Yossef Elizarov

The long term effect of Radiofrequency Ablation on the Intrinsic Nerve System of the Myocardium

Master’s thesis

Thesis supervisor
Lekt. Kristina Rysevaite-Kyguoliene

Kaunas 2017
Table of Contents

1. Summary .................................................................................................................. 3
2. Conflict of Interest .................................................................................................. 4
3. Permission issued by the ethics committee ............................................................. 5
4. Abbreviations ......................................................................................................... 6
5. Introduction ............................................................................................................. 7
6. aim .......................................................................................................................... 8
7. objective .................................................................................................................. 8
8. Literature Review ................................................................................................... 9
   5.1 Extrinsic cardiac innervation ............................................................................ 9
   5.2 Intrinsic cardiac nerve system (ICNS) .............................................................. 9
   5.3 The heart conduction system .......................................................................... 11
   5.4 The interconnection of the intrinsic cardiac system and the conduction system. ......................... 12
   5.5 Atrial fibrillation ............................................................................................. 12
   5.6 Cardiac frequency ablation ............................................................................ 14
   5.7 Catheter radiofrequency ablation in atrial fibrillation. ................................. 14
   5.8 Radiofrequency ablation clinical results ......................................................... 14
   5.9 Radiofrequency ablation consequence on nerve fibers. ............................... 15
9. Research methodology and methods .................................................................. 16
10. Results .................................................................................................................. 18
   7.1 ChAT marker results ..................................................................................... 18
   7.2 TH marker results ......................................................................................... 19
   7.3 PGP 9.5 marker results ................................................................................. 21
11. Discussion .............................................................................................................. 23
12. Conclusions .......................................................................................................... 26
13. References .............................................................................................................. 27
1. SUMMARY

**Aim** - The aim of the study is to investigate the impact of radiofrequency catheter ablation on the intrinsic nerve system of the myocardial layer of a sheep, by measuring and comparing three types of neuronal markers population using immune histochemical methods.

**Objectives**

1) To determine if there is difference in the nerve fibers population in all heart locations as a whole, between experimental group and control

2) To determine if there is difference in the nerve fibers population in dorsal aspect of the heart between experimental group and control

3) To determine if there is difference in the nerve fibers population in ventral aspect of the heart between experimental group and control

4) To determine if there is difference in the nerve fibers population in 4 different location (dorsal apex, dorsal middle, dorsal base, ventral) in the heart between experimental group and control

**Methodology** - 5 sheep undergo a catheter radiofrequency ablation, three other sheep didn’t undergo the procedure and were used as a control group. After 5 months all sheep were euthanized, and their hearts were removed, and the hearts were cut into separate regions, tissue samples frozen and cut using cryomicrotomy into samples, and then the samples undergo a immune histochemistry for general, cholinergic and adrenergic neural markers using antibodies, photos of the samples were taken and analyzed by axiovesion program to determine the percent of the marker from the total area of each frame, the data were collected and compared the experimental group vs the control group.

**Results** – the study found a clear change in the levels of the neural markers, levels of PGP and TH Marker were lower in the experimental group, while the levels of ChAT were higher in the experimental group. The changes observed in 4 types of comparisons all regions of the heart as a whole, dorsal to dorsal, ventral and specific regions, though in some tables the difference was not statistically significant.

**Conclusions** - catheter radio frequency ablation in the root of the pulmonary veins decrease the levels of nerve fibers in the myocardium and thus has a clear impact on the intrinsic system in the myocardium layer of the heart. The changes in the nerve fiber population is a decrease in the overall number of nerve fibers and decrease in the sympathetic nerve system fibers, while there is increase in the levels of the parasympathetic nerve fibers.
2. CONFLICT OF INTEREST

The author reports no conflicts of interest related to this study
3. PERMISSION ISSUED BY THE ETHICS COMMITTEE

