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Spectroscopic studies of the human heart conduction system *ex vivo*: Implication for optical visualization

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ABSTRACT

Fluorescence excitation and emission spectra of the heart tissues specimens have been measured *ex vivo* with the aim of finding out the optical differences characteristic for the human heart conduction system (the His bundle) and ventricular myocardium.

The optimal conditions enhancing the spectral differences between the His bundle and myocardium were found by recording the fluorescence signal in the range from 420 nm to 465 nm under the excitation at wavelengths starting from 320 nm to 370 nm. In addition, the spectral differences between the His bundle and the connective tissue, which is often present in the heart, could be displayed by comparing the ratios of fluorescence intensities being measured at above 460 nm under the preferred excitation of elastin and collagen. The left and right branches of the His bundle were visualized *ex vivo* in the interventricular septum of the human heart under illumination at 366 nm.

1. Introduction

Optical spectroscopy techniques nowadays find applications in many areas of diagnostic medicine due to its non-invasive approach and instantaneous assessment of diagnostic data. Various spectroscopic methods were used to study tissue composition and to determine the differences between healthy and pathologic tissues [1–8].

The conduction system of the heart is the means by which the heartbeat is initiated and propagated quickly into the different parts of the myocardium in a regular, orderly fashion resulting in the most effective heart contraction. The conduction system is not morphologically homogenous and its main parts (sinoatrial and atrioventricular nodes, the bundle of His and the Purkinje fibres) possess distinctive features. Accidental surgical interference with the conduction system tissue may result in occurrence of dangerous post-operational complications.

There is no explanation what makes the propagation of a heartbeat in the conduction system to be about 10 times faster than in the myocardium tissue. The propagation speed of the pulse is the fastest in the bundle of His, therefore this part of the conduction system was chosen for spectroscopic studies.

Spectroscopic investigations of heart tissues taken from experimental animals are rather sparse. Fluorescence response of canine heart tissue was studied by Aziz et al. [9] in an attempt to localise atrioventricular node of the conduction system by excitation at 308 nm and 364 nm. No difference between the nodal conduction system and surrounding myocardial tissue was found. In order to evaluate the potential of cardiovascular tissue characterisation using near-infrared spectroscopy and laser-induced fluorescence by the excitation at 337 nm, spectra of various pig heart tissues were recorded *in vitro* [10]. The spectra were analysed by the method of principal component analysis, revealing several characteristic features suitable for tissue classification.

Argon-ion laser stimulated fluorescence was used for the characterisation of myocardial tissue [3]. Fluorescence spectra of specimens from 20 sheep hearts were measured under excitation at 457.9 nm. The differences in shape and intensity were detected between the fluorescence spectra of atrial and ventricular tissues. Authors concluded that spectroscopic methods might prove useful in the detection of pathologic myocardial states or tissue rejection in the case of a transplanted heart, enhancing the biopsy results and in some cases replacing this procedure.

In the study on fluorescence identification of sinoatrial and atrioventricular nodal conduction tissue of human hearts [11], differences in the spectra of nodal tissue and adjacent atrial endocardial tissue were detected. Spectra recorded from nodal tissue were distinguished by a notable decrease in fluorescence emission intensity at wavelengths from 440 to 500 nm, as well as by a

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significant decrease both in peak width and area when compared with adjacent atrial endocardial tissue. Nodal conduction tissue was also distinguished from ventricular endocardium by an increase in fluorescence emission at 430–550 nm. Authors concluded that fluorescence measurements could help to differentiate nodal conduction tissue from atrial and ventricular endocardium and may provide a new diagnostic tool for the recognition and subsequent ablation of nodal conduction tissue.

In the present study, the His bundle and ordinary myocardium tissues of human heart were for the first time examined in human necropsy specimens by means of fluorescence excitation and emission spectroscopy. Spectral differences found between the tissues of myocardium and the conduction system could be of a practical value for the establishment of a reliable method for optical visualization of the heart tissues based on the fluorescence spectroscopy.

2. Materials and methods

2.1. Tissues

The human heart samples from 18 subjects (of different age without respect of pathology) were obtained at autopsy (12–24 h post mortem) by the pathologist in the National Centre of Pathology. Due to the muscular origin of both heart conduction system (HCS) and myocardium (MC) it is complicated to distinguish these two tissues visually. Just the fact that the HCS is surrounded by the connective tissue allowed us to separate it microscopically from the ordinary myocardium. Anatomic atlases can be used as a reference for approximate detection of HCS in the heart, though its precise location, especially in various pathologic cases, still remains very problematic.

In obtained samples the artery of the atrioventricular (AV) node branched from the right coronary artery on the same level as the posterior interventricular sulcus. It was separated up to the middle level of the septal tricuspid leaflet close to the front of the coronary sinus. On this level the artery penetrates the back pole of the AV node or gets into a posterior part of the interventricular septum under the node. From this step of preparation a Carl Zeiss biological stereomicroscope with 12–36× magnification was used.

