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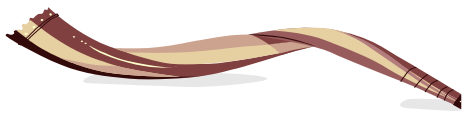

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List of Abbreviation

Abb.	Meaning
CVD	Cardiovascular disease
ECM	Extracellular matrix
EMT	Epithelial-to-mesenchymal transformation
GAGs	Glycosaminoglycans
LDL	Low density lipoprotein
MMPs	Matrixmetalloproteinases
VECs	Valve endothelial cells
VICs	Ventricularintersititial cells

Effect of Ageing on Aortic Valve of Albino Rat

Thesis

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Introduction

Aging is the natural phenomenon, which is the process of growing old and is usually defined as the gradual biological impairment of normal function which has direct impact on the functional ability of organs and on the biological systems. These irreversible series of changes inevitably end in death **(Hedden and Gabrieli, 2004).**

The aortic cusps are thin, flexible structures that come together to seal the valve orifice during diastole. The aortic valve is normally composed of three cusps or leaflets. The individual cusps are attached to the aortic wall in a semilunar fashion, ascending to the commissures (where adjacent cusps come together at the aorta) and descending to the basal attachment of each cusp to the aortic wall; this anatomical structure is also called the aortic valve annulus. A portion of the annulus is attached to cardiac muscle, while the other half is continuous with the fibrous leaflets of the mitral valve. The functional unit of the aortic valve includes the cusps and their respective aortic sinus complexes, also called the aortic root **(Thubrikar et al., 1986; Katayama et al., 2008).**

The structure of the aortic valve cusps is organized into three layers: (i) the zona ventricularis, closest to the left ventricle chamber and composed largely of elastin, which can extend in

diastole and recoil in systole to minimize cusp area; (ii) the zona fibrosa, closest to the outflow surface, rich in densely packed collagen organized in radial and circumferential direction, which provides the strength and stiffness of the cusps and is mainly responsible for bearing diastolic stress; and (iii) the centrally located zona spongiosa, which consists mainly of glycosaminoglycans (GAGs) that accommodate shear forces of the cuspal layers, and absorbs shock during the valve cycle **(Schoen, 2008).**

The prevalence of valvular aortic stenosis increases with age and is due to stiffening, scarring and calcification of the aortic valve leaflets. Aortic valve calcification is present in 80% of old people with atherosclerosis, explaining the increased risk of myocardial infarction and death **(Kurz et al., 2006)**.

Calcific aortic valve disease ranges from focal, irregular thickening of aortic valve leaflets with no hemodynamically significant to severe calcification referred to as aortic stenosis. The paradigm of aortic stenosis has shifted from being considered a degenerative aging process; it is now recognized as a dynamic inflammatory process with features similar to atherosclerotic plaque. These features include endothelial disruption, focal deposition of low density lipoprotein (LDL) cholesterol and lipoprotein A, accumulation of macrophages and T lymphocytes, and calcification **(Moura et al., 2007).**

Aim of the Work

To study the histological structure of the aortic valve underlying the physiological ageing process, which may ultimately help in the understanding and prevention of age-related problems and diseases.

Review of Literature

Ageing, in its broadest sense is the continuous and irreversible decline in the efficiency of various physiological processes once the reproductive phase of life is over. The word "Ageing" does not give a good feeling to most of us because of problems and diseases associated with ageing. History of the world is replete with tales of individuals trying to stave off aging and death **(Balcombe and Sinclair, 2001).**

The ageing of population is often measured by increases in the percentage of retirement ages. The definition of retirement ages may vary but a typical cutoff is 65 years, and nowadays a society is considered relatively old when the fraction of the population aged 65 and over exceeds 8-10%. By this standard, the percentage of elderly people in the United States 12.6% in 2000, compared with 4.1% in 1900 and a projected increase to 20% by the year 2030 **(De Grey et al., 2002).**

Gerontology distinguish between 3 subgroups: younger older people (60-74 years), older people (75-85 years) and very old people (>85 years). The fact that a considerable decrease in physical and mental efficiency occurs much more frequently beyond the age of 80, clinicians distinguish between 2 subgroups of older patients: those under and those above that age **(Schwartz and Zipes, 2007).**

Ageing become an important part of all human societies reflecting the biological changes that occur, but also reflecting cultural and societal conventions. Roughly 100,000 people worldwide die each day of age-related causes.