Permission number 33ĮV-62 issued by the State Service for Food and Veterinary at 2014 02 05
4. ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>ChAT</td>
<td>Choline Acetyltransferase</td>
</tr>
<tr>
<td>PGP 9.5</td>
<td>Protein Gene Product</td>
</tr>
<tr>
<td>TH</td>
<td>Tyrosin Hydroxylase</td>
</tr>
<tr>
<td>INS</td>
<td>Intrinsic Nerve System</td>
</tr>
<tr>
<td>AF</td>
<td>Atrial Fibrillation</td>
</tr>
<tr>
<td>RFA</td>
<td>Radio frequency ablation</td>
</tr>
<tr>
<td>AV</td>
<td>Atrioventricular</td>
</tr>
<tr>
<td>SA</td>
<td>Sinoatrial</td>
</tr>
<tr>
<td>SVC</td>
<td>Superior vena cava</td>
</tr>
</tbody>
</table>
4. INTRODUCTION

Radiofrequency ablation is widely used method for a drug resistant atrial fibrillation, though the procedure has good outcomes in the short term [1], studies that investigate the long term impact found high rate of recurrence [2]. These outcomes are especially important considering that the developments in procedure lead to more extensive ablated areas in order to ensure better results [3].

These findings made the scientific community to wonder weather the procedure is efficient as expected, and thus lead the Institute of Anatomy to an hypothesis that the ablation in the root of the pulmonary veins may cause an irreversible damage to the intrinsic system of the heart and by that compromising the conduction system of the heart and contractility all which lead to pathological outcomes, while morphological studies have been done on the issue, this study aim to compare quantitative data between two group, in the study a experimental group of sheep that underwent catheter radiofrequency ablation and a control group.
5. AIM

The aim of the study is to investigate the impact of radiofrequency catheter ablation on the intrinsic nerve system of the myocardial layer of a sheep, by measuring and comparing three types of neuronal markers population using immune histochemical methods.

6. OBJECTIVE

1) To determine if there is difference in the nerve fibers population in all heart locations as a whole, between experimental group and control
2) To determine if there is difference in the nerve fibers population in dorsal heart between experimental group and control
3) To determine if there is difference in the nerve fibers population in ventral heart between experimental group and control
4) To determine if there is difference in the nerve fibers population in 4 different location (dorsal apex, dorsal middle, dorsal base, ventral) in the heart between experimental group and control
7. **LITERATURE REVIEW**

7.1 **EXTRINSIC CARDIAC INNERVATION**

The heart is innervated by the autonomic nerve system (ANS) that compose of the sympathetic and the parasympathetic nerve systems. The Parasympathetic nerve system regulated in the medulla by 3 vagal nerve nuclei. The parasympathetic efferent regulation reaches the heart by 3 branches of the vagal nerve (superior, inferior and thoracic) vagus nerve that synapses at ganglion adjacent to the heart and send short post synaptic fibers the form plexuses located at the cranial and dorsal heart [4].

The sympathetic nervous system (SNS) descends to the intermediolateral and intermediomedial cells in the thoracolumbar regions of the spine, extending from T1 to T4 rami. Fibers leave the spinal cord and synapse in 3 - cervical ganglia and first 3 or 4 thoracic ganglia, that send the cardiac superior nerve, middle cardiac nerve and the inferior cardiac nerve that reach the heart and form intrinsic cardiac ganglionated plexuses [5].

7.2 **INTRINSIC CARDIAC NERVE SYSTEM (ICNS)**

Fibers from ANS systems forms the Intrinsic cardiac nerve system, a system that receive input from the ANS and regulate the heart rate, contractility features, conduction and the coronary blood flow [6]. The ANS enters the heart thorough the heart hilum at 3 locations and reach atriums and ventricles from medial side of cranial vena cava and around left azygos vein and from between the aorta and pulmonary trunk that reach directly the ventricles [7].

The ICNS of the human and sheep is regulated heart function via 7 sub plexuses [8] [7] presented in figure 1:

1. Left coronary – between the aorta and pulmonary trunk
2. Right coronary – between the aorta and pulmonary trunk
3. Ventral right atrial – superior intra-arterial sulcus
4. Ventral left atrial – between superior intraatrial sulcus and left atrial nerve fold
5. Left dorsal – at left atrial nerve fold
6. Middle dorsal – between the right and left superior pulmonary veins
7. Dorsal right atrial – between superior vena cava and right superior pulmonary vein
FIGURE 1: schematic ventral (a) and dorsal (b) views of the pressure-inflated lamb heart. The heart surface is subdivided into regions for quantitative analysis of ganglia and representation of the course and innervation regions of the seven epicardial subplexuses. Inset (c), cardiac zone around the roots of the aorta and the pulmonary trunk. Dotted lines, limits of the hh; thick grey semitransparent arrows, course of neural subplexuses; blue shadowed areas, main areas with the highest density of subplexal ganglia.

