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Collagen Accumulation in Rat Heart Tissue in Relation to Ageing

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Abstract : Structural studies were undertaken to reveal the sites and morphology of collagen accumulation with different age group of rat. In the present study, collagen has been analysed in myocardium as a function of growth and aging. Present study concluded that the heart continues to accumulate collagen even after the organ has ceased to grow in size. In myocardial tissue, the total amount of collagen rises steadily until the rats reach up to high age. This study also shows that concentration of collagen also increases with age increases. Histology shows a relative increase in the fibrous component of tissue which distinctly invaginated interchordal space in adult heart. The precise magnitude of invasion its accumulation and change in collagen parameters may be of great significance in cardiac hypertrophy, heart failure and other pathogenesis of cardiac diseases.

Key words : Heart, Age, Collagen, Myocardium, Histology,

Introduction :

Alternation in normal myocardial performance is a major problem associate with aging. The change in collagen concentration affects the mechanical properties of myocardial tissue. It also affects the myocardial performance. Age related alteration of collagen is likely to be related to the overall metabolic activity of the animals. A correlated assessment of the amount and structure of collagen is thus of considerable importance to understanding of change in myocardial performance with aging. Several studies have shown that there are different growth rates of collagen and non-collagenous proteins of the myocardium during normal postnatal heart growth. It is also of interest to know the extent of the concentration of collagen among different age groups of normal heart.

The interstitial space of the myocardium is composed of nonmyocyte cells and a highly organized collagen network which serves to maintain the architecture and mechanical behavior of the myocardial walls. It is the myocardial collagen matrix that determines myocardial stiffness in the normal and structurally remodelled myocardium. In hypertensive heart disease, the heterogeneity in myocardial structure, created by the altered behavior of nonmyocyte cells, particularly cardiac fibroblasts which are responsible for collagen synthesis and degradation, explains the appearance of diastolic and/or systolic dysfunction of the left ventricle that leads to symptomatic heart failure. Several lines of evidence suggest that circulating and myocardial renin-angiotensin systems (RAS) are involved in the regulation of the structural remodelling of the nonmyocyte compartment, including the cardioprotective effects of angiotensin converting enzyme (AChE) inhibition that was found to prevent myocardial fibrosis in the rat with reno-vascular hypertension. In cultured adult rat cardiac fibroblasts angiotensin II was shown to directly stimulate collagen synthesis and to inhibit collagenase activity, which is the key enzyme for collagen degradation, that would lead to collagen accumulation. In the spontaneously hypertensive rat, an appropriate experimental model for primary hypertension in man, left ventricular hypertrophy could be regressed and

abnormal myocardial diastolic stiffness due to interstitial fibrosis could be restored to normal by inhibition of the myocardial RAS. These antifibrotic or cardioreparative effects of ACE inhibition that occurred irrespective of blood pressure normalization may be valuable in reversing left ventricular diastolic dysfunction in hypertensive heart disease (Brilla *et al.*, 1993).

Hence, structural studies were undertaken to reveal the sites and morphology of collagen accumulation with different age group of rat. In the present study, collagen has been analysed in myocardium as a function of growth and aging.

Materials and methods :

Rat (*Rattus norvegicus*) bred in breeding centre of J.N.V. University duly licensed and registered for the purpose by expert committee of social justice, Govt. of India under prevention of animal cruelty act were subjected to study from day 1 of the birth. The rats were maintained under identical condition. All animals were given standard diet with free access to drinking water. New animals were obtained after breeding. A total of 12 recently bred animals were selected. Tagging of date of birth was also done. For the study purpose, animals were obtained according to their date of birth. Age matched healthy and disease free animals were used for the study purpose. Investigations were performed on the rats bred in rigid condition at fix time of life cycle. Those are infant group (1 to 2 months), young group (5 to 6 months) and adult group (12 months and above).

For histological studies and routine paraffin procedure, 10% buffered neutral formalin is used as fixative. The paraffin sections of the different tissues were cut 3 to 5 micron thick with the help of microtome. Paraffin sections were used for various histological investigations. collagen fibre of heart tissue were stained by Van Gieson Method (1889) as described by Lynch *et al*. in Lynch's medical laboratory technology, Page 803, W.B. Saunders Co., Philadelphia, 1983.

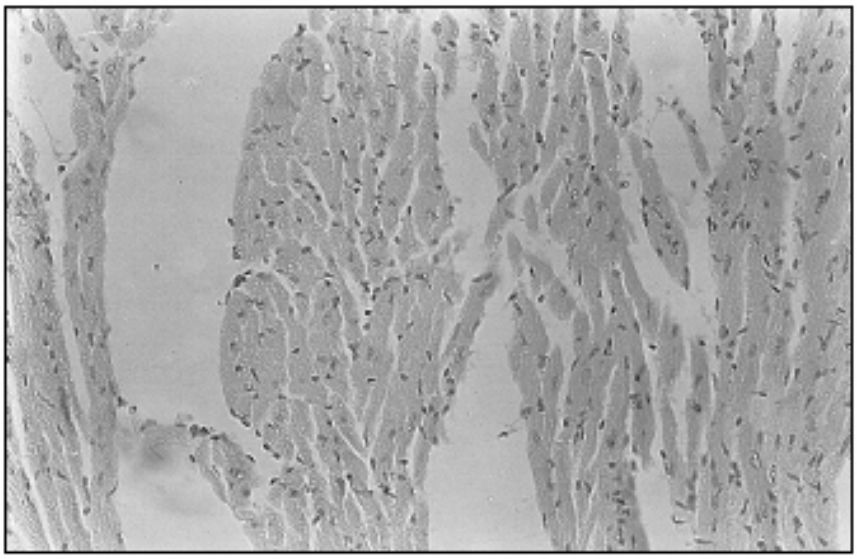

Fig. 1 : Microphotograph shows fibrous component of tissue distinctly invaginated interchordal space (Van Geison stain, 200 x Age – Infant group).

