



Assessment of radiofrequency ablation technique in development of aortic valve stenosis in rabbits

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Summary

Purpose: To develop a minimally invasive and reproducible model of aortic stenosis in rabbits using radiofrequency ablation technique (RFA).

Material and methods: Eleven rabbits were studied. A radiofrequency ablation catheter was introduced via the femoral artery and advanced to the aortic valve area under fluoroscopic control. In three rabbits radiofrequency energies, at 5 W, 10 W and 15 W respectively, were applied thrice for 90 sec. In eight rabbits, energy of 15 W was applied for the same time periods. The velocity of the blood through the aortic valve was determined by color Doppler ultrasound immediately before and after ablation and after six weeks. After six weeks the rabbits were sacrificed and the aortic valve was examined macroscopically.

Results: Peak systolic velocity (PSV) was similar at the time of ablation and after six weeks in eight of the nine surviving rabbits, and had increased from 1.1 to 1.75 m/s in one rabbit. Two rabbits developed aortic insufficiencies visualized by color Doppler. No macroscopic changes were seen at the aortic valve area 6 weeks after ablation.

Conclusion: In the current study we did not succeed in inducing aortic valve damage/fibrosis using different RFA energies. Inadequate RFA power or inappropriate positioning of the RFA catheter could be limitations of our study.

Introduction

Blood serotonin in patients with serotonin-producing neuroendocrine tumors (carcinoid tumors) is considered to cause valvular heart disease (Robiolio et al. 1995). During the last decade, several drugs acting on serotonin receptors have been recognized to induce cardiac valvulopathy. Appetite-suppressants such as fenfluramine were the first drugs described to cause valvular heart disease (Rothman et al., 1999). Serotonin induces heart valve disease in rats after long-term serotonin administration (Gus-

tafsson et al., 2005) and terguride, which has serotonin antagonist activity, decreases heart disease in rats exposed to long-term hyperserotoninemia (Hauso et al., 2007). Despite this evidence, it is unclear whether serotonin may also play a role in the development of common valvular heart disease. The fact that people with normal, but bicuspid aortic valves, develop progressive valvular disease (Ward, 2000) indicates that flow disturbances may provoke valvular disease. Hemodynamic disturbances with accelerated and turbulent flow probably activate platelets and

may cause release of serotonin from dense granules (Brandt et al., 1992). Our hypothesis is that platelet-derived serotonin may be a crucial factor in the development of valvular heart disease. Therefore a suitable animal model of aortic stenosis is required to investigate disease mechanisms and potential therapies. Most of the experimental animal models for this disease have been based on the development of atherosclerotic plaques which have the disadvantage of being time-consuming (Johnson & Jackson, 2001; Riedmuller et al., 2010) and also involve non-mechanical changes. Mechanical techniques to produce aortic stenosis, including ligation of aortic leaflets (Copeland et al., 1974) and ascending aorta banding (Taylor & Whamond, 1975), have been used. However, these methods often result in aortic rupture in adult animals or valvular insufficiency. Presently, there is no good method to induce aortic valvular stenosis in small animals.

The aim of the current study was to develop a minimally and reproducible model of aortic stenosis in rabbits by using the radiofrequency ablation technique (RFA).

RFA has been established as one of the treatment options for cardiac tachy-arrhythmias (Nath et al. 1994). It has also been used for treatment of varicose veins (Marsh et al., 2010).

Recently a new rabbit model of arterial luminal stenosis was reported, by endovascular application of radiofrequency (RF) energy in the aorta below the level of the renal arteries (Lazoura et al., 2011). In the present study, we wanted to establish a new experimental model based on a similar technique using RFA on rabbit aortic valves to provoke valvular damage/fibrosis. The intention was to induce blood flow disturbances which would possibly activate platelets and in the end lead to the development of progressive valvular aortic stenosis.

Materials and methods

The National Animal Welfare Committee approved the study. Eleven New Zealand White male rabbits (Harlan Laboratories, UK), 14-16 weeks old, weighing 3-4 kg were housed individually in cages. Concerning their health and microbiological status, positive results for *Eimeria spp* and *Passalurus ambiguus* have been found in the past, but not in the last 18 months.