Abbreviation used in the figure: ao – aorta; pt – pulmonary trunk; ocs – opening of coronary sinus; gcv – opening of great cardiac vein; olav – opening of left azygos vein; veins: gcv – great cardiac; lv – left pulmonary; mpv – middle pulmonary; rpv – right pulmonary; lav – left azygos; subplexuses: dra – dorsal right atrial; lc – left coronary; ld – left dorsal; md – middle dorsal; rc – right coronary; vla – ventral la; rv – right ventral; regions: clav – cardiac portion of the lav; cs – coronary sinus; dira – dorsal inferior right atrial; dla – dorsal la; dlv – dorsal left ventricular; drv – dorsal right ventricular; dsra – dorsal superior right atrial; hh – heart hilum; islau – inferior surface of left auricle; israu – inferior surface of right auricle; lc – left side of coronary groove;logo – post-aortic; preao – pre-aortic; postca – region laying behind the conus arteriosus; preca – region laying in front of the conus arteriosus; rcs – right side of coronary groove; rcv – root of the cranial caval vein; sslau – superior surface of left auricle; ssrau – superior surface of right auricle; vila – ventral inferior la; vira – ventral inferior right atrial; vlv – ventral left ventricular; vrv – ventral right ventricular; vsla – ventral superior la; vsra – ventral superior right atrial.

Points (1-23): (1) approximate mid-point of terminal groove; (2) cranial end of free margin of right auricle; (3) cranial dorsal bend of ventral interatrial groove; (4) middle of ventral interatrial groove; (5) base of ventral interatrial groove; (6) mid-point of the aorta and pulmonary trunk on the level of hh; (7) ventral edge of the bed of the root of the right coronary artery; (8) branching site of the first branches from the right coronary artery to the conus arteriosus; (9) right lateral edge of aorta;
(10) dorsal edge of the bed right coronary artery root; (11) basal midst of conus arteriosus; (12) cranial end of ventral interventricular groove or ventral edge of the left coronary artery root; (13) left lateral edge of pulmonary trunk; (14) dorsal edge of the bed of the left coronary artery root; (15) ventral end of the free margin of left auricle; (16) lateral edge of left azygos vein; (17) point of juncture of left azygos vein and great cardiac vein; (18) point of juncture of left azygos vein and coronary sinus; (19) lower edge of caudal caval vein; (20) medial edge of left azygos vein; (21) upper Figure is taken from :The Epicardial Neural Ganglionated Plexus of the Ovine Heart: Anatomical Basis for Experimental Cardiac Electrophysiology and Nerve Protective Cardiac Surgery. Inga Saburkina1, Kristina Rysevaite1, Neringa Pauziene1, Karl Mischke2, Patrick Schauerte2, José Jalife3, and Dainius H. Pauza.

On study of ventricular innervation of rabbits heart neurons of the intrinsic nerve system of the heart were found at the epicardium at the site of conus arteriosus but the majority were located on another site, within the adventitia of the pulmonary trunk root [9]. It is important to note that the cardiac ganglia and their associated nerves embedded primarily in the epicardiume fat, but distributed in atrial and ventricular tissues as well.

Nerve fibers from the plexuses in the epicardial layer extend to the ventricles. Fibers reach ventrally from the arterial aspect of the heart hilum. And dorsally from the venous aspect of the heart hilum [9].

7.3 The heart conduction system

Sinoatrial node (SA) is innervated by postganglionic adrenergic and cholinergic nerve terminals located in the right atrium laterally to sinus venarum, at the junction of superior vena cave (SVC) merge the right atrium. Its located at the myocardium layer of the heart approximately 1 mm from the epicardium.

The node composed of pacemaker cells – those are specialized cells that characterized by automaticity and thus provoke action potential independently by ion channels. The result is constant heart beat that its rate influenced by the autonomic nerve system [10].

