ABSTRACT

The conduction fibers and Purkinje network of the ventricular myocardium have their peculiar location and immuno-histochemical characteristics. The bundle of His is located at the inferior border of the membranous septum, where the single trunk ramifies into the left and right bundle branches. The left bundle branches are clearly visible at the surface. The right bundles are hidden in the septal myocardium and it is not easy to recognize them. The cellular characters of the conduction bundles are modified myocardial cells with less cytoplasmic filaments. Myoglobin is expressed at the contractile part, whereas CD56 is expressed at the intercalated disc. A fine meshwork of synaptophysin positive processes is noted particularly at the nodal tissue. C-kit positive cells are scattered, but their role is not well understood. Purkinje cells are a peripheral continuation of bundles seen at the immediate subendocardium of the left ventricle.

Key words: conduction system, Purkinje network, pathology, arrhythmia, electrophysiology

Introduction

The functional assessment of abnormal cardiac rhythm and a targeted treatment based on electrophysiologic studies are successful advances in cardiology.1 Morphological assessment or confirmation of hearts with such abnormalities is rare, not only due to the limited availability of human hearts but also inherent technological limitations of existing technology.2 Classical morphological approaches and immuno-histochemical or molecular biologic assessment on a heart transplant recipient will be a unique chance to study the conduction system of the human heart. In this brief review, the histological characteristics of conduction cells, stained by conventional and immuno-histochemical staining, are demonstrated in the second part of the review.3

The characteristic location of the ventricular conduction system

The atrioventricular node is situated in its subendocardial location at the triangle of Koch. A cluster of short spindle cells are arranged compactly around the AV nodal artery. The long axis of the AV node is approximately 0.7 cm. Fatty tissue and some nerve fibers are seen around the node. The bundle of His is 0.5~0.7 cm long and 0.1 cm in diameter (Figure 1, 2). The bundle has a short traversing segment to the anterior superior direction and then ramifies into the
right and left bundles (Figure 2). The left bundle and its anterior and posterior fascicles are recognized histologically at the subendocardium of the left ventricular surface of the ventricular septum. The right bundle is located between the thick bundles of the right ventricular myocardium and the true septal myocardium. The histological verification of the right bundle is not easier than the left side.

Masson trichrome staining is one of the convenient methods to differentiate the conduction tissue from collagenous stroma and pathologic fibrous changes of the myocardium are demonstrable as well through the trichrome staining (Figure 3). It is understood that bundles and fascicles are covered with fibrous sheath so that rapid conduction from the myocardium is insured, although electrical conduction between cells needs a special gap junction protein or intercalated disc as revealed by electron microscopy. Side to side contact by a parallel alignment of muscle bundles is not conductive. It is therefore important to have an axial alignment to propagate contractile stimulation.

**Histological and immuno-histochemical characteristics of the conduction system**

Cells of the conduction system are mainly myocardial cells. The cytoplasm contains myofibrils and they do not have cytoplasmic processes as seen in neural cells. The conduction cells have less myofibrils than myocardial cells and intimate contact with nerve endings are noted. Conduction cells at the microscopic level are spindle cells with smaller cytoplasm. The immuno-histochemical reaction against myoglobin is positive (Figure 3). The intensity of staining by immuno-histochemistry is often influenced by fixation and other factors so that visual intensity of staining may not be an indicator of the amount of protein. Neural cell adhesion molecule (NCAM, CD56) is a member of the immunoglobulin super family, mediating intercellular adhesion in the nervous system and skeletal muscle and CD56 was up-regulated at the ischemic myocardium in human and animal models. There was a strong positive reaction for CD56 at the

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**Figure 1.** Left ventricular surface of the ventricular septum. The initial cut was made at the commissure of right and non-coronary cusps (RC, NC) of the aortic valve. After that, 4 cm long tissue blocks, 2 cm above and below the aortic valve, were designed to make 9 pieces of vertical sections, each with 4 mm thickness (#1-9). Block #9 is toward the anterior wall of the left ventricle, while block #4 toward the posterior wall. The rest of the septal surface was sectioned horizontal to make blocks (#10-21).