Room temperature was $24\pm 1^{\circ}\text{C}$ with a 12-hour light/dark cycle. A commercial pellet diet (Scanbur, Karlslunde, DK), dried grass and water were supplied *ad libitum*. Before all procedures, each animal

was premedicated with Hypnorm (Vetapharma Ltd, Leeds UK) 0.15 ml/kg sc.

RF generator and catheter

We used a Medtronic Atakr II radiofrequency generator and a Medtronic Mariner 5 Fr, 35 mm reach radiofrequency ablation catheter with a 4 mm platinum tip. The high frequency current passed between the electrode and an ablation pad applied to the shaved back of the rabbits.

Rabbit model development

We aimed to induce significant valvular aortic stenosis using the minimum amount of energy, without vessel rupture or any other complications.

After premedication, each animal was anesthetized with a high dose of isofluran (5%, 3 to 5 minutes) until deep anesthesia, continuing with 1.5 to 2% isofluran and oxygen / nitrous oxide in a ratio of 40/60 during the procedure. The chest was carefully shaved. Using a GE Vingmed Vivid 7 ultrasound scanner and a GE Vingmed M4s 1.5-4.0 MHz phase arrayed ultrasound probe the peak velocity of the aortic valve blood flow was measured as the mean of 5 cycles of continuous Doppler recordings. The rabbit was placed in the supine position. Following shaving, a sterile drape was applied to right ventral femoral area and a ~2-cm vertical incision was performed. The right femoral artery was dissected free and ligated at its distal end, followed by dripping papaverin upon the vessel to reduce spasm tendency. The right femoral artery was then cut open to place a 4 Fr introducer sheath (Introducer II, Radiofocus) for dilation, followed by the insertion of a 5 Fr heparinized (5000 E heparin/100 ml water) introducer sheath. The 5 Fr heparinized RF catheter was then advanced to the aortic valve, away from the septum to reduce the risk of AV block. The procedure was performed under radiological control by X-ray and the aortic catheter position was verified by echocardiography.

In 3 rabbits temperature controlled (55 degrees C) radiofrequency energy was applied at 5 W, 10 W and 15 W respectively 3 times for 90 sec. In the next 8 rabbits a similar procedure using 15 W was applied. Following completion of the procedure, the sheath was removed, the common femoral artery was ligated and the muscle layers and skin were closed with 3.0 Vicryl resorbable sutures. Color Doppler ultrasound was also performed after the procedure, to measure the blood flow velocity at the aortic valve. Finally Temgesic (Reckitt Benckiser, Berkshire, UK,