Intranodal conduction consist of 3 pathways:

1) anterior intranodal pathway – leave the SA anteriorly, curves SVC anteriorly, enters anterior intratrical band (Bachmann bundle), travel to the left atrium responsible for left atrium contraction

2) middle intranodal tract – leaves the SA node from posterior superior margins, curves SVC posteriorly, reach the interatrial septum, descent through the septum to the AV node.

3) Posterior intranodal tract - leaves the SA node from posterior margins, curves SVC posteriorly along the crista terminalis, reach the interatrial septum, reach the AV node posteriorly [11].
Atrioventricular node (AV)- Located between the atrium and the ventricle, posteroinferiorly to the interatrial septum, anterior to the ostium of coronary sinus. Bundle of his - Are fibers that leave the distal part of the SA node, perforate the fibrous body. this fibers transmit impulses from the AV node to the bundle branches, that give rise to Bundle branches that Divided into two branches the right and the left. The left bandal branch subdivided to left anterior fascicles and left posterior fascicles that give rise to the Terminal purkinje fibers [12].

7.4 THE INTERCONNECTION OF THE INTRINSIC CARDIAC SYSTEM AND THE CONDUCTION SYSTEM.

The interconnection of the intrinsic cardiac system and the conduction system of the heart is very complex and composed of overlapping, thus complete map of this interconnection still doesn’t exist. The intrinsic cardiac nerve system innervates not only the SA node, but also the AV node, his bundle. It supplies it with adrenergic and cholinergic innervation and thus influencing hemodynamic characters of the heart [5]

Its also known that the the AV node receive parasympathetic innervation that reach the heart at the junction of the inferior vena cava and the inferior aspect of the left atrium, close to the coronary sinus ostium. The SA node receive signals from ANS predominantly from the right sympathetic nerves and right vagal nerve, whereas the AV node receive its from the left. Thus the stimulation of the right cardiothoracic ganglion provokes sinus tachycardia since its affect primarily the SA and have milder effect on AV node. Whereas stimulation of the right vagal nerve slows the SA node rate, and the stimulation of the left vagal nerve prolongs AV nodal conduction period and refractoriness [7]. Bundle of his in the other hand doesn’t stimulated by neither of ANS [5] [13].

7.5 ATRIAL FIBRILLATION

Atrial fibrillation (AF) is characterized by disorganized atrial electrical activation and uncoordinated atrial contraction. Classified as paroxysmal last less then 7 days and persistent that last more than 7 days, while permanent is resistant to cardioversion or other attempts to terminate the arrhythmia. Epidemiological evidence show that atrial fibrillation is the most common arrhythmia require treatment with 2.2 – 5 million patients in the US and 4.5 in Europe. And even this numbers may be an under estimation since most of patients are asymptomatic [14].

Pathophysiology: atrial fibrillation is associated with variety of predisposing factors like hypertension, heart failure, coronary disease, valvular disease. At the moment three mechanism are known to trigger the arrhythmia:
1. Triggering foci of rapidly firing cells - Usually located in the sleeves of atrial myocytes that extending into the pulmonary veins. This is most common mechanism of atrial fibrillation. This foci present abnormal conduction characteristics such reduced refractory period, conduction delay and conduction block between the PV and the LA. These properties can trigger reentry with in the pulmonary vein itself. The most common site are the foci with the SVC, ligament of Marshall and adjacent to pulmonary sinus. Foci can also be at the LA and along crista terminals in the right atrium. atrial fibrillation due to PV foci present with primarily increase in adrenergic tone follow by increase in vagal tone prior to the onset of the atrial fibrillation [15] [16]

2. Multiple wavelet hypothesis - Multiple waves can occur when refractory period, conduction velocity or anatomical obstacle are present [17].

3. Fibrosis of the atria - Fibrosis of the atria result in inhomogeneity of conduction within the atria. Fibrosis can be caused due to stretch of the atria as a result long lasting atrial fibrillation result in a loss of atrial myofibrils and other architectural changes that result in atrial dilatation [18].

Hemodynamic effect

1. Loss of atrial contraction
2. Rapid ventricular rate
3. Irregular ventricular rhythm
4. Loss of AV mechanical synchrony impure the filling of the ventricles affecting the cardiac output
5. Thromboembolism that is the main cause of stroke – the thrombi are the result from turbulent rather then laminar flow of blood [19].

