Isolation, characterization, and effects of exosomes in an isolated rat heart model after circulatory death

Anja Müller, BMA 18-21 B

College of Higher Education

Educational Program for Biomedical Scientists

Departement of Cardiovascular Surgery, Inselspital

Departement for BioMedical Research, University of Bern

1. Abstract

The treatment of choice in end-stage heart failure is heart transplantation. Donor heart availability is insufficient to meet the demand. Donation after circulatory death (DCD) could increase the number of cardiac grafts, but one concern is the warm ischemia following circulatory arrest, which can lead to graft damage. Exosomes are nanoparticles that are released from a multitude of cell types. They are important regulators of the cellular niche and suggest their importance for diagnostic and therapeutic applications and as drug delivery vehicles. The aim was to isolate and characterize exosomes from reperfusion buffer of ischemic and non-ischemic (sham) hearts using several centrifuge steps, Nanoparticle Tracking Analysis (NTA) technology and western blot (WB) with exosome specific markers. An isolated, working rat heart model of DCD was used to find out more about the role and effects of exosomes during ischemia and reperfusion. Macrophage-derived exosomes were administered intravascularly to the hearts during reperfusion and their effect on cardiac recovery and metabolism were determined. Results showed that ischemic hearts released more exosomes than sham hearts. In the WB, nanoparticles could not be clearly identified. Data showed that macrophage-derived exosomes affected cardiac recovery and metabolism. In conclusion, the isolation protocol must be revised. Further, the size of the experimental groups needs to be increased and the effects of macrophage-derived exosomes on cardiac recovery need to be confirmed.

2. Introduction

The treatment of choice in end-stage heart failure is heart transplantation. Donation after circulatory death (DCD) could increase the insufficient number of available cardiac grafts when used in addition to conventional donation after brain death (DBD). [1] One major concern of DCD is the warm ischemia following circulatory arrest, which can lead to rapid and severe graft damage. To limit the ischemic injury to the warm ischemia in the donor, all DCD heart transplantations requiring graft transport have used normothermic machine perfusioin (NMP) instead of the conventional transport on ice, but reperfusion with NMP may cause further damage. [2]

Exosomes are small (40 – 150 nm) extracellular vesicles (EV) that are released from a multitude of cell types and of endosomal origin. EV execute diverse cellular functions including intracellular communication, antigen presentation and they are transporters of molecules such as proteins, lipids and mRNA. [3] Exosomes can mimic the function of parental cells, if they are injected into acutely infarcted porcine hearts, they reduce scar size and improve cardiac function. This paves the way to the development of exosome-based, cell-free treatments for heart disease. [4] Data showed that circulating EV after myocardial infarction not only promote and exacerebate inflammatory response but also contribute to reduced heart function by directly inducing cardiomyocyte death. [5]

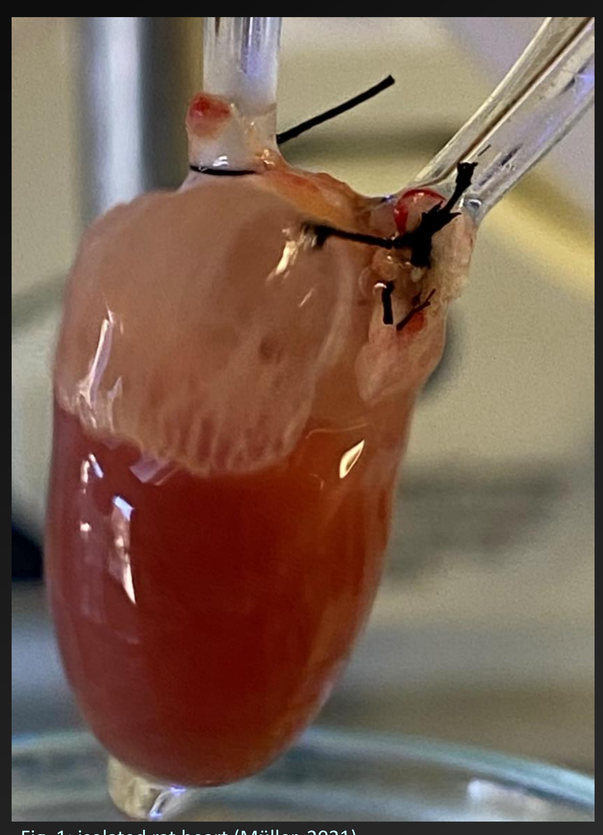

Fig. 1: isolated rat heart (Müller, 2021)

3. Aims and leading questions

Aim:

- Determine the effects of intravascular administration of macrophage-derived exosomes during early reperfusion on cardiac recovery of isolated rat hearts.

Leading question:

- Do macrophage-derived exosomes affect cardiac recovery when administred at early reperfusion in ischemic and non-ischemic rat hearts?

4. Material, methods, procedure

To simulate a DCD situation, rat hearts were made to beat with a perfusion system. Hearts were excised and then perfused through the aorta and pulmonary vein.