MV; mitral valve, NC; non-coronary cusp, RC; right coronary cusp
Figure 2. A. histological section at piece #3 of Fig 1 showing the atrioventricular node (AV) over the fibrous annulus at the atrial surface. B. A section at piece #2 of Fig 1 showing the non-branching His bundle (HB) at the inferior border of the membranous septum, where the posterior fascicle (long open arrows) ramifies. C. A section at piece #1 of Fig 1 showing a non-branching bundle (HB), the anterior fascicle of the left bundle (long arrows) at the surface and the intramyocardial right bundle (short arrows). D. A section at piece #7 of Fig 1 showing the right part of the ventricular septum extending to the infundibulum (INF) of the right ventricle. Short arrows indicate the plane between myocardial layers where the right bundle is located (Masson’s trichrome stain).

AO; aorta, MV; mitral valve, RC; right coronary cusp of aortic valve, TT; tendon of Todaro, TV; septal leaflet of tricuspid valve

intercalated disc of the ventricular myocardium (Figure 4). The atrioventricular node and bundles did not show such strong reaction. It is also noted that the length of myocytes represented as the length between two intercalated discs was longer at the subendocardial left bundles. Synaptophysin, another marker for neural cells is negative at the myocardial cells, but a fine meshwork of synaptophysin positive fibers is seen around the individual conduction cells. The staining pattern of synaptophysin at the atrial myocardium was a slender network between the myocytes (Figure 5). This pattern was the most prominent at the atrial myocardium and AV node. His bundle is faintly stained and ventricular myocardium is the weakest. C-Kit is known to be one of the markers of stem cells and interstitial Cajal-like cells related with atrial arrhythmia. The C-Kit positive cells were rare isolated cells at His bundle (Figure 5). Atroventricular
Figure 3. A. Histological section at piece #16 of Fig 1 showing a cross section of the distal part of the subendocardial left bundle forming Purkinje fibers (PF) (arrow) separated from the underlying myocardium (MC). B. A section at piece #16 of Fig 1 showing subendocardial smooth muscle nodules overlying left bundles (open arrow & dot line). C. A section at piece #1 of Fig 1 showing thick and continuous subendocardial smooth muscle fibers (open arrow & dot line) stained violet with Masson trichrome but appear negative for myoglobin as shown in D. (A–C; Masson’s trichrome stain, D; Myoglobin)

node has some, but rest of area show few positive cells.

Previous studies on the conduction system of the heart showed subsarcolemmal SR (sarcoplasmic reticulum), but no T-tubule or corbular SR. Connexin 45 and neurofilament M (NF-M) were found, but connexin 43 and atrial natriuretic peptide were lacking.1,8

Myocytes of the sinoatrial and atrioventricular (AV) nodes characteristically have small, dispersed gap junctions predominantly composed of Cx45. The His bundle co-expresses Cx45 with Cx43, while its downstream branches prominently express Cx40. Cx43 becomes abundant in the more distal portions of the system, while Cx45 is expressed continuously from the AV node to the ends of the Purkinje fibers.9

Purkinje network

The Purkinje fibers are part of the ventricular
Figure 4. A. An intense positive reaction for CD56 at intercalated discs (arrows) of ventricular septal myocardial cells. B. A less intense positive reaction for CD56 at bundle branches (double head arrow) and long interval intercalated discs.

Figure 5. A. The staining pattern of synaptophysin at atrial myocardium was a slender network between the myocytes. B. The staining pattern of C-Kit positive cells at His bundle. (x400) (A; Synaptophysin, B; C-kit)
conduction system and were originally discovered by Tawara.\textsuperscript{10,11} These fibers conduct excitation (electrical activation) rapidly from the bundle of His to the ventricular myocardial tissue. The Purkinje fibers are myocardial cells with vacuolated cytoplasm located in the ventricular walls of the heart, just beneath the endocardium.\textsuperscript{12} Purkinje fibers, being modified myocardial cells, contain some contractile protein, but the amount is small and glycogen and other organelles occupy the cytoplasm. It has been argued that vacuolated Purkinje cells are only seen in the ungulate heart, but the vacuolated character is noted in human heart too. There are, however, muscular cells, smooth muscle cells, other than Purkinje fibers found in the subendocardium (Figure 3C), particularly in hearts with failure.

From the point of view of ultrastructural composition, the cells of different parts of the cardiac conduction system are partly similar. In contrast to the heart contractile cardiomyocytes, the cells of the cardiac conduction system including Purkinje fibers have a small amount of myofibrils, small mitochondria, lighter cytoplasm and higher glycogen content, but no T-tubular system.