Treatment options

1. Anticoagulants – given in order to prevent thrombus formation due to the turbulent blood flow. Anticoagulants are proven to reduce mortality.
2. Antiarrhythmic drugs – are prescribed as first line of treatment according to the ACC/ AHA/ ESC guidelines, flecainide, prepafenon or sotalol are the drugs of choice. Second line drugs are amiodarone or dofetilide [19].
3. Cardioversion by using antiarrhythmic drugs or direct current approach [20]
4. Catheter ablation – eliminating triggering foci using radiofrequnce ablation [21].
7.6 CARDIAC FREQUENCY ABLATION

The essence of ablation of heart tissues relay on the fact that myocytes possess conduction properties, and thus damaging them eliminating the current conduction from that particular spot, weather its an ectopic spot, a spot with impaired conduction properties or an accessory pathway. The first time cardiac frequency ablation were technique were developed at the 1960s’ and in 1968 its practicality was proven by eliminating an accessory pathway– Wolff – Parkinson White syndrome. The procedure performed by reaching the heart by catheter from the femoral vein (its possible to place the catheter in the subclavian or internal jugular vein as well) and has two parts, the first part is intracrdial signal recording and data assembling the goal is to map and identify the souse / path of the arrhythmia, the success of the treatment relays on the accurate identification of the target site of ablation [3]. Once the site of ablation is decided specialized ablating catheter with an electrode is introduced and positioned in direct contact with the desired site. Radiofrequency energy is introduced with a frequency of 500 - 750 Hz current that create thermal energy that result in 5 mm depth tissue damage.

This part of the procedure takes about 30 - 60 sec. Modern current ablation system includes temperature monitoring and control to prevent unintentional damage. And addition tools for better control of the procedure [3].

7.7 CATHETER RADIOFREQUENCY ABLATION IN ATRIAL FIBRILLATION.

The goal is to eliminate foci of rapidly firing cells at the pulmonary veins or reentry current pathways. The main complication was pulmonary vein stenosis due to the damage to the vassals, thus circumferential pulmonary vein technique was developed in order to isolate the focus rather then eliminating it [22].

7.8 RADIOFREQUENCY ABLATION CLINICAL RESULTS

There is controversy about the recurrence rate because different studies examine recurrence after different period of time. In a study that examined physical capacity in the 6 months after the ablation found improvement and improvement in the quality of life, and 76% of patients presented with no recurrence when examined on Holter monitor for 24h [1].

In another study that examined patients for 6 years found that 77% were free of arrhythmia for the first year and so they were examined for the next 5 years totally 41% had reoccurrence of the arrhythmia after passing the first year without arrhythmia. Thus it is clear that in long term the reoccurrence rate is significant [2].
7.9 Radiofrequency Ablation Consequence on Nerve Fibers.

Studies that examined long term affect of the radiofrequency ablation at pulmonary veins on epicardial nerves located distally from the ablation site found no change in the population of acetylcholine esterase under histochemical staining however there was a clear reduction in choline acetyltransferase and tyrosine kinase histochemical staining, which reflect decrease decreased population of nerve fibers distal to the ablation site. Thus suggesting that radiofrequency ablation damaging the intrinsic nerve system, which arguably may explain the high rate of recurrence [23]. In another study was examined the levels nerve growth factors after ablation, it is important to note that these factors are important for nerve regeneration after damage, like the damage that occur after ablation, but is the study they find that the nerve growth factor levels dose not increase significantly and thus its safe to assume that the nerves tissue that are damaged by the ablation will not regenerate [24].
8. RESEARCH METHODOLOGY AND METHODS

Five black-faced sheep (weight 22±3 kg) of either sex were used in accordance with local, state and European Commission guidelines for the care and use of experimental animals (permission no. 33JV-62). Three other sheep did not undergo an RFA procedure and were used as control. Experimental sheep were sedated, anesthetized then left lateral thoracotomy was performed and epicardial ablation at the site between left pulmonary vein and left cranial vein was performed.

