MEDICAL UNIVERSITY OF VIENNA

Ludwig Boltzmann Cluster for Cardiovascular Research

S-Nitroso-Human-Serum-Albumin Administration to Donor Prior to Organ Procurement Attenuates Cardiac Isograft Fibrosis and Alters Myocardial Micro-RNA-126-3p Expression in a Murine Heterotopic Heart Transplant Model

Anne Schaefer[1], Attila Kiss[1] Andre Oszwald[2], Matthias Hackl[3], Renate Kain[2], Bruno K. Podesser[1]

[1]Center for Biomedical Research, Medical University of Vienna, Ludwig Boltzmann Cluster for Cardiovascular Research, Vienna, Austria. [2]Clinical Institute of Pathology, Medical University of Vienna, Vienna, Austria, [3]TAmiRNA GmbH

Background

Cardiac allograft injury impairs survival after heart transplantation. Cold ischemia during transportation and ischemia-reperfusion (I/R) injury are cornerstones of its development. S-nitroso-human-serum-albumin (S-NO-HSA) has shown potential to attenuate I/R injury. GATA2, miR-126-3p as well as miR-92a are considered to be involved in vascular and endothelial injury. A recent study has shown downregulation of myocardial GATA-2 and miR-126-3p in murine cardiac isografts compared to non-transplanted myocardium.

Aims

To assess whether S-NO-HSA attenuates the long-term development of isograft fibrosis, and whether it influences the expression of GATA2, miR-126-3p and miR-92a.

Material and Methods

- **Isogenic transplantation** was performed in male **C57BL/6 (8-9 weeks old) mice**
- Donors received infusion of S-NO-HSA (0.1 µmol/kg/h) for 20 min (**SNO, n=5**), or normal saline (**CTRL, n=6**). Donor hearts were preserved in HTK-N solution for 12h before heterotopic transplantation, anastomosing the donor's ascending aorta to the abdominal aorta and donor's pulmonary artery to the recipient's inferior vena cava. 60 days later, tissue samples were collected.
- **miRNA and mRNA quantification:** RNA from tissues was isolated using the miRNeasy Mini Kit. For qPCR cDNA was mixed with SYBR Green master mix and LNA-enhanced miRNA primer for U6 snRNA, **miR-126-3p or miR-92a-3p**. For qPCR cDNA was mixed with SYBR, nuclease-free water and Primer for ß-actin or **GATA2**. Results were normalized to U6 snRNA for miRNA and ß-actin.
- **Histology:** Native and transplanted hearts were formalin fixed and embedded in paraffin. Sections of 10 µm were taken and stained with hematoxylin and eosin as well as Sirius red for the assessment of fibrosis. fibrosis quantified in areas of the right ventricle (RV) and the interventricular septum (IVS).

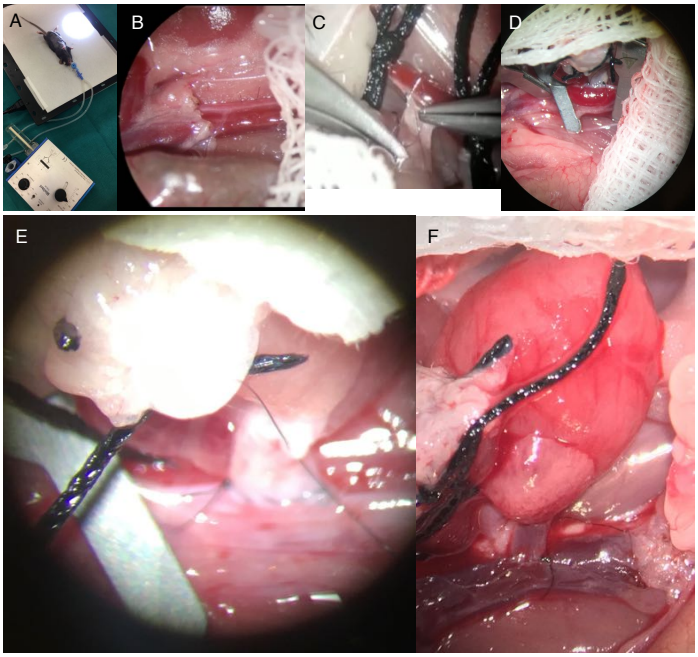

Figure 1. Operative setting during implant surgery:
A) Intubated and ventilated recipient mouse. **B)** The infrarenal aorta and vena cava are dissected and cross-clamped. **C)** The first anastomosis is created between the donor's ascending aorta and the recipient's infrarenal aorta. **D)** Finished arterial anastomosis.
E) The graft is turned and the second anastomosis is created between the donor's pulmonary trunk and the recipient's inferior vena cava.
F) Heart isograft in the recipient's abdomen after reperfusion.