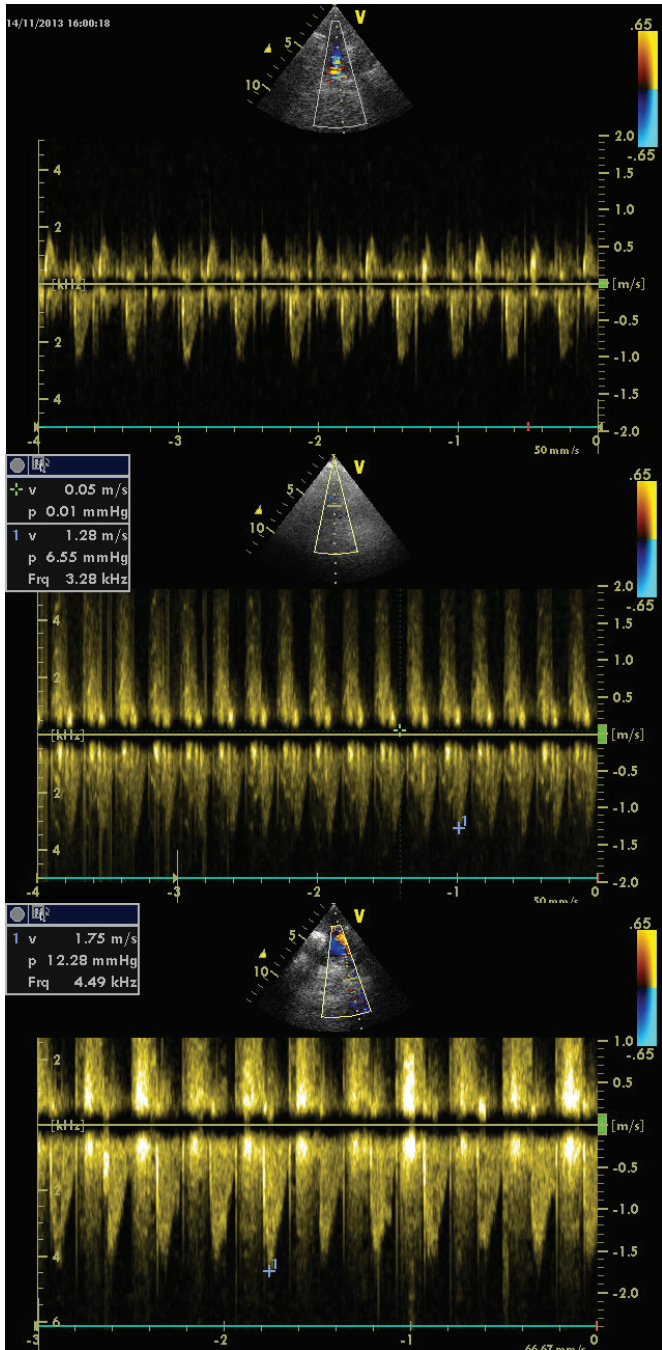


Figure 1: Continues wave Doppler in rabbit No.4, A. before RFA (Velocity~ 1.1 m/s) B. Immediately after RFA (Velocity= 1.28 m/s), C. six weeks after RFA (Velocity=1.75 m/s).

0.02 mg/kg) was administered as an analgesic. Animals showing any discomfort suggesting pain were given another dose of Temgesic 3-4 hours after the procedure. The surviving rabbits were examined by color Doppler ultrasound six weeks after the procedure and sacrificed to look for macroscopic changes.

The rabbits were euthanized with phenobarbital 30 mg/kg i.v at the termination of study (or directly after a failed procedure) or earlier if the rabbits suffered from breathlessness, signs of pain or developed progressive weight loss. The rabbits were monitored several times daily by the personnel of the animal department.

Echocardiography and Macroscopic examination

The rabbits were examined by echocardiography before, during and after the procedure as well as during follow up. Two-dimensional color-flow Doppler in the parasternal long -and short axis views was used to visualize aortic flow and regurgitation, and the peak systolic velocities (PSV) through the aortic valve were measured. Continuous-wave Doppler recordings of rabbit No.4 are shown in Figure 1.

Sodium pentobarbital, 30 mg/kg i.v was administered intravenously six weeks after the procedure in all rabbits. We then performed a median sternotomy, carefully removed adhering tissue around the myocardium, and excised the hearts with the aortic root (Figure 2).

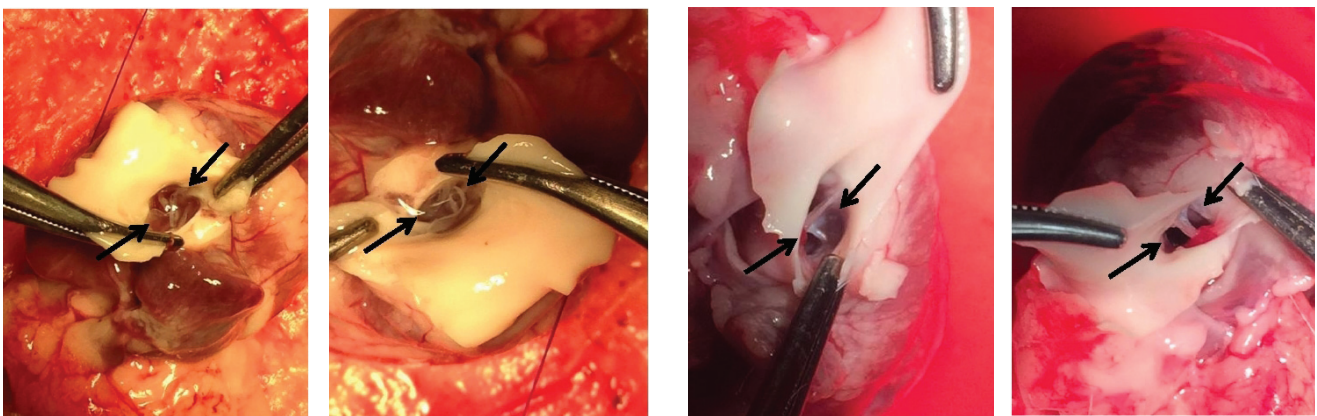


Figure 2: Macroscopic view of aortic valve in rabbits, A- Control, B- at RF power of 15 W, 6 weeks after RFA.