After 4-5 months postoperatively, animals were euthanized by a lethal dose of sodium thiopental (100 mg/kg). The heart was removed from the chest, perfused with phosphate buffered saline (PBS; 0.01M), prefixed with 4% paraformaldehyde (PFA) solution in PBS. The pericardium was removed and both ventricles were separated from the atria. Ventricles were cut into three blocks: the upper third part or the basal level; the middle third part or the middle level; and the lower third part or the apical level of the ventricles. From each level some tissue samples were taken. Tissues were fixed for 40 min in 4% PFA solution and washed 3 times 10 min in PBS. These blocks were cryoprotected by immersion in PBS containing 25% sucrose and 0.05% sodium azide (4 °C, 24 h). Following cryoprotection, the tissue samples were frozen using a tissue-freezing medium (Triangle Biomedical Sciences, USA). Tissue samples were cut using a microtome (cryomicrotome HM 560 (Microm, Germany) at 22 °C), mounted onto Superfrost Plus microscope slides (Menzel Glaser, Germany) and air-dried at room temperature for 1h.

Immunohistochemistry for general, cholinergic and adrenergic neuronal markers was performed, using polyclonal primary guinea pig anti protein gene product 9.5 (PGP9.5 dilution 1:300, ab 10410, Abcam, Cambridge, United Kingdom), mouse anti-tyrosine hydroxylase (TH, dilution 1:500, 32-2100, ZYMED, Laboratories Inc., Carlsbad, CA, USA), rabbit anti-choline acetyltransferase (ChAT; dilution 1:500; P3YE8, from prof. M. Schemann's lab) antibodies for 24 hours at +4°C. Washed 3 times 10 min in PBS, then applied species specific secondary antibodies were conjugated, respectively, to Alexa Fluor 488 (dilution 1:200, A21202, Invitrogen Corp., Carlsbad, CA, USA) and Cy3 (Jackson ImmunoResearch Laboratories, West Grove, PA, USA) for 2 hours, then again washed with PBS. The immune histochemically stained sections were analysed and photographed utilizing a fluorescent microscope AxioImager Z1 (Carl Zeiss, Jena, Germany) equipped with Apotome (Carl Zeiss, Jena, Germany). The photos were analysed by axiovesion program to calculate the percent of the marker area from the total frame.
Statistical analysis – The statistical analysis was performed using Microsoft Excel. For evaluation of variable distributions and groups correlations the Student T-Test was used. The statistical difference between two groups is defined by $p < 0.05$. 
9. Results

In order to receive clear results about the influence of radiofrequency ablation on nerve population of the heart, three different markers were used TH, ChAT, PGP 9.5. The markers areas were measured in each frame and the data was collected as the percentage of the area that the marker occupies in the frame of marker from the all frame area. The data was collected in this manner from the experimental group and the control group. The measurement were then compared in three ways:

1. comparing mean on the area percent of each heart as a whole;
2. comparing means of each heart aspect: mean of area percent of experimental hearts; dorsal aspect compare to control hearts and same about ventral hearts;
3. comparing 4 specific areas of the heart: dorsal base, dorsal middle, dorsal apex and ventral heart.

7.1 ChAT Marker Results

Mean of ChAT area percent were found to be higher when compared frames of the dorsal aspect of the heart and when comparing the ventral heart separately. Same results were found when means of the hearts as whole were compared (figure 2).

When specific zones were compared (figure 3) same result were received, the percentage of area percent was clearly higher in the experimental group where compared to the control group except in the dorsal apex area that didn’t show statistical difference when the means were compared in T test.
In overall ChAT result show that percent’s of ChAT is higher in the hearts with cardiac frequency ablation in all regions except dorsal apex area that show that the difference isn’t statistically significant.

**FIGURE 3: ChAT marker, means of area percent comparison**

**FIGURE 4: immuno histochemistry slide of ChAT (red color) marker from base dorsal of dorsal heart control group (left), experimental group (right).**

**7.2 TH MARKER RESULTS**

TH measurements results (figure 5) found that that comparison of mean of area percent from all regions of the heart therefor when hearts of experimental and control group were compared as a whole the TH marker percent was higher in the control group with statistical difference when compared by T test.

