Cardiovascular Pathology 34 (2018) 28–37

Contents lists available at ScienceDirect

Cardiovascular Pathology

Increased Calcific Aortic Valve Disease in response to a diabetogenic, procalcific diet in the LDLr$^{-/-}$ApoB$^{100/100}$ mouse model☆

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ARTICLE INFO

Article history:
Received 23 August 2017
Received in revised form 5 February 2018
Accepted 6 February 2018
Available online xxxx

Keywords:
Calcific aortic valve disease
type II diabetes mellitus
aortic stenosis
valve interstitial cells

ABSTRACT

Objective: Calcific aortic valve disease (CAVD) is a major cause of aortic stenosis (AS) and cardiac insufficiency. Patients with type II diabetes mellitus (T2DM) are at heightened risk for CAVD, and their valves have greater calcification than nondiabetic valves. No drugs to prevent or treat CAVD exist, and animal models that might help identify therapeutic targets are sorely lacking. To develop an animal model mimicking the structural and functional features of CAVD in people with T2DM, we tested a diabetogenic, procalcific diet and its effect on the incidence and severity of CAVD and AS in the, LDLr$^{-/-}$ApoB$^{100/100}$ mouse model.

Results: LDLr$^{-/-}$ApoB$^{100/100}$ mice fed a customized diabetogenic, procalcific diet (DB diet) developed hyperglycemia, hyperlipidemia, increased atherosclerosis, and obesity when compared with normal chow fed LDLr$^{-/-}$ApoB$^{100/100}$ mice, indicating the development of T2DM and metabolic syndrome. Transthoracic echocardiography revealed that LDLr$^{-/-}$ApoB$^{100/100}$ mice fed the DB diet had 77% incidence of hemodynamically significant AS, and developed thickened aortic valve leaflets and calcification in both valve leaflets and hinge regions. In comparison, normal chow (NC) fed LDLr$^{-/-}$ApoB$^{100/100}$ mice had 38% incidence of AS, thinner valve leaflets and very little valve and hinge calcification. Further, the DB diet fed mice with AS showed significantly impaired cardiac function as determined by reduced ejection fraction and fractional shortening. *In vitro* mineralization experiments demonstrated that elevated glucose in culture medium enhanced valve interstitial cell (VIC) matrix calcium deposition.

Conclusions: By manipulating the diet we developed a new model of CAVD in T2DM, hyperlipidemic LDLr$^{-/-}$ApoB$^{100/100}$ that shows several important functional, and structural features similar to CAVD found in people with T2DM and atherosclerosis including AS, cardiac dysfunction, and inflamed and calcified thickened valve cusps. Importantly, the high AS incidence of this diabetic model may be useful for mechanistic and translational studies aimed at development of novel treatments for CAVD.

1. Introduction

Calcific aortic valve disease (CAVD) is the underlining pathology leading to the clinical manifestation of aortic stenosis (AS), which can lead to heart failure and death if untreated [1]. CAVD is characterized by the accumulation, over time, of calcium-phosphate nodules within the fibrous matrix of the aortic valve leaflets resulting in a dysfunctional and narrowed valve opening. As CAVD is a progressive disease, patients are frequently free of symptoms for several decades, however ~30% of the aging population is affected with aortic sclerosis that is the early asymptomatic manifestation of the disease. A smaller group of this population will progress to the symptomatic AS, ~2% by age 65 and ~4% by age 85. CAVD accounts for 50% of cardiac valve disease and is the third most common cardiovascular disease following coronary disease and hypertension [2,3]. In symptomatic CAVD patients, the narrowed valve opening results in obstruction of left ventricular outflow, reduced cardiac output, and increased blood velocity through the valve opening eventually leading to left ventricular dysfunction and heart failure [4,5].

CAVD risk factors include congenital malformation, age, male sex, smoking, hypercholesterolemia, hypertension, and diabetes mellitus [6]. Patients with type II diabetic mellitus (T2DM) not only have a heightened risk for CAVD but also a significantly increased "new" incidence compared to those without [7,8]. In addition, Nishimura et al. have recently reported that diabetes and moderate/severe calcification score at diagnosis predicted first year rapid progression [9]. Metabolic syndrome was also associated with faster disease progression and worse outcome in patients with AS [10]. At the tissue level, histopathological assessment showed greater calcification in diseased aortic valves from T2DM patients compared to nondiabetic patients [11].

☆ Conflict of Interest: None.

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https://doi.org/10.1016/j.carpath.2018.02.002
1054-8807/

To date, there are no pharmacological treatments available to reverse or retard the progression of CAVD. Traditional cardiovascular drugs like cholesterol-lowering therapies (statins) and renin–angiotensin system blocking drugs have been the major pharmacological agents under active investigation in clinical trials, but have proven to be unsuccessful in slowing the progression of CAVD [12–15]. These findings imply that despite the similarity in risk factors between vascular and valvular disease and the coexistence of CAVD and cardiovascular disease in patients, different mechanisms underlie their development and progression [16–18]. Thus, as there is no effective drug therapy for CAVD, AS is the second most common indication for cardiac surgery. Surgical methods to repair or replace the aortic valve include open-heart or transcatheter aortic valve replacement [19]. Both are associated with risk of adverse events and substantial healthcare costs [4]. Given that the burden of diabetes and CAVD will continue to increase worldwide in the coming decade, a pharmacological method to reverse or slow the progression of CAVD is greatly needed.