Results :

Van Gieson is a stain in which acid media is produced by picric acid and stain is acid fuschin. In the microphotographs acid-fast components of cytoarchitecture are visible as dark zone while the picric acid stained background tissue takes yellow stain (light colour) .Yellow stain cords or clumps are evident as representative of cardiocytes while the dark stained are either the nuclear component or cytoplasmic component of fibrous element (collagen fibre). In addition the nuclear component, endothelium of artery is also evident (Fig. 2).

A comparison indicates a gradual reduction in nuclear density while increase in dense coloured thin fibrous zones (collagen fibre) in the interchordal space confirming that as age advances there is a relative increase in the fibrous component of the tissue distinctly invaginating interchordal space. Here it is to be clarified that the cord has been used as roughly homogenously stained cardiocytes which are fibrous in nature, arranged in bundles but the nucleus was not distinct. It reflects that the heart continues to accumulate collagen even after the organ has ceased to grow in size. In myocardial tissue, the total amount of collagen rises steadily until the rats reach up to higher age.

Discussion :

Present study shows that collagen accumulates in the myocardial tissue during growth and aging of rats .Study also supports that concentration of collagen fibre also increases with age. Although the heart itself grows consistently with age, there are various observations in the growth and development of collagen fibres. Collagen in the myocardial tissue is shown to accumulate in nearly linear fashion during the life span of animals, resulting in a continuous increase in size and perhaps number of several kinds of discrete interstitial collagenous structures.

The extracellular matrix of the myocardium contains an elaborate structural matrix composed mainly of fibrillar types I and III collagen. This matrix is responsible for the support and alignment of myocytes and capillaries. Because of its alignment, location, configuration and tensile strength, relative to cardiac myocytes, the collagen matrix represents a major determinant of myocardial stiffness. Cardiac fibroblasts, not myocytes, contain the mRNA for these fibrillar collagens. In the hypertrophic remodelling of the myocardium that accompanies arterial hypertension, a progressive structural and biochemical remodelling of the matrix follows enhanced collagen gene expression. The resultant significant accumulation of collagen in the interstitium and around intramyocardial coronary arteries, or interstitial and perivascular fibrosis, represents a pathologic remodelling of the myocardium that compromises this normally efficient pump. Hence, the structural nature, biosynthesis and degradation of collagen in the normal and hypertrophied myocardium can be differentiated. It suggests that interstitial heart disease, or the disproportionate growth of the extracellular matrix relative to myocyte hypertrophy, is an entity that merits greater understanding, particularly the factors regulating types I and III collagen gene expression and their degradation (Eghbali and Weber, 1990).

In the older animals, accumulation of collagen also occurs in fibrotic region. Eghbali *et al*. (1989) also observed a relatively more accumulation of collagen component in the hearts of older animals. The distribution of collagen in the heart has

Fig. 2 : Microphotograph shows that fibrous component (collagen fibres) are more concentrated and figure also shows higher collagen concentration around the artery.
(Van Geison stain , 200 x Age – Young group)

Fig. 3 : Microphotograph shows a relative increase in the fibrous component of tissue distinctly invaginated interchordal space. (Van Geison stain , 200 x Age – Adult group)

been reported in different parts at different age by various researches (Madugorac, 1980). Madugorac and Jacob (1983) reported a preponderance of collagen in right ventricle and the left ventricle was found to have lower collagen content than even that in the septum. Same group reported that ventricular connective tissue of young rats contains non numeric alpha third chain collagen in adult rats while the hearts of aged rats revealed only traces of alpha third chain. Age related changes in collagen composition have also been observed in human and calf skin effect of which is evident by morphological inspection. Annoni *et al*. (1998) reported augmented collagen deposition in interstitium and paracoronary arteries space in the heart with aging. Same case is also evident in present study (Fig 2). Earlier Carbonin *et al*. (1990) and Mays *et al* (1988, 1991) also reported augmentation of the collagen deposition coupled with alterations in collagen metabolism.

The passive elastic properties of myocardium and thereby mechanical function of heart are influenced by myocardial collagen content and composition. We, therefore, studied the total amount and structure of myocardial collagen as function of age in heart of rat. The precise magnitude of and change in collagen parameters may be of great significance in cardiac hypertrophy, heart failure and other pathogenesis of cardiac diseases.

Clenbuterol is known to have therapeutic potential in ameliorating muscle atrophy because of its presumed anabolic effects. Increased lipids in the heart by clenbuterol infer towards its deleterious effects on heart. Antagonist butoxamine had stimulatory effects similar to clenbuterol initially where an increase in the lipid levels was observed (Garg and Sharma, 2006). Heart disease risk factors like hypertension, smoking and diabetes were found to increase antioxidant enzyme levels. Rise in antioxidant enzyme levels in blood samples of the patients were independent of sex and age. Electrophoretic separation of SOD on native gels confirmed antioxidant enzyme stimulation in patients. Surprisingly low levels of MDA content were characteristically recorded in the patients (Sundal *et al*., *2005*). Aging mimics to a greater extent all such disorders and present study reveal some of the fundamental changes in collagen fibres accumulation in relation to age.

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