The same results were also when dorsal hearts were compared and ventral as well. Comparison of TH levels in different heart location were performed as well, and the results (figure 6) present the same trend as in the comparison of dorsal and ventral hearts (figure 5), except at the comparison of dorsal middle area of the heart there was no statistical difference.
FIGURE 5; *TH* marker, means of area percent comparison

FIGURE 6; *TH* marker, means of area percent comparison

FIGURE 7; *immuno histochemistry slide of TH (green color) marker from base dorsal of dorsal heart control group (left), experimental group (right).*
7.3 PGP 9.5 Marker Results

Measurement of PGP 9.5 markers showed that general comparisons like comparing dorsal side of the hearts or ventral side of the hearts or when whole hearts mean were compared (figure 8) the area percent of PGP 9.5 in the control group is higher, but when specific regions were compared (figure 9) the data are inconclusive because though dorsal base of the heart and ventral heart showed that the means of area percent are higher in the control group with clear statistical difference when were compared with T test.

The measurements from the dorsal middle location showed the opposite and the dorsal apex showed that the difference is not statistically significant.
FIGURE 10: immuno histochemistry slide of PGP marker from base dorsal of dorsal heart control group (left), experimental group (right).
10. DISCUSSION

The research aim was to investigate the long-term effect of radiofrequency ablation on the cardiac intrinsic nervous system, three different markers were used to reflect the changes of the intrinsic nerve system by measuring their population in different sites.

The measurement showed dramatic change in the levels of the markers, though the markers didn’t show same trend of change. The measurements of the ChAT marker show that the experimental group - the hearts that undergo a cardio frequency ablation, had higher levels of ChAT. The measurements found as well that the levels of TH were and PGP where higher in the control group sheep - those that didn’t undergo radio frequency ablation. Even though the measurements found some irregularities in middle layer of the dorsal aspect of the heart in the TH marker, and middle and apex layer of the dorsal aspect of the heart in the PGP marker, the overall conclusion of the measurement of all three markers showed clear difference between the experimental and control group.

The ChAT measurements show that marker population were higher in the experimental group, in a contrary to what was found in another study about the epicardial layer [23]. This contradicting finding raise the question regarding to what might be reason of such a difference between the two layers? The first hypothesis relied on the assumption that the measurements are correct, and that the population of ChAT might increased only in the myocardial layer as a compensatory mechanism for ablation of the ganglions near the left dorsal subplexus, which might reflect a remodeling process of the hearts intrinsic nerve system, and suggest that RFA in the root of the pulmonary veins lead to a compensatory mechanism that promote the parasympathetic nerve system which represented by ChAT marker. Studies have showed that myocardial infarction cause changes in the composition of the nerve fibers of the sympathetic nerve system or parasympathetic nerve system that represented by TH and ChAT markers respectively and those changes associated with an increased risk for spontaneous ventricular arrhythmia and sudden death [24], the effect of radiofrequency ablation should be the same, considering the fact that both processes eventually cause damage to the cardiac muscles and the intrinsic nerve system. Other studies shown that cells with different neurochemical identity reflect different functional specialization [25], which indicate that remodeling of the heart population of nerve fibers and the balance between the sympathetic and the parasympathetic nerve system will affect the heart normal function.

Another reason that might explain the increase levels of ChAT in the myocardial layer in a contrary to the pericardial layer is the histological differences of the two layers. Studies have showed that the epicardial layer has significantly higher level of ChAT than the myocardium [9] [28]. The fact that the two layers have different histological structure in the first place indicates that they have different
functional designation and thus doesn’t necessary should demodulate in the same manner. In another study it was shown that as part of the remodeling process following myocardial infarction can cause an increase of the sympathetic nerve system nerve fibers [29], means that remodeling process following an injury may cause an increase of certain aspects of the intrinsic nerve system.

One more reason that may explain the difference in the adrenergic and ChAT population after RFA may be the fact that the site of the ablation is at the roots of the left and the middle pulmonary veins (the site is also known as the ligament of Marshall). In Ligament of Marshall the sympathetic nerves of the intrinsic nervous system are more dominant which may suggest that ablation at that site may cause a different impact on the sympathetic and the parasympathetic nerve system [29] The difference may be reflected by difference in the nerve fiber population, as was found in our results.

Those three theories relies on the first hypothesis, that the high ChAT levels in the experimental group are correct. These three theories based on information from other articles that study the effect of myocardial infarction on the intrinsic nervous system because there is no morphological studies about the affect of RFA on the intrinsic nerve system of the heart, thus those three theories should be investigated furthermore and the study should be investigated in more detail to find out if the measurements are correct, and how cardiac ablation in certain site affect different layers of the heart as a result of their different histological composition.

