Regenerative medicine therapy: adipose derived extracellular vesicles in viral myocarditis

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Abstract

Objective: Myocarditis, inflammation of the heart muscle, is an autoimmune heart disease that can be caused by viruses, bacteria and toxins. Myocarditis can lead to dilated cardiomyopathy (DCM) and heart failure. Currently there are no disease-specific therapies for treating myocarditis or preventing progression to DCM. Adipose Extracellular Vesicles (AEVs) are lipid bilayer nanoparticles that are released into the outside environment of adipocytes and provide promising regenerative potential for inflammatory diseases like myocarditis.

Methods: Lipoaspirate was obtained from women and men and AEVs isolated from the lipoaspirate using tangential flow filtration. We injected wild type male BALB/c mice with 250uL AEVs (1x10^10 EV/mL) intraperitoneally or sucrose control on day -1, 0, 1 with viral infection on day 0. Mice were harvested on day 10 post infection at the peak of myocarditis.

Results: We found that male mice treated with AEVs from a female patient had a significantly higher body weight (p=0.0003), less calcification in the gut (p=0.001) and less myocardial inflammation (p=0.007) than controls. Mouse hearts analyzed by qRT-PCR revealed that AEV treated mice had significantly lower relative gene expression of cell markers for total immune cells (CD45, p=0.002), macrophages (CD11b, p=0.002, F4/80, p=0.0004); specifically M2 macrophages (Chi313, p=0.003), as well as CD3+ (p=0.007) and CD4+ T cells (p=0.01) than controls. Additionally, we found that mice treated with AEVs from a male patient also had significantly less myocardial inflammation (p=0.01).

Conclusion: AEVs could provide an innovative therapy to reduce cardiac inflammation and decrease the risk of developing DCM following myocarditis.

Methods

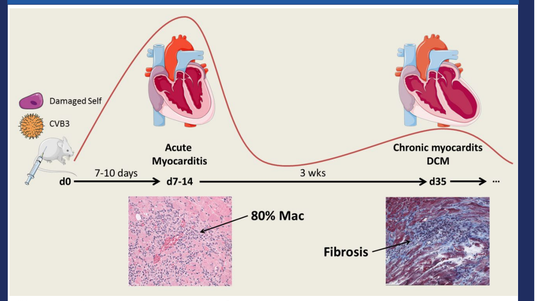


Figure 1. Disease progression for viral myocarditis in animal model

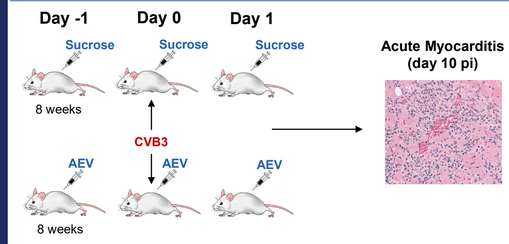
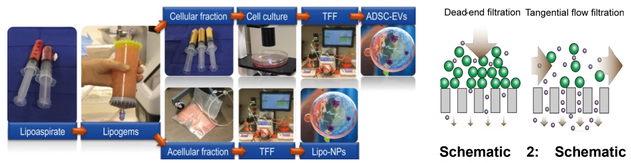


Figure 2. Study design for AEV treated mice during myocarditis.

Results



Schematic 1. Procedure for obtaining AEVs by processing human lipoaspirate.

Schematic 2: Schematic illustrating the difference between dead-end filtration and tangential flow filtration (TFF).

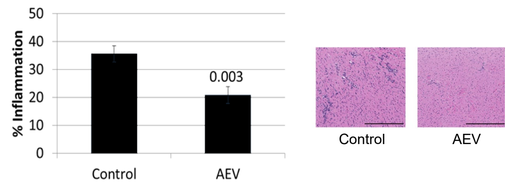


Figure 3. AEV treated mice have significantly reduced inflammation compared to sucrose treated controls. Inflammation (%) scored using H&E stain and eye piece grid normalized to the size of the heart.