Anti-ageing therapies become most attracted subject in newspapers and magazines. It has attracted high level of attention from the United Nations, who is focusing its research agenda on ageing for the 21st century **(Laura, 2005; Aubrey and de Grey, 2007).**

Ageing arises from two possibly related effects: increasing longevity and declining fertility. An increase in longevity raises the average age of the population by increasing the numbers of surviving older people. A decline in fertility reduces the number of babies, and as the effect continues, the numbers of younger people in general also reduce.

Although etiology of ageing is important to understand, but it is equally important to differentiate the normal physiological changes from those associated with diseases. The clinician's inability to recognize these differences may result in unnecessary testing, misdiagnoses and mismanagement of the elderly person **(Giacomoni, 2005; Lutz et al., 2008).**

Effect of ageing on cardiovascular system:

The incidence and prevalence of CVD increases dramatically with advancingage in both men and women **(Lakatta and Levy, 2003).**

With ageing there are changes in the cardiovascular system, which result in alterations in cardiovascular physiology. The changes in cardiovascular physiology must be differentiated from the effects of pathology, such as coronary artery disease, that occur with increasing frequency as age increases. The changes with age occur in everyone but not necessarily at the same rate, therefore accounting for the difference seen in some people between chronologic age and physiologic age **(Cheitlin, 2003).**

The Changes in the cardio-vascular system which occur with ageing include normal atrophy of the heart muscle, especially in the left ventricle, calcification of the heart valves, loss of elasticity in arterial walls (arteriosclerosis) and intra-arterial deposits(atherosclerosis). Also there is decrease in cardiac output,baroreceptor sensitivity and SA node automaticity result in decrease inrenal and hepatic function **(Terman and Brunk, 2005; Levin et al., 2007).**

The cardiac structure and function remarkably similar among mammalian species, and the use of animal models has been extremely helpful in developing treatment strategies for

alleviating heart disease in humans. Extending animal studies beyond young adult ages to very old ages may provide similar benefits to cardiovascular health of the growing aged population **(Timothy et al., 2005).**

The total number of ventricular myocytes decreases with age in the rat heart, likely as a result of an increase in necrotic and apoptotic cell death **(Apostolos et al., 2010).**

Effect of ageing on blood vessels:

According to study of Baltimore Longitudinal Study on Ageing of human, there are macroscopic and microscopic structural changes in the peripheral vessels that influence the blood supply of tissues and cardiac function. Macroscopically the main changes observed are: 1) dilation and convolution of large arteries (dilation is more severe in the aorta proximal to the myocardium and in its large branches, but smaller in the muscular arteries); and 2) enlargement of the vascular lumen and thickening of the vascular wall. However, the factors that are implicated in the progressive thickness of tunica intima and median aging are not well known. Microscopically, endothelial cells become irregular in shape and increase in height. In addition there is also a decrease and fragmentation of elastin as well as calcification. The amount of extracellular matrix is increased and

becomes rich in glycosaminoglycans, thus decreasing the vessels compliance **(Fleg et al., 1995; Seals, 2003).**

Embryology of heart valve:

During normal development of the heart in human, the heart tube consists of endocardium and myocardium separated by a cellular extracellular matrix (ECM) called cardiac jelly. After the completion of heart looping, the valve cusps/leaflets originate from mesenchymal outgrowths known as endocardial cushions, the precursors of valves and the cardiac septa. A subset of endothelial cells in the cushion-forming area, driven by signals from the underlying myocardium, change their phenotype to that of mesenchymal cells and migrate into the cardiac jelly to form ventricular interstitial cells. This phenotypic/functional transformation of embryonic progenitor endothelial/endocardial cells to mesenchymal cells is termed transdifferentiation or epithelial-to-mesenchymal transformation (EMT) **(Armstrong and Bischoff, 2004; Butcher and Markwald, 2007).**