The second hypothesis relies on the assumption that the higher result in the experimental group are not correct, the reason that this hypothesis was suggested in because in another study that studied the influence of RFA in the ligament of Marshall on the epicardial layer showed decrease levels of ChAT in the epicardiume of the experimental group [23], while the TH levels that were decreased in this study as presented in this study as well.

TH marker showed that their population of nerve fibers is decreased in the experimental group, thus may suggest that radiofrequency ablation cause in a decrease in the adrenergic nerve fibers in the long term. Another study of the epicardial layer showed the same impact of on TH levels following a radiofrequency ablation [23].

The protein gene product 9.5 (PGP 9.5) is a markers used in immunohistochemistry technique to mark neuronal-tissue, as the other markers in the study we used it in both groups to study the impact of cardio frequency ablation on the intrinsic nervous system but unlike the ChAT and TH, the PGP 9.5 isn’t specific to the sympathetic nor the parasympathetic nerve system, it marks all neural tissues of the heart, and thus show us the impact of ablation on the intrinsic nerve system as a whole.
The results showed a decrease in the PGP 9.5 marker area percent which indicate that there is a decrease in the nerve fiber population in the hearts of sheep that underwent ablation. Same results were observed as well in the epicardial layer study [23]. I found that the study of the epicardial layer is the only one that study the morphological impact of catheter ablation And thus I decided to compare my results with studies that investigated the effect of myocardial infarction on the intrinsic nerve system, considering the fact, that both processes causing damage to cardiac tissues. These studies show that hearts that undergo myocardial infarction and were investigated by immunohistochemistry, showed a decrease in cholinergic nerve fibers but no change in the adrenergic fibers following an episode of the myocardial infarction [28].

The aim of the study was to investigate the long term impact of the radiofrequency catheter ablation in the roots of the pulmonary veins on the intrinsic nerve system, and the overall results show that the procedure dose has an impact on the intrinsic nerve system. This results support the suggestion, that this changes might be a remodeling process take place in the heart much like the remodeling process following a myocardial infarction. This process in the long term cause functional abnormalities in all types of tissues, and in the intrinsic nerve system as well, studies showed that nerve signal from infarcted region to remote sites of the intrinsic nerve system was diminished in comparison to nerve signals from non-affected areas this changes in nerve signal transduction reduced the capacity to transduct changes in the preload of the heart, moreover, they found that this changes reduces the response of intrinsic nerve system to ventricular pacing [28] [30]. And another study found association between spontaneous ventricular arrhythmia and increase in the sympathetic system nerve fibers density that were found in periphery of areas of injury [25] [29].

This data may suggest that injury to heart tissue cause remodeling to the intrinsic nerve system of the heart that in turn lead to functional changes in the intrinsic nerve system that can lead to spontaneous arrhythmia, this chain of event was the hypothesis of the this study that might explain the high recurrence rate arrhythmia following a cardiac radio frequency ablation [2].
11. CONCLUSIONS

After measuring and comparing the levels of the neuronal markers in the experimental group and the control group we can determine that

1. radio frequency ablation has an impact on the intrinsic nerve system.
2. radio frequency ablation causes a decrease in the PGP 9.5 marker levels thus indicating a decrease of nerve fibers population as a whole.
3. radio frequency ablation causes a decrease in the TH marker levels thus indicating a decrease of sympathetic nerve fibers population.
4. radio frequency ablation causes an increase in the ChAT marker levels thus indicating an increase of parasympathetic nerve fibers population.
5. The changes were found to be consistent in all types of comparisons such as comparing the marker population in all heart locations as a whole, comparing dorsal heart, ventral heart or specific location in the hearts of the two groups.
6. This change supporting the hypothesis that radiofrequency ablation has a clear impact on the intrinsic nerve system of the heart and it leading to high recurrence rate of arrhythmia.
12. REFERENCES


12. Moore D. Anatomy 137-139


16. Chen SA, Hsieh MH, Tai CT, Tsai CF, Prakash VS, Yu WC, Hsu TL, Ding YA, Chang MS. Initiation of atrial fibrillation by ectopic beats originating from the pulmonary veins: electrophysiological characteristics, pharmacological responses, and effects of radiofrequency


