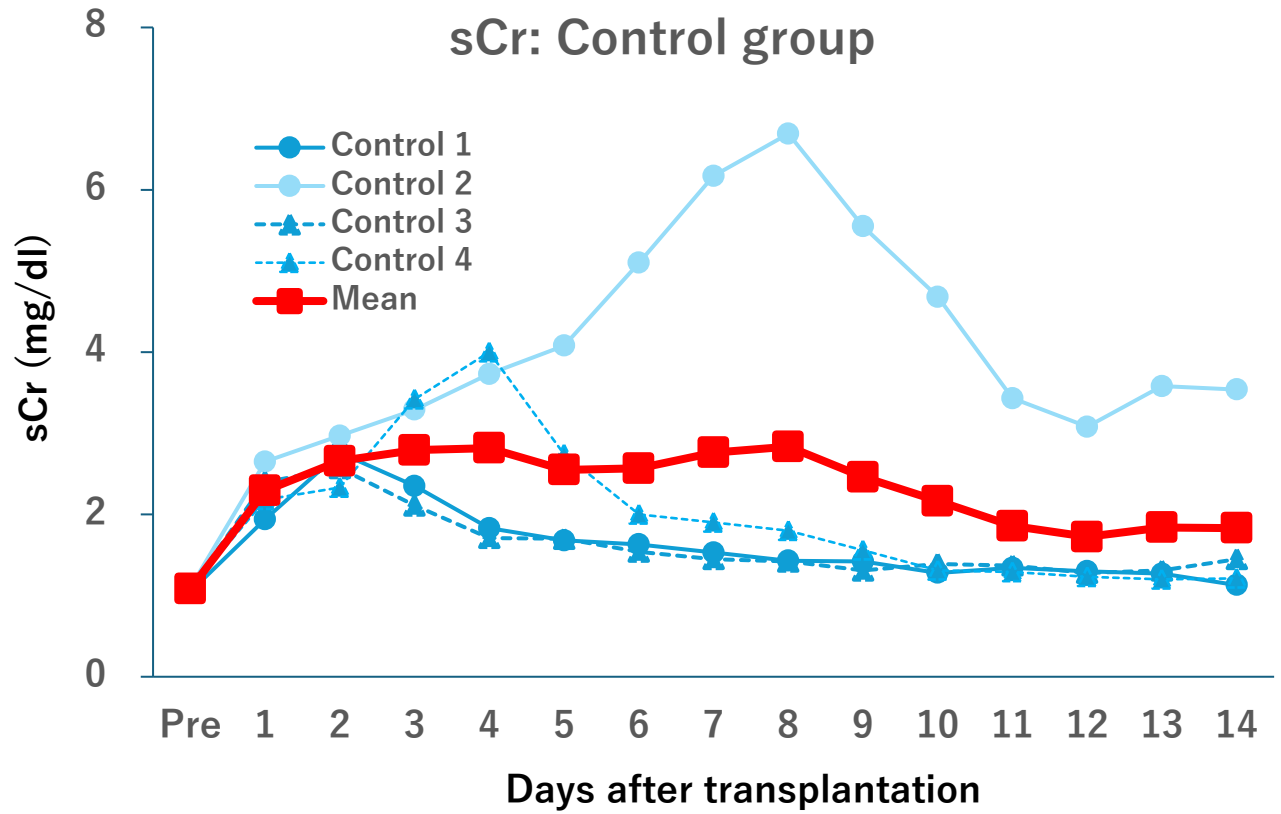
The proximal tubular injury at 1 hour after KTx was observed in all cases of Control group.