Results

Nine rabbits survived the experimental procedure. Two rabbits died instantly after the procedure because of complete atrioventricular block. Information about the animals and Doppler flow measurements for each application of RF energy of aortic valve is detailed in Table 1.

Rabbit No.3 developed cardiac arrest due to ventricular fibrillation during the second 90 sec ablation period (power of 15 W). After resuscitation, by use of heart compressions, the rabbit lived for one more week. The cause of death was probably heart failure caused by large amounts of cardiac effusion (found on autopsy) possibly secondary to a myocardial rupture. In rabbit No.6 we planned to apply RFA power of 20 W, but at 15 W maximum temperature was achieved and the system automatically stopped delivering RFA energy.

Aortic insufficiency was found 6 weeks after ablation with 15 W in two rabbits. PSV through the aortic valve at six weeks after ablation was similar in all animals except in one rabbit (No.4) with increased PSV and which also had aortic insufficiency (Table 1). No changes were identified by macroscopic examination.

Discussion

In the current study, we aimed to develop a minimally invasive and reproducible model of progressive aortic stenosis in rabbits using RFA. We did not succeed in inducing aortic valve stenosis using different RFA energies. In one rabbit there was increased blood flow velocity through the aortic valve which,

however, most probably was secondary to a large aortic insufficiency. We may have applied too little energy to induce valvular changes. The RFA procedure, however, induced ventricular fibrillation in one rabbit and two rabbits died during the procedure due to complete heart block. A further increase in RFA energy levels seemed futile. Furthermore, application of high levels of energy may result in vessel rupture (Zacharoulis, 2011). We did only eleven examinations since we felt that we could not proceed further both from an economical as well as an ethical point of view. However, we cannot exclude the possibility that including more animals may have given a different result.

Lazoura et al. (2011) developed a new rabbit model of aortic luminal stenosis between the origins of the renal arteries and the aortic bifurcation, based on endovascular RFA (Lazoura et al., 2011). The optimal RFA power to induce significant stenosis in their study was 24-36 W for 1.5 min which was higher than we applied.

Another possible reason for our failure to induce aortic valve stenosis may be incorrect placement of the RFA energy. Localization of the aortic valve area is difficult, and in another study an intracardiac catheter was used for accurate catheter placement (Doi et al., 2003). This technique was used in a canine model and may be difficult to apply in small animals like rabbits.

Therefore inadequate RF power and inappropriate guidance to place RF catheter could be the limitations of our study investigating a less invasive method to induce aortic valve stenosis in a rabbit model. Nevertheless, it may be possible to further explore and develop RFA into a method to initiate

Table 1. Radiofrequency ablation (RFA) of the aortic valves (power, time and numbers of applications) and peak systolic velocity determined by color Doppler ultrasonography before, immediately after RFA and 6 weeks after RFA.

Rabbit No	Power (Watt)	Time (s)	¹ RF applications (time)	² PSV before RF (m/s)	PSV after RF (m/s)	Follow-up PSV (m/s)
1	5	90	3	1.1	1.1	1.0
2	10	90	3	1.2	1.05	0.9
3	15	90.73	3	1.0	0.95	-
4*	15	90	3	1.1	1.28	1.75
5*	15	90	3	0.9	1.0	1.0
6	15	90	3	1.18	1.15	1.0
7	15	90	3	1.1	1.15	1.2
8	15	90	3	1.1	1.1	1.0
9	15	90	3	0.95	0.95	1.0

¹RF: Radiofrequency, ²PSV: Peak systolic velocity, *Aortic insufficiency

progressive aortic valvular stenosis. Such a method is required to develop and test new drugs with the ability to stop progression of valvular disease.

Competing interests

The authors declare that they have no competing interests. The authors alone are responsible of the content and writing of the paper.

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