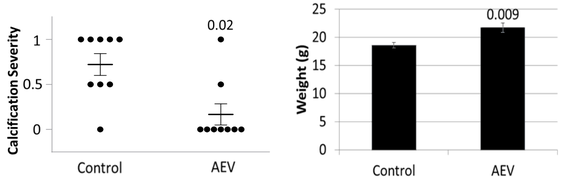


Figure 4. AEV decreased disease severity. AEV treatment reduced calcification (*left*) and increased body weight (*right*)- both measures of improved disease.

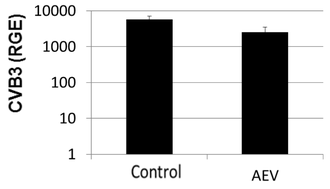


Figure 5. AEV treatment does not significantly alter CVB3 viral genome number in the heart. Y-axis is log10 scale; RGE determined using qRT-PCR. These findings suggest that reduced cardiac inflammation is not related to reduced viral levels in the heart.

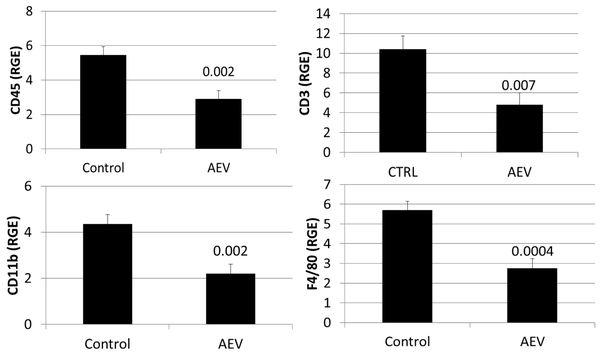


Figure 6. AEV treatment reduces immune cell markers compared to control. AEVs decrease total immune cells (CD45+), Total T cells/T helper cells (CD3), CD11b (neutrophils, macrophages, mast cells and dendritic cells), and F4/80 (activated macrophages).

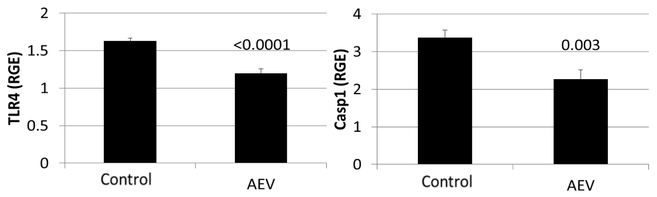


Figure 7. Hearts of AEV treated mice have decreased expression of TLR4 (left) and caspase-1 (right), which increase inflammation and remodeling in the heart.

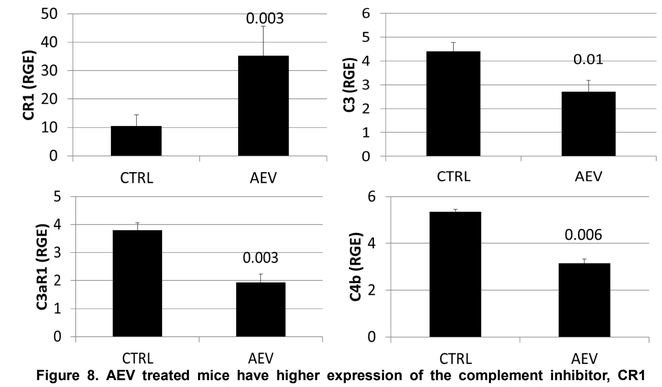


Figure 8. AEV treated mice have higher expression of the complement inhibitor, CR1 which lowers expression of activated complement pathway genes C3, C3aR1, and C4b and reduces inflammation.

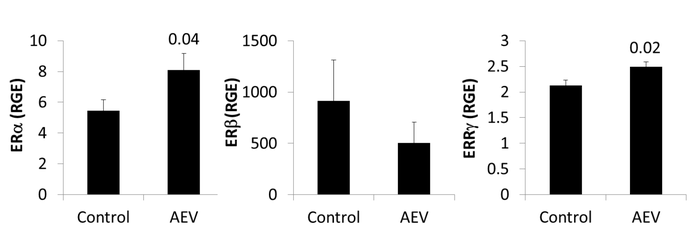


Figure 9. AEVs altered hormone expression in the heart of mice.