Part of the reason for the lack of therapies to treat CAVD in diabetes is the paucity of animal models mimicking the structural and functional features of human diabetic CAVD. Structurally human diseased valve leaflets show severe fibrosis, calcific nodules, neoangiogenesis, inflammation, bone metaplasia with or without hematopoiesis, adipose metaplasia, and cartilaginous metaplasia [20]. Further, valve leaflets derived from diabetic patients have statistically more calcium nodules and overall calcification [11]. In the present study, we report the development of a new mouse model of lipid-driven, diabetic CAVD that recapitulates the functional features of CAVD found in patients as well several of the structural features of human diseased valve leaflets including fibrosis, calcification deposits, inflammation and cartilaginous metaplasia. To achieve this we used the atherosclerosis prone *LDLr$^{-/-}$:ApoB$^{100/100}$* mice that when fed a custom diabetogenic procalcific (DB) diet developed T2DM and metabolic syndrome, high incidence of CAVD characterized by more calcium deposits on the valve leaflets than control diet mice, and had a higher incidence of hemodynamically significant AS than any diet induced animal models to date. Finally, left ventricular function of these mice was also diminished [21]. This new diet and genetic model combination should aid in testing new mechanistic hypotheses regulating CAVD in diabetes and therapies in general.

2. Material and Methods

2.1. Animals

Male *LDLr$^{-/-}$:ApoB$^{100/100}$* male mice, 10-12 weeks old (≥20g), were randomly assigned to two groups fed either a diabetogenic, procalcific diet (DB; Bio-Serv., 1.25% cholesterol, 57.5% kcal fat, 27.4% kcal carbohydrate) [21] to induce CAVD or normal chow (NC) as dietary control. Body weight and fasted blood glucose levels were recorded before challenging with the diets and every five weeks until 20 weeks of diet. A total of 13 mice per group were used for echocardiography and histological analysis of aortic valves. Mice were euthanized via intraperitoneal injection of pentobarbital (150 mg/kg) followed by exsanguination through cardiac puncture to collect sera. All animals were maintained in a specific pathogen-free environment and genotypes were determined as described [21,22]. All protocols are in compliance with the NIH Guideline for the Care and Use of Laboratory Animals and have been approved by the Institutional Animal Care and Use Committee, University of Washington.

Table 1
Serum biochemistry at 14 months for NC and DB diet fed LDLr−/−ApoB100/100 mice. Data are normally distributed. Data analyzed with Unpaired t-test, *p<0.001 DB vs. NC. Mean ± S.E.M., n=13.

	NC diet	DB diet
Triglyceride (mmol/L)	2.26 ± 0.10	3.51 ± 0.27*
Cholesterol (mmol/L)	10.71 ± 0.49	33.00 ± 3.65*
Phosphate (mmol/L)	2.49 ± 0.14	2.87 ± 0.10*
Calcium (mmol/L)	2.11 ± 0.16	2.83 ± 0.06*
Urea Nitrogen (mmol/L)	7.28 ± 0.45	8.57 ± 0.68

2.2. Echocardiography

Transthoracic echocardiography was performed in isoflurane anesthetized mice with a heart rate of ~450-500 beats/min using high-resolution *in vivo* ultrasound imaging system for small animals equipped with a 40-MHz transducer (Vevo 2100™, VisualSonics Inc.). Aortic valve function was assessed using Pulse Wave Doppler-mode that measures aortic valve peak velocity and gradient. In brief, Doppler flow velocity spectrum of the ascending aorta of each mouse was recorded at three locations, the anterior, middle, and posterior parts of the aortic lumen. A minimum of three cardiac cycles at each location were traced to obtain an average for aortic peak velocity and gradient using Vevo 2100™ software. Images were taken from the upper right parasternal long axis view and the angle of the transducer was maintained at 55 degree. B-mode and M-mode images of the parasternal long axis and short axis views were used to measure left ventricular dimensions and volumes, fractional shortening, and ejection fraction. The cardiac package provided by VisualSonic was used to measure and calculate several parameters including the aortic jet

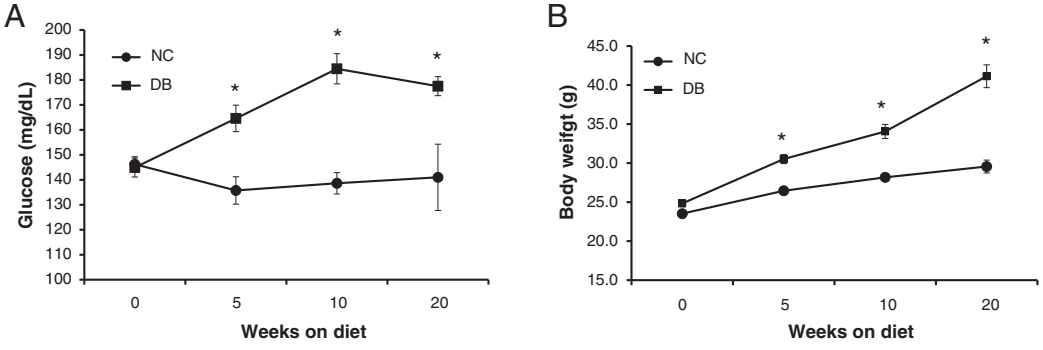

Fig. 1. Characterization of LDLr−/−ApoB100/100 mice fed a customized type 2 diabetes mellitus inducing procalcific diet. LDLr−/−ApoB100/100 mice were challenged with a customized type 2 diabetes mellitus inducing procalcific (DB) and a control normal chow (NC) diet. T2DM development was monitored by blood glucose level (A) and body weight (B). Data are normally distributed. Data were analyzed by two-way ANOVA with repeated measure and Bonferroni's test, Mean ± S.E.M., n=8–13. *p<0.001 DB vs. NC for both glucose and body weight.