Semi lunar valve formation begins during the fourth week of gestation. At this time, opposing dextrosuperior and sinistroinferiorendocardial cushions appear in the cephalad portion of the truncusarteriosus. Simultaneously, 2 additional intercalated endocardial cushions form, each located 90º from

dextrosuperior and sinistroinferiorendocardial cushions **(Misfeld and Sievers, 2007).**

The dextrosuperior and sinistroinferior cushions fuse and, in doing so, form the truncal septum. The truncal septum undergoes a complex process of differentiation, eventually forming the right and left aortic valve cusps and 2 leaflets of the pulmonic valve. Of the 2 intercalated endocardial cushions, the right cushion eventually forms the posterior aortic valve cusp, whereas the left forms the anterior pulmonic valve leaflet. This occurs during the counterclockwise rotation and caudal shift of the conotruncus. During this time, the endocardial cushions also undergo dedifferentiation from a myosin-heavy chain to an alpha-smooth muscle actin phenotype, resulting in mature arterial valvular leaflets. The improper fusion or the incomplete dedifferentiation of the previously mentioned endocardial cushions is thought to be responsible for the formation of anatomically and structurally congenitally abnormal aortic valves **(Malouf et al., 2008).**

Anatomy of heart valves:

The topographic features of the rat's heart and its valves are similar to those other mammals. The heart of the rat is a four chambered organ, it is enclosed in thin transparent pericardium that is attached to the major arteries and veins. The aortic and

pulmonary valves have three leaflets while the mitral has anterior and posterior leaflets and tricuspid has posterior septal leaflet and two lateral leaflets **(Mark et al., 2005).**

The aortic, pulmonary, mitral and tricuspid valves are positioned in a plane, the so-called 'base' of the heart. It is this area which was named by early French anatomists the 'fibrous skeleton' of the heart. It consists of densely collagenous fibres and remains almost stationary in contrast to the dynamic movements of the myocardium, leaflets and arteries. The positions of the valve orifices defined by the fibrous skeleton of the heart also demonstrate the close relationship of the four heart valves to each other. However, each heart valve itself has its own anatomical features and histological structures, this allows each valve to function in its individual environment. The aortic and pulmonary valves are challenged by different pressures but positioned in the same direction as flow. On the other hand, the atrioventricular valves are exposed to different flow features as the flow changes its direction when it is ejected by the ventricles. The mitral and tricuspid valve design consider to these specific flow characteristics **(Misfeld and Sievers, 2007).**

Anatomy of aortic valve:

The aortic valve is part of the aortic root. The latter one connects the heart to the systemic circulation and plays a major

role in the function of the heart and cardiovascular system. It also maintains optimal coronary perfusion and plays a role in the maintenance of a laminar flow in the vascular system. The structure of the aortic root has its individual histological profile and anatomical architecture. The crown shape annulus, the three sinuses of Valsalva and interleaflet triangles, as well as the sinotubular junction, commissures and the aortic valve leaflets interact with each other in a certain way to maintain optimal function **(Yacoub et al., 1999; Anderson, 2000).**

The aortic valve is composed of three semilunar cusps (also called leaflets") attached to a fibrous annulus connected to the distal end of the left ventricular outflow tract. A portion of the annulus is attached to cardiac muscle, while the other half is continuous with the fibrous leaflet of the mitral valve. The aortic valve annulus is a collagenous structure. It is shaped like a crown and extends to the level of the aortic sinuses. It attaches to the aortic media distally and the membranous and muscular ventricular septum proximally and anteriorly. The functional unit of the valve includes the cusps and their respective aortic sinus complexes, they collectively called the aortic root **(Underwood et al., 2000).**

The aortic root is a bulb-shaped fibrous structure to which the aortic cusps, or leaflets, are attached. The sinuses of Valsalva are three elliptical depressions in the aortic wall opposite each