Experiments were performed with the perfusate passing through the hearts or with powdered heart tissue.
Experimental groups:
Group 1: non-ischemic (sham) hearts
Group 2: ischemic hearts
Group 3: ischemic hearts, intravascular administration of macrophage-derived exosomes M0 (inactivated)
Group 4: ischemic hearts, intravascular administration of macrophage-derived exosomes M1 (proinflammatory)

Left ventricular work, cardiac output and coronary flow represented heart recovery. An ELISA kit was used to measure cardiac Troponin I (cTnI) as a necrosis marker. For metabolism, glycolysis measurements were performed by measuring radioactive ^3H-H_20 generation during ^3H-Glucose degradation using an anion exchange column.

5. Results

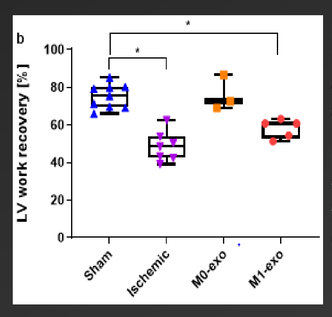

Fig. 2 shows that the LV work recovery of the ischemic group and the M1-exo group was significantly worse than the recovery of the sham group. Further, the M0-exo group tended to a better recovery than the other ischemic groups. (Müller, 2021)

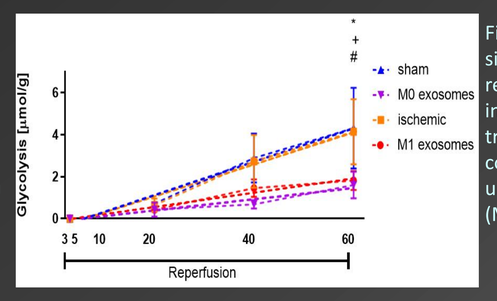

Fig. 3 shows significantly reduced glycolysis in exosome treated hearts compared to untreated groups. (Müller, 2021)

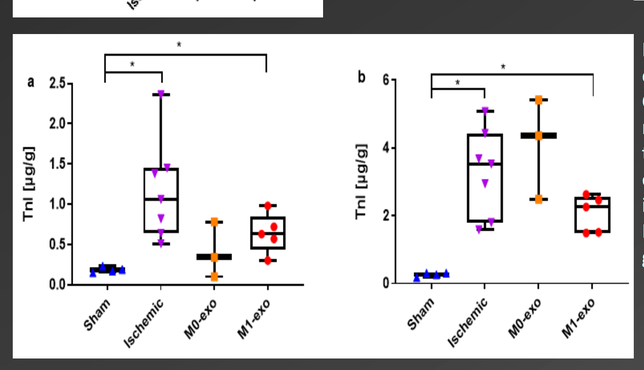

Fig. 4 shows the release of the cell death marker cTnI. The content of cTnI in sham hearts was low after 20 and after 60 min of reperfusion. CTnI level in ischemic and M1-exo group is significantly higher after 20 min of reperfusion than in sham hearts. After 60 min of reperfusion there was a threefold increase of cTnI in both groups. Further, the M0-exo group tended to suffer less cell death after 20 min than the other ischemic groups. After 60 min, there was a massive increase in cTnI of M0-exo group, but without significant difference from other ischemic groups. (Müller, 2021)

6. Discussion

Data have shown that MΦ-derived exosomes can affect cardiac recovery and metabolism. However, the pathophysiological background remains unclear. One assumption is, that the use of a different energy source or the activation of salvage pathways counteract reactive oxygen species generated in ischemia-reperfusion injury. CTnI levels at 20 min are thought to better represent ischemic damage because they are closer to the ischemia time. This was confirmed by a previous study, which stated that troponin levels in early reperfusion correlate with cardiac recovery.

References

[1] Farine et al., (2016). Controlled Reperfusion Strategies Improve Cardiac Hemodynamic Recovery after Warm Global Ischemia in an Isolated, Working Rat Heart Model of Donation after Circulatory Death (DCD). *Frontiers in Physiology*, *7*. https://doi.org/10.3389/fphys.2016.00543

[2] Yellon & Hausenloy, (2007). Myocardial Reperfusion Injury. *The New England Journal of Medicine*, 15.

[3] Greening et al., (2015). A Protocol for Exosome Isolation and Characterization: Evaluation of Ultracentrifugation, Density-Gradient Separation, and Immunoaffinity Capture Methods. In A. Posch (Ed.), *Proteomic Profiling* (Vol. 1295, pp. 179–209). Springer New York. https://doi.org/10.1007/978-1-4939-2550-6_15

[4] Barile et al., (2017). Beneficial effects of exosomes secreted by cardiac-derived progenitor cells and other cell types in myocardial ischemia. *Stem Cell Investigation*, 93–93. https://doi.org/10.21037/sci.2017.11.06

[5] Biemmi et al., (2020). Inflammatory extracellular vesicles prompt heart dysfunction via TRL4-dependent NF-κB activation. *Theranostics*, *10*(6), 2773–2790. https://doi.org/10.7150/thno.39072

Figures

Fig. 1 Müller, A. (2021). Isolated rat heart. Own figure.
Fig. 2 Müller, A. (2021). LV work. Own graph.
Fig. 3 Müller, A. (2021). Glycolysis. Own graph.
Fig. 4 Müller, A. (2021). cTnI. Own graph.