Pulmonary Myocardium Assessed in Medical School Cadaveric Material

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Introduction

The heart is basically a unidirectional double-pump. In order to fulfill it's purpose, it needs a coordinately contracting myocardial network and some valves. The coordination of the myocardial contraction is assured by the size of the connexins in the gap junctions between each cardiomyocyte. The gap junctions are channels formed by connexons, which in turn are built up by connexins. The size of the connexins will determine the speed of the electrical impulse crossing the gap junctions – smaller the connexins are, faster the speed. Thus, we have the fastest junctions/smaller connexins in the conduction system, then atria; the biggest connexins/slower junctions in the ventricles. The particular arrangement of the connexins in the ventricular myocardium (slow conduction) and conduction system (fast conduction) allows the ventricles to start contracting from their furthest point from the valves.

With the development of the electrophysiology, it became possible to map the path of the electrical impulse within the cardiac muscle. Soon, it was observed that very high percentage of the atrial fibrillations (94%) is actually originating from outside the heart, from the pulmonary veins [1,2]. With the help of different ablation methods, now these type of fibrillations are successfully remediated. The reason why ectopic electrical impulse can come from the pulmonary veins is that during the embryonic development the left atrial myocardium outgrows onto the pulmonary veins. It was shown that this outgrowing pulmonary myocardium/myocardial sleeves act as a fully functional atrial myocardium – meaning that the cardiomyocytes are interconnected with gap junctions.

The goal of our study was to start determining the prevalence and extent of ectopic atrial myocardium growth into the pulmonary veins in cadaveric material.

Methods

Sample collection:

- We have arbitrarily chosen 5 cadavers from our anatomy lab, which had comparatively normal hearts.
- Our samples are from both males and females, with no specific indication of heart disease in their cause of death.
- We started our study by arbitrarily choosing the superior pulmonary veins, which were dissected out from the lungs until their third branching,
- 3-5 mm rings were excised from the main segment and subsequently from the 1st-, 2nd- and 3rd branching.

Histology, immunohistology:

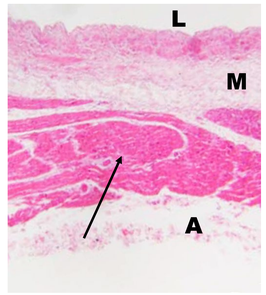
- The excised samples were thoroughly washed and rehydrated in phosphate-buffered saline.
- Then processed for paraffin embedding and sectioning – 5 µm sections were cut on a rotary microtome, then collected on regular glass slides.
- Some of the slides were processed for hematoxylin-eosin (H&E) staining, the rest of them for immunohistochemistry.
- For immunohistochemistry we used the anti-human cardiac myosin heavy chain which is myocardium specific.

Biochemistry:

- Some of the rehydrated samples were processed for protein extraction.
- The tissue lysates were normalized based on their protein content.
- 20 µl of samples were loaded into the membrane.
- Each sample was run 3 times and labeled with anti-connexin 43 antibody.

Results

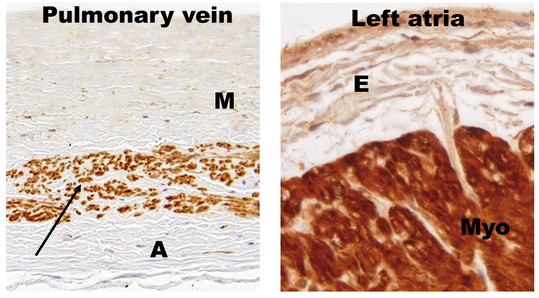
Figure 1. **Identification of the myocardial-like tissue in the wall of the pulmonary veins**



Cadaver	Atrial Control	Main Pulmonary Vein	1st branching	2nd branching	3rd branching
1	+	+	+	+	
2	+	+	+	+	+
3	+	+	+	+	+
4	+	+	+	+	
5	+	+	+	+	+

Representative image of the wall segment of the main pulmonary vein (left). On the adventitial side (A) thick bundle of myocardial-like cells (arrows) are seen. Hematoxylin-eosin staining. L-lumen, M- media. The table on the right shows the number of cadavers we isolated pulmonary veins, and the levels we collected samples (main, 1st-, 2nd-, and 3rd branches). The + indicates where we find the myocardial-like bundles. As a control, we used a sample from the left atria (histology not shown).

Figure 2. **Identification of the myocardium by immunohistochemistry**



Representative images of DAB-developed indirect immunohistochemistry of the pulmonary vein (left) and atrial wall (right). The anti-human cardiac myosin heavy chain antibody labels the bundles identified with the H&E staining between the media (M) and adventitia (A). In the atrial wall, the staining is restricted to the myocardium (Myo) underneath the epicardium (E).

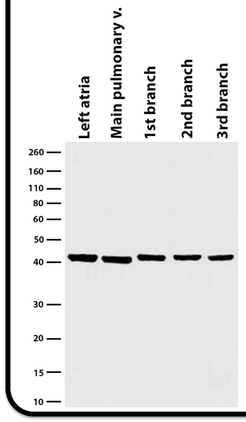


Figure 3. **Presence of the Connexin 43 in the samples indicates functional atrial myocardium in the pulmonary veins**

Western blot analysis of the left atrial and pulmonary vein sample lysates. Samples were normalized on the protein amount. The bands labeled by the anti-Connexin 43 antibody are localized at the 43 kD level.

Conclusions

We have successfully processed cadaveric material from the anatomy lab for histology, immunohistochemistry and biochemistry.

We were able to confirm immunohistologically that the myocardial-like tissue in the pulmonary veins at different levels is indeed myocardium, similar to that found in the atrial wall.

We also were able to determine the presence and type of the connexins in our sample by western blotting.

The presence of the Connexin 43, the building block of the fast-conducting gap junctions, explains why this ectopic myocardium can potentially act as an ectopic location for impulse conduction, and potentially overtake the major impulse generator, the sinus node.

The major limitations of our study are the low number of samples, and the very limited markers we used.

In the future, we intend to collect samples from more cadavers, as well to use more specific markers for characterizing the pulmonary myocardial outgrowths. It is also our plan to collect samples more targeted, taking in consideration the cause of death (specially indicating cardiac arrythmias).

Our findings support the existing theory of atrial fibrillation.

The potential outcome of our study, by fully characterizing these pulmonary myocardial outgrowths, is to provide a platform for targeted pharmaceutical intervention for prevention and cure of the majority of the atrial fibrillations. The pharmaceutical approach may prevent the secondary/adverse effects of the different ablation methods – namely the subsequent scarring and constrictions in the pulmonary vein diameters.

References

1. Khan R. Identifying and understanding the role of pulmonary vein activity in atrial fibrillation. Cardiovascular Research. 2004;64(3):387-394. https://doi.org/10.1016/j.cardiores.2004.07.025.
2. Haïssaguerre M, Jaïs PJ, Shah DC, et al. Spontaneous initiation of atrial fibrillation by ectopic beats originating in the pulmonary veins, N Engl J Med. 1998;339:659-666. https://doi.org/10.1056/NEJM199809033391003

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This work is exempt from IRB as we are working with completely deidentified cadavers, which do not qualify as human subject material.