The electrophysiologic demonstration of the Purkinje network showed a propagation of excitation starting from the apex, continuing rapidly towards the base of the ventricle resulting in a contractile motion from the apex to the base.\textsuperscript{12} Another study using electromechanical wave imaging visually confirmed that artificially created sinus rhythm and right-atrial pacing, consisted of a contraction wave that started at the apex right at the beginning of the QRS complex, propagated along the septum and then the left-ventricular posterior wall and finally to the base.\textsuperscript{13}

Ventricular pacing, on the other hand, revealed a disharmonized contraction of the ventricle, confirming that Purkinje fibers have a crucial role in the synchronization of ventricular contractions.\textsuperscript{13} A richly trabeculated endocardium, in which the endocardial invaginations carry the Purkinje tissue partway into the left ventricular endocardium, further increases the speed and area of early ventricular activation. This is a factor in ventricular synchronous activation together with the distribution of the conduction branches and the rapid velocity of the conduction system.\textsuperscript{14}

Rapid ventricular arrhythmias in post-myocardial infarction in the canine heart arise from ectopic foci (triggered) within the Purkinje fiber network located in the subendocardium of the infarct zone in the left ventricle. These spontaneously occurring arrhythmias predictably occur between 24 to 48 hours after occlusion. In Purkinje cells dispersed from the subendocardium of the infarct zone (Purkinje cells from a 48-hour infarcted heart), the density and function of several sarcolemmal ion channels are altered compared to normal Purkinje cells. Little work has been done on remodeled Purkinje cells, particularly Purkinje cells involved in the initiation and perpetuation of cardiac arrhythmias in diseased hearts.\textsuperscript{15}

The Purkinje fibers are distinguished from heart muscle cells by a distinct pattern of gene expression. They up-regulate Cx42, a conduction cell-specific gap-junction protein, unique ion channels, and genes typically expressed in neuronal cells. In addition, conduction cells induce a unique set of myofibrillar protein genes, such as slow-twitch skeletal muscle myosin heavy chain (sMyHC), atrial myosin heavy chain (aMyHC), and skeletal muscle-type myosin binding protein-H (MyBP-H). Purkinje fibers also down-regulate heart muscle-specific myofibrillar proteins, such as troponin and myosin binding protein-C (cMyBP-C), which are essential for normal heart muscle contractility.\textsuperscript{14} Studies on experimental animals with ischemic heart disease demonstrate alteration in the distribution of gap junction immunolabel to the sides of the myocyte, called ‘lateralization’. Gap junction mediated passage of ionic/molecular signals appears responsible for the spread of the ischaemia-reperfusion injury from myocyte to myocyte that leads to rigour contracture and cell death.

Gap junction channels are almost absent in the sinus node, there are few of them in the AV node, but there are a lot of them in fast conducting muscle cells such as His and Purkinje fibers. This gives rise to the anisotropic propagation of depolarization along the
cardiac muscle fiber orientation.15

Heterogeneity of gap junction distribution combined with reduced Cx43 levels appears to act co-operatively to create an arrhythmogenic substrate at less severe levels of overall gap junction reduction than predicted in theoretical models. The findings in mouse models lend considerable support to the view that the nature and extent of the Cx43 reduction in the failing human ventricle is, in practice, of sufficient magnitude to increase susceptibility to arrhythmia.9

The Cx43 gap junction arrangement is particularly disordered in hypertrophic cardiomyopathy, the most common cause of sudden cardiac death due to arrhythmia in young adults. In cardiac-restricted Cx43 knockout mice selected for longer term survival, reduction of coupling resulting from declining ventricular Cx43 leads to propagation of the impulse across numerous Purkinje/working ventricular myocyte junctions that normally remain dormant, thereby creating abnormal activation patterns and wave-front collisions in the ventricular myocardium.

**Conclusion**

It is important that the His bundle and the left bundle branches form a continuum with the distal part of the ventricular conduction system, named the Purkinje network. This will allow myocardial contraction to begin from the apex after the activation of the Purkinje network. The key morphological feature of Purkinje fibers in ungulate hearts is vacuolated cytoplasm, which is a manifestation of small numbers of contractile elements and a large amount of glycogen. Molecular characterization of the activity of connexin 43 is one of the important factors involved in arrhythmias in heart failure, myocardial infarcts and hypertrophic cardiomyopathy.

**References**

1. Boyett MR, Dobrzynski H. The sinoatrial node is still setting the pace 100 years after its discovery. Circ Res. 2007;100:1543-1545.