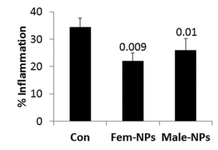


Figure 10. Lipo-NPs (AEVS) from two different women and one man decrease CVB3 myocarditis when given to male BALB/c mice.

Conclusions and Future Studies

Conclusions

- AEV treatment decreases myocarditis severity (decreases % inflammation in the heart, increases body weight, and decreases calcification)
- AEV decreases immune cell populations in the heart (total immune cells, neutrophils, mast cells, and dendritic cells, macrophages and T cells
- AEV treated mice have higher expression of CR1, the primary regulator of the complement system. AEV also decreases expression of main complement cascade components
- AEVs decrease proinflammatory and profibrotic TLR4 and caspase-1
- AEVs altered hormone expression, specifically ERα and ERRγ in the heart of mice

Future Studies

- Determine whether AEV treatment can reduce or prevent DCM and chronic heart failure if given during myocarditis- a clinically relevant time-point
- Perform flow cytometry to better characterize immune cell populations
- Determine the internal and external composition of AEVs
- Compare ip to iv routes of administration
- Investigate anti-inflammatory drug loaded AEVs effect on myocarditis and DCM

References

1. Arslan, F., Lai, R. C., Smeets, M. B., Akeroyd, L., Choo, A., Aguor, E. N. E., ... de Kleijn, D. P. (2013). Mesenchymal stem cell-derived exosomes increase ATP levels, decrease oxidative stress and activate PI3K/Akt pathway to enhance myocardial viability and prevent adverse remodeling after myocardial ischemia/reperfusion injury. *Stem Cell Research*, *10*(3), 301–312. https://doi.org/10.1016/j.scr.2013.01.002
2. Eirin, A., Riester, S. M., Zhu, X. Y., Tang, H., Evans, J. M., O'Brien, D., … Lerman, L. O. (2014). MicroRNA and mRNA cargo of extracellular vesicles from porcine adipose tissue-derived mesenchymal stem cells. *Gene*, *551*(1), 55–64. https://doi.org/10.1016/j.gene.2014.08.041
3. Tian, M., Ticer, T., Wang, Q., Walker, S., Pham, A., Suh, A., Busatto, S., Davidovich, I., Al-Kharboosh, R., Lewis-Tuffin, L., Ji, B., Quinones-Hinojosa, A., Talmon, Y., Shapiro, S., Rückert, F., Wolfram, J., Adipose-Derived Biogenic Nanoparticles for Suppression of Inflammation. Small 2020, 16, 1904064. https://doi.org/10.1002/smll.201904064
4. Eirin, A., Zhu, X. Y., Puranik, A. S., Tang, H., McGurren, K. A., van Wijnen, A. J., … Lerman, L. O. (2017). Mesenchymal stem cell–derived extracellular vesicles attenuate kidney inflammation. *Kidney International*, *92*(1), 114–124. https://doi.org/10.1016/j.kint.2016.12.023
5. Elamm, C., Fairweather, D. L., & Cooper, L. T. (2012). Republished: Pathogenesis and diagnosis of myocarditis. *Postgraduate Medical Journal*. NIH Public Access. https://doi.org/10.1136/postgradmedj-2012-301686rep
6. Fairweather, D. L., Cooper, L. T., & Blauwet, L. A. (2013). Sex and Gender Differences in Myocarditis and Dilated Cardiomyopathy. *Current Problems in Cardiology*, *38*(1), 7–46. https://doi.org/10.1016/j.cpcardiol.2012.07.003
7. Fairweather, D., Stafford, K. A., & Sung, Y. K. (2012). Update on coxsackievirus B3 myocarditis. *Current Opinion in Rheumatology*. https://doi.org/10.1097/BOR.0b013e328353372d
8. Poe, A. J., & Knowlton, A. A. (2017). Exosomes as agents of change in the cardiovascular system. *Journal of Molecular and Cellular Cardiology*. NIH Public Access. https://doi.org/10.1016/j.yjmcc.2017.08.002
9. Schultheiss, H. P., Khl, U., & Cooper, L. T. (2011). The management of myocarditis. *European Heart Journal*. Oxford University Press. https://doi.org/10.1093/eurheartj/ehr165