Results

Myocardial Interstitial Fibrosis

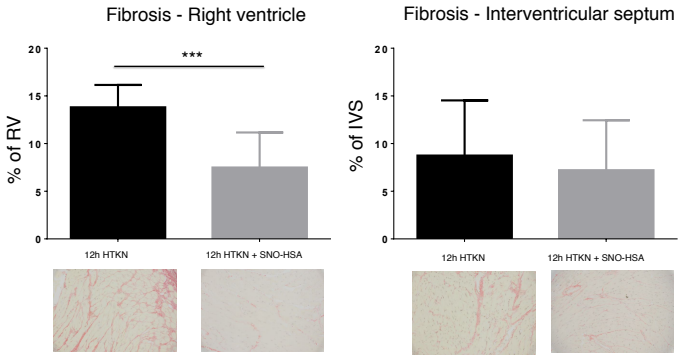

Figure 2. Fibrosis was reduced in right ventricular (RV) myocardium of transplanted hearts in the SNO group (SNO: 7.49+-3.67% vs. CTRL: 13.8+-2.35%, p=0.012, left) There was a trend towards reduced fibrosis in the SNO group in areas of the IVS (SNO: 7.21±5.24% vs. CTRL: 8.74 ±5.79%, n.s., right)

Expression of GATA-2, miR-126-3p and miR-92a

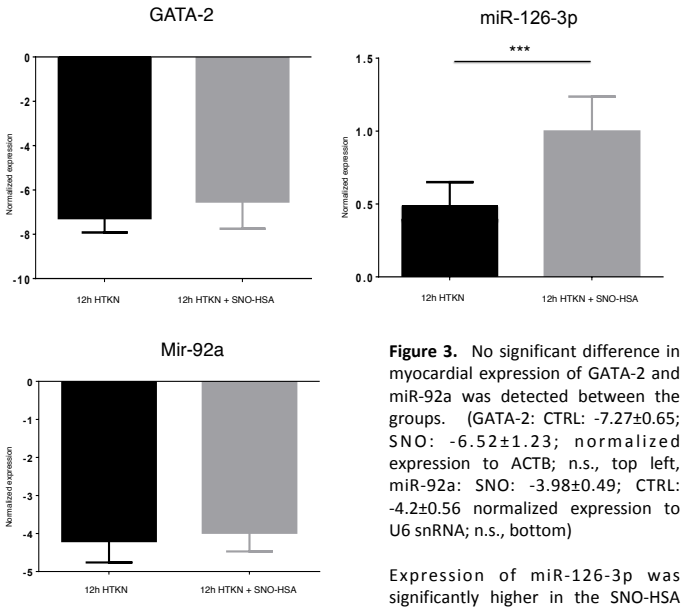

Figure 3. No significant difference in myocardial expression of GATA-2 and miR-92a was detected between the groups. (GATA-2: CTRL: -7.27±0.65; SNO: -6.52±1.23; normalized expression to ACTB; n.s., top left, miR-92a: SNO: -3.98±0.49; CTRL: -4.2±0.56 normalized expression to U6 snRNA; n.s., bottom)

Expression of miR-126-3p was significantly higher in the SNO-HSA group (SNO: 1.0±0.27 vs. CTRL: 0.51±0.38; normalized expression to U6 snRNA; p=0.038, top right.)

Conclusion

S-NO-HSA, when administered to the donor prior to organ procurement, can maintain levels of miR-126-3p and reduces isograft fibrosis 60 days after heterotopic heart transplantation in mice. Further studies are needed to identify mechanisms by which S-NO-HSA administration alteres myocardial miR-126-3p expression, and attenuates long-term development of fibrosis.

SNO-HSA administration to the donor could be a potential novel therapeutic approach to limit myocardial fibrosis in transplanted hearts.

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