Bar: 25μm
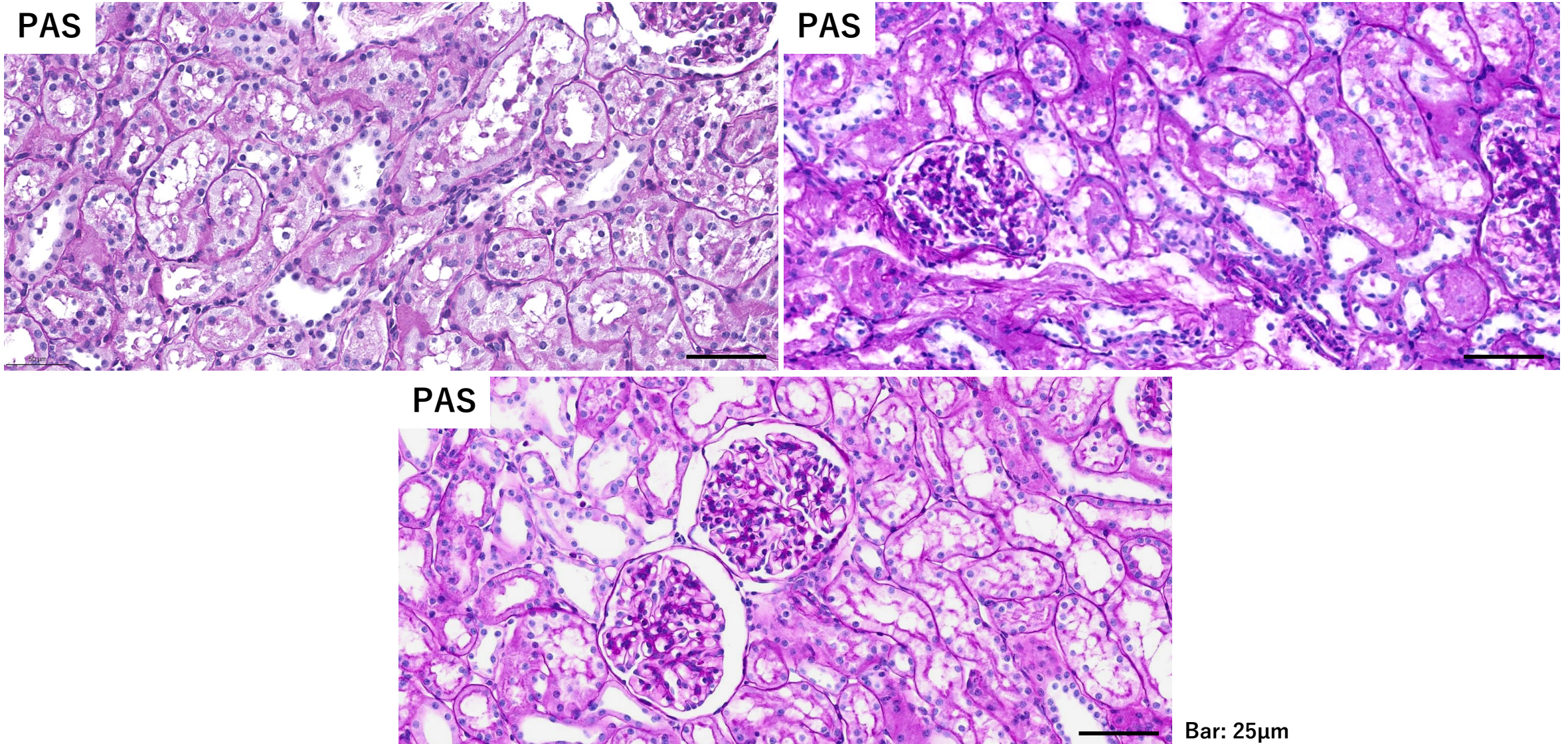
Results ② Effect of Ar inhalation in porcine IRI-KTx model

In the Ar group (yellow line), no significant difference in sCr and metabolic acidosis was observed compared to the control group. Tacrolimus serum levels were similar in both control and Ar groups and remained within the target range.

sCr

Days after transplantation　　（Mean±SEM）

BE

HCO₃⁻

Hours/Days after transplantation　　（Mean±SEM）

The tacrolimus concentrations in all cases were maintained within target range throughout POD14.