To find the anterior pole of AV node the connection between a central fibrous body and a fibrous ring of the tricuspid valve had to be disclosed. To perform this, endocardium, soft tissues and myocardium had to be separated from the central fibrous body. Going down, the connection between the right slope of the central fibrous body and tricuspid ring was found. It corresponds to the level of anterior commissure of tricuspid valve. Then the basement of the fibrous ring of the septal tricuspid leaflet was separated from the soft tissues till the back node pole, thus finishing the artery preparation. In this way the right edge of the node was discovered. The preparation was eased by the fact that the connective tissue coating the AV node is joined to the tricuspid fibrous ring. This allowed precise separation of the right edge of the node. To find the left edge of the node the soft tissues covering AV node were separated 4–6 mm in width (it corresponds to the node width). So the whole AV node was disclosed completely together with the atrial part of the His bundle (HB), which is located close to the junction of the right tricuspid fibrous ring with central fibrous body or 1–1.5 mm behind it. A penetrating part of the bundle can be easily found after destruction of a junction between the central fibrous body and the fibrous ring of the tricuspid valve. To achieve this the following steps were performed: one blade of the scissors was introduced under the right edge of the central fibrous body close to the junction with the tricuspid fibrous ring and the junction was cut. The bifurcation of the HB is located on the border between membranous part of the interventricular septum and the crest of muscular interventricular septum part (Fig. 1). Firstly, the top right borders of the HB ventricular part were separated from surrounding tissues, and then the left edge (with the very thin left branch) was found. There were no difficulties to disclose the HB branches by their fixed location.

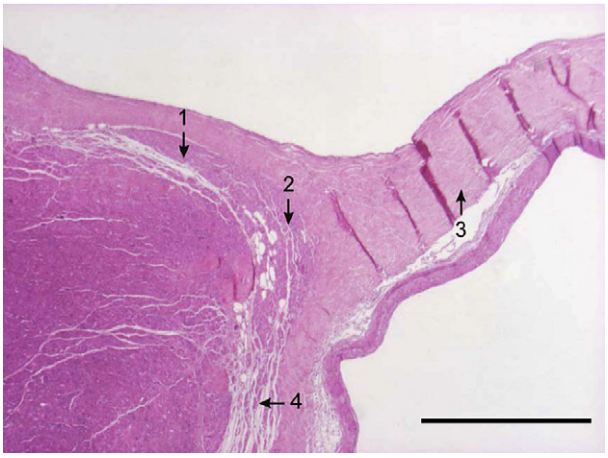

Fig. 1. Cryosection of the His bundle (Haematoxylin-Eosin stain, ×20). 1 – right branch of the HB; 2 – bifurcation of the HB; 3 – membranous part of the interventricular septum; 4 – left branch of the HB. The scale mark is 1 mm.

The samples of the conduction system tissues used for the spectroscopic studies were abstracted from the site of the HB bifurcation and a proximal part of the left branch by the micropreparation method during the autopsy. The samples for the microscopic visualization experiments (2–3 cm in size) were prepared from the left branch of the HB and taken together with surrounding tissues of the endomyocardium. The samples prepared from a whole interventricular septum were taken for the macroscopic visualization experiments. All specimens were fixed in 10% neutral buffered formaldehyde.

2.2. Instrumentation

Fluorescence excitation and emission spectra of the heart tissue specimens were recorded on a LS 50B spectrofluorimeter (Perkin–Elmer, USA). Since the comparison between the spectra measured from both fixed and non-fixed tissue specimens revealed no significant differences, only fixed specimens were used for further studies. Every specimen was put between two quartz slides, placed into the sampling chamber of the instrument and fastened to the black background turned at 55° with respect to the excitation beam for the detection of the emission signal from the surface of the specimens. The installed long-pass filters were used optionally to cut off the scattered excitation light. Because the fluorescence intensity of tissue samples was low, the values of the slit widths for the excitation and emission monochromators were set at spectral resolutions of 5 nm and 7 nm, respectively.

The presence and location of the branches of the HB in the specimens was investigated by means of the fluorescence microscope Olympus BX 60 under illumination at 366 nm using an UV excitation fluorescence mirror unit U-MWU2 (exc: 330–385 nm, dichr: 400, em: 420 nm) and the images obtained with a digital camera Olympus D 50 were compared with the histological data. Some of the specimens were selected for further fluorescence measurements and the fluorescence spectra were registered by means of the microscope-spectrofluorimeter MC ΦY (Russia) under the same illumination conditions as before using the analogous combination of filters.

During the macroscopic visualization experiments the spectral region around 366 nm of high-pressure mercury lamp ДРШ 250-