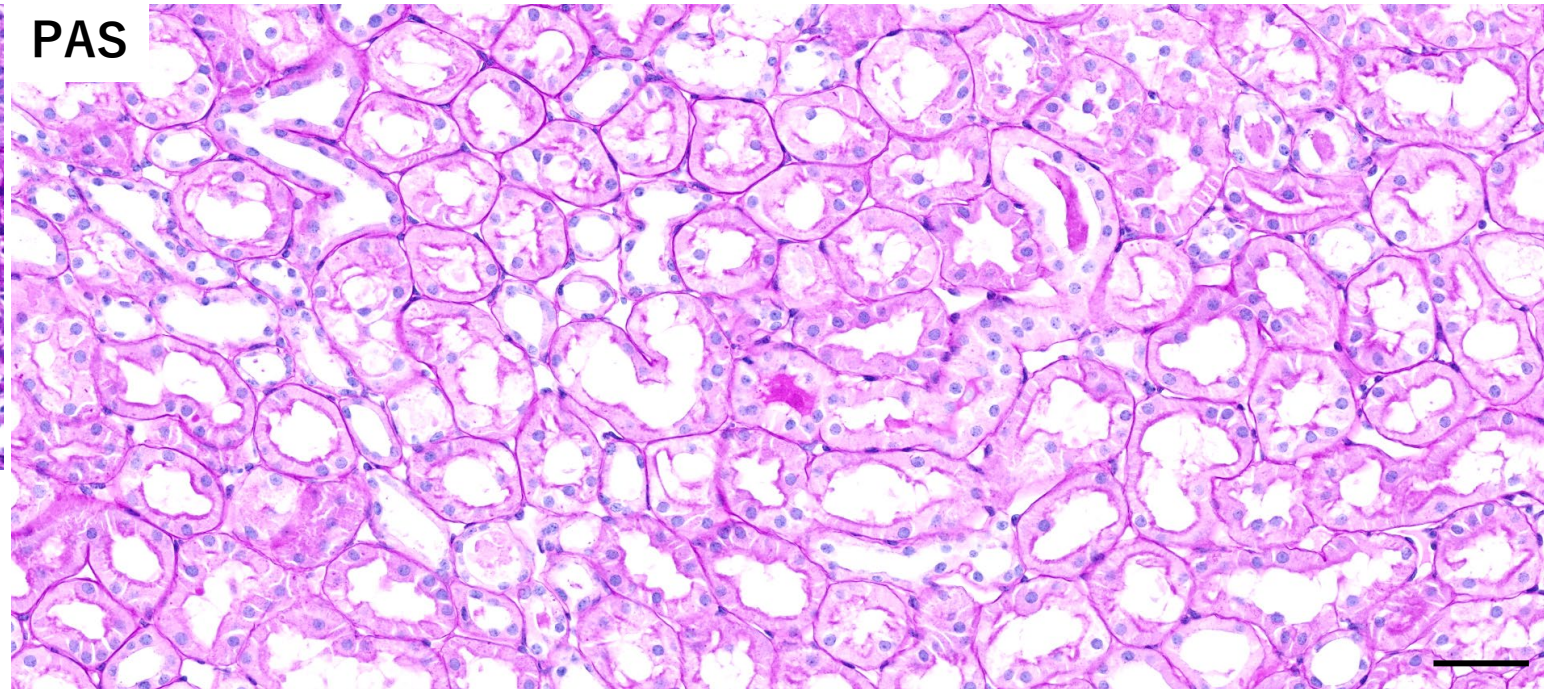| Tacrolimus (ng/ml)	
Control 1	19.2 ± 1.9
Control 2	20.9 ± 1.7
Control 3	20.2 ± 0.6
Control 4	20.5 ± 1.2
Ar 1	18.9 ± 1.7
Ar 2	18.4 ± 1.5
Ar 3	18.3 ± 1.4

（Mean±SEM）
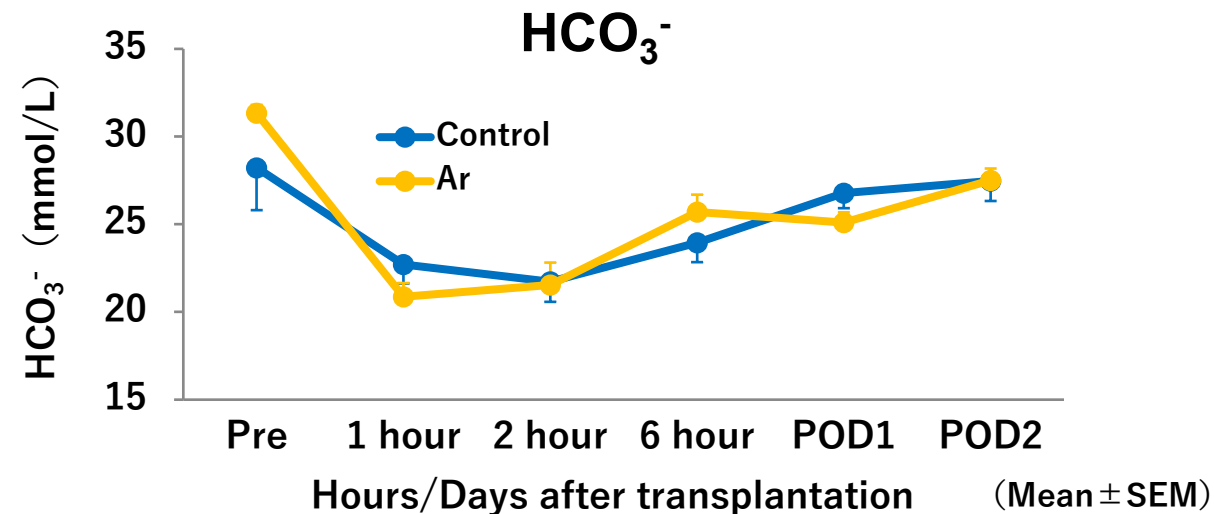
Renal graft biopsy at 1 hour after reperfusion showed a tendency for less severe tubular injury in the Ar group compared to the Control group
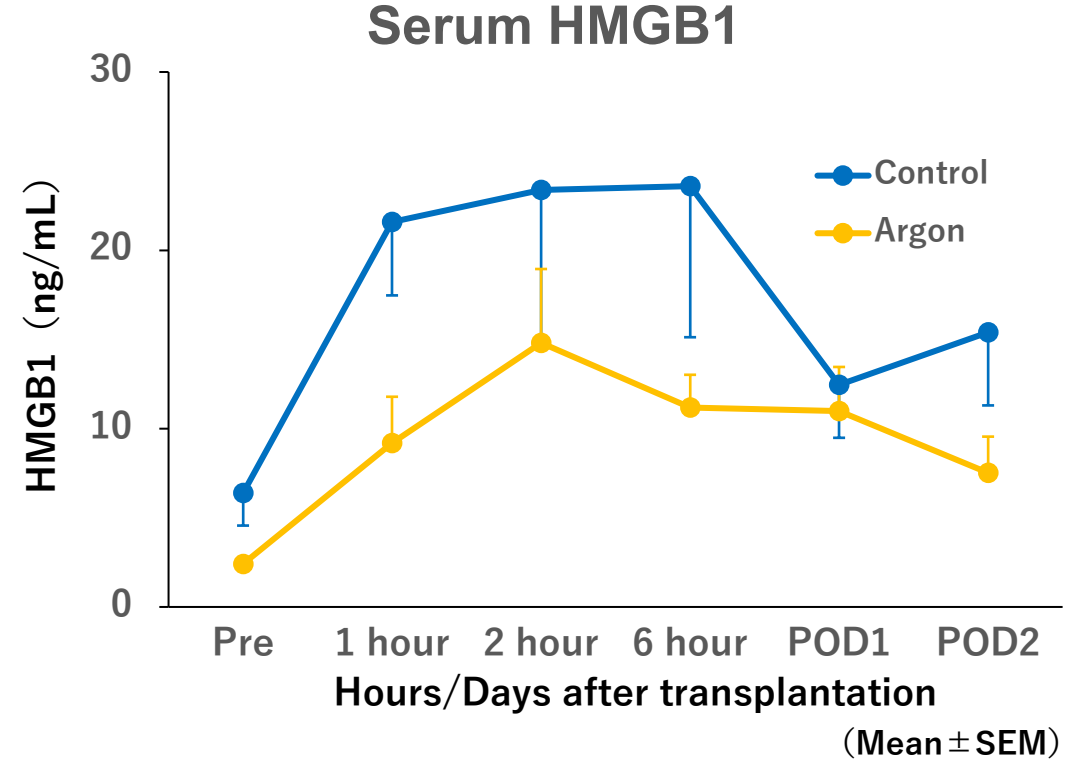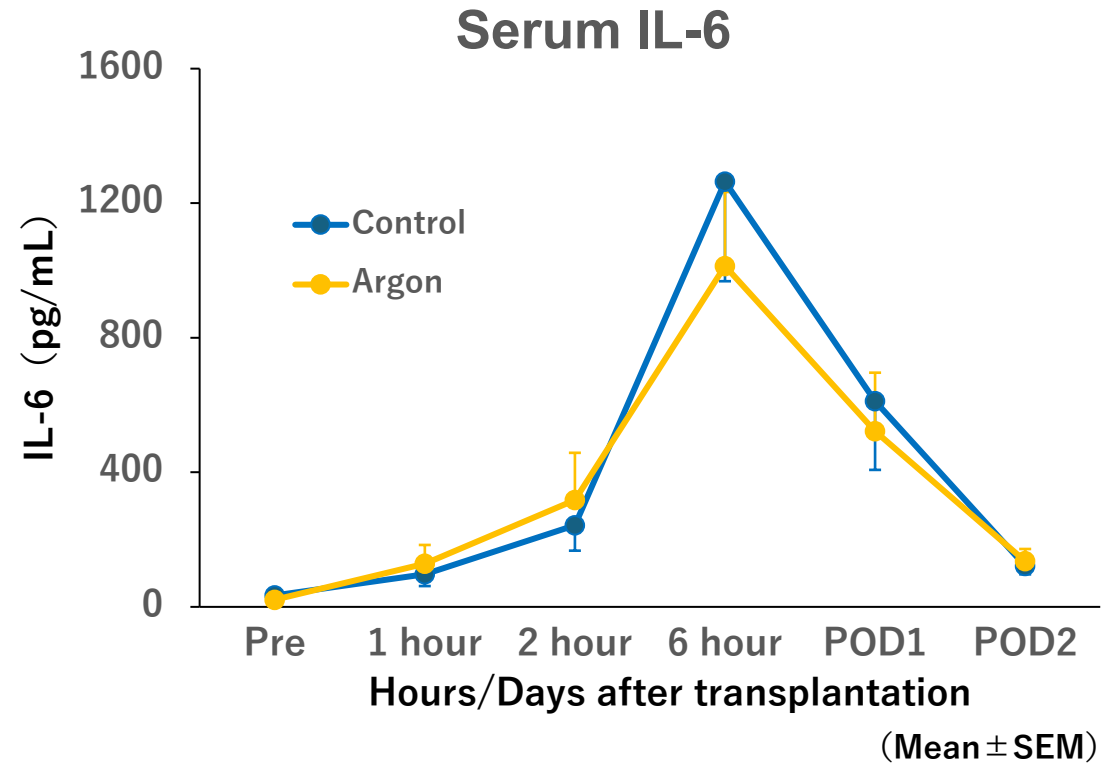
Control group
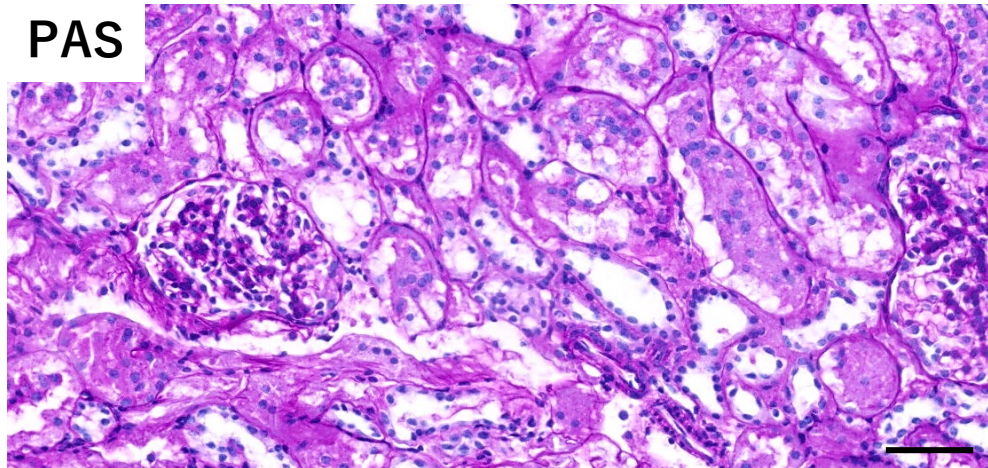
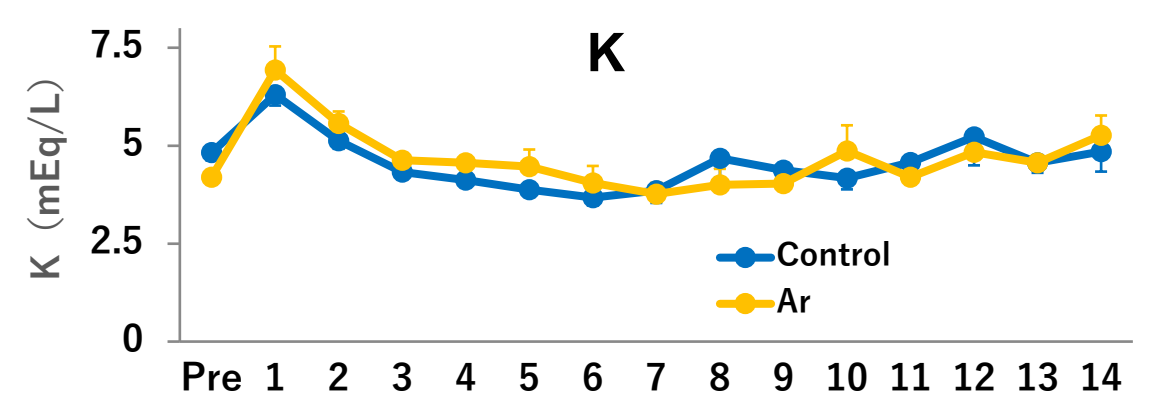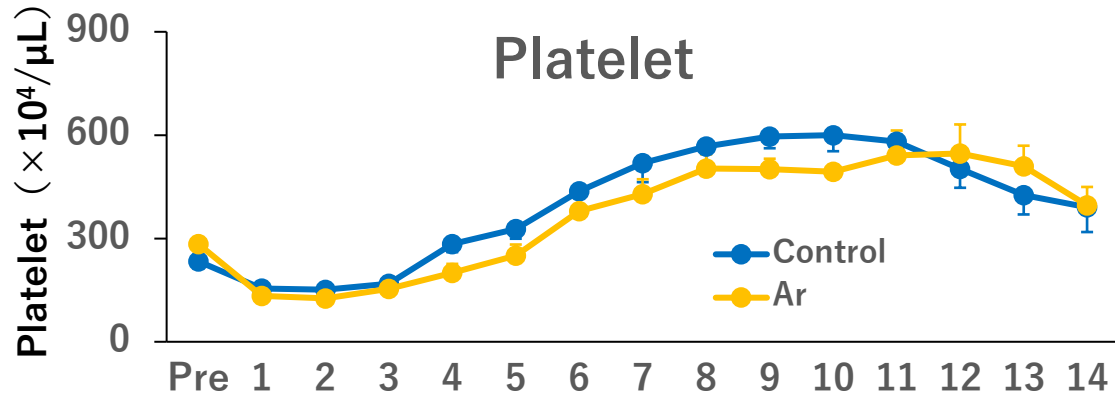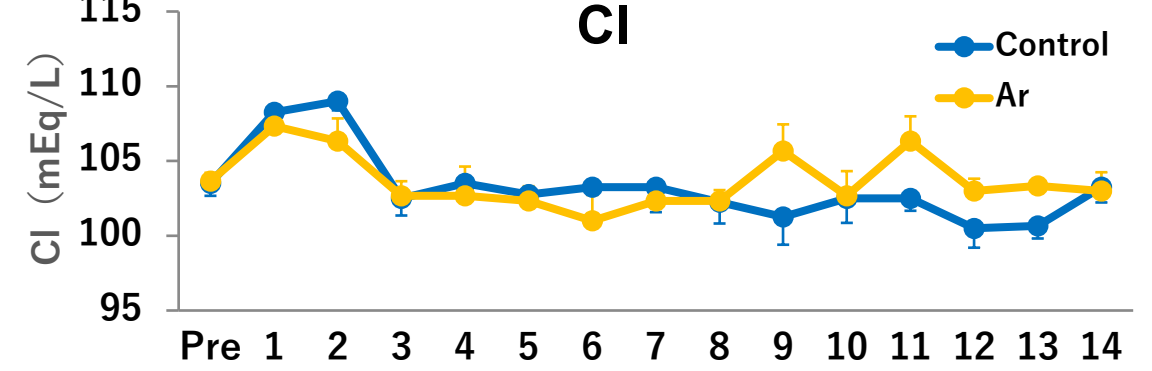**Ar group: 1-hour posttransplant biopsy of the renal graft**
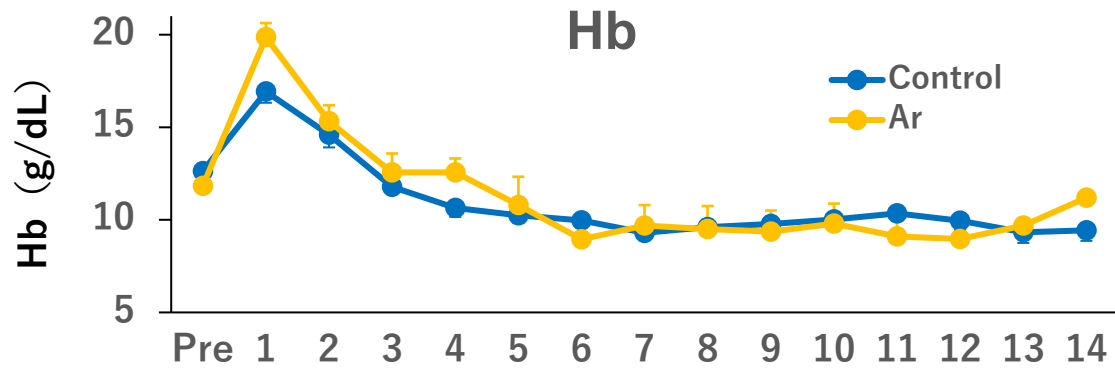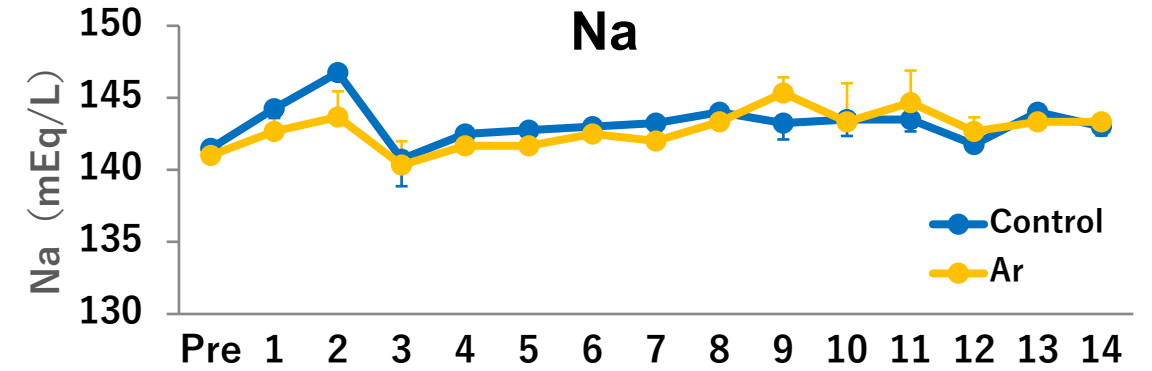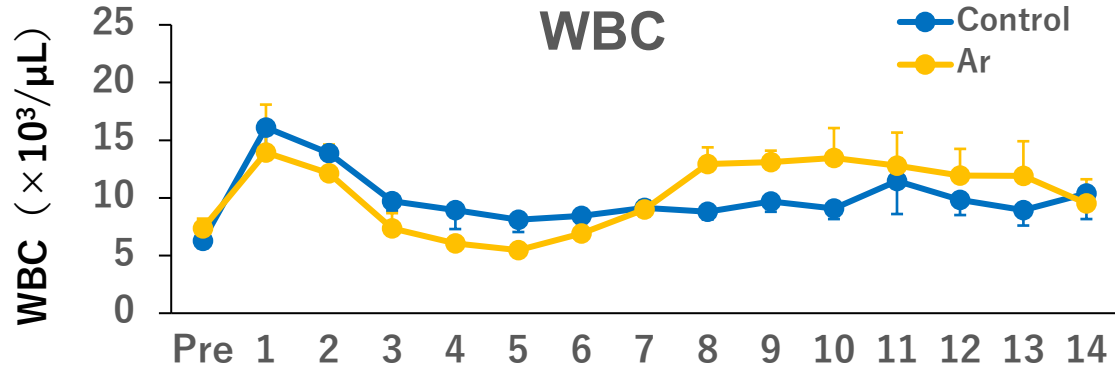
PAS

PAS

Bar: 25μm

Serum cytokine levels of IL-6 and HMGB-1 did not show a significant difference between Ar and Control groups.

No obvious side effects of Ar: No significant difference between Ar and Control groups in complete blood count and electrolytes

Summary

✓ **Control group:** MHC-matched porcine IRI-KT model was successfully induced by 5 hours of cold preservation of the kidney. In half of the Control group, sCr levels did not recovered until POD5 and 9. Acute tubular injury was observed in graft biopsy at 1 hour after reperfusion.

✓ **Ar group:** Perioperative 6-hour Ar inhalation for the recipients showed the possibility of mitigating tubular injury histologically.
     However, no significant therapeutic effect was observed on sCr, metabolic acidosis, or inflammatory cytokines (IL-6 and HMGB1).

✓ Although the efficacy of Ar was demonstrated in the porcine lung IRI model in our study, there was no effect on reducing IRI in the porcine KTx model. Various factors are being considered to evaluate, including (i) differences in methods of IRI induction, (ii) mechanisms of injury progression in different organs, (iii) timing of Ar administration (for donor/recipient), and (iv) differences in the effects of inhalation in different organs.