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Research article

Structure of the heart wall and existence of the blood cells in the heart of the dog-faced water snake *Cerberus rynchops* (Schneider, 1799)

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Abstract

A plurality of investigations on the heart structure of reptiles has been extensively reported; however, it has yet to conduct studies in *Cerberus rynchops*, the so-called common estuarine water-snake. In this current study, we histologically started to examine the heart wall of the atrium, ventricle chambers, and study the existence of the blood cells in the heart of sexually mature females. The selected samples were those *C. rynchops* from the Paknam Pranburi Estuary in Thailand using accurate histochemical techniques. The results showed that the heart structure of *C. rynchops* consisted of three primary compartments (sinus venosus, atrium, and ventricle). All compartments were surrounded by the heart wall, which was typically classified into three layers (the epicardium, the myocardium, and the endocardium), as likely seen in underlying patterns of reptilian heart. In particular, the myocardium of the ventricle in this snake was the thickest layer of the heart wall. Major components of the cardiac muscle fibers in the myocardium with green/ pinkness colors were associated with Masson's trichrome and Periodic Shift reagent methods, indicating to present various fibers and glycoproteins. Consequently, several blood cells were also detected in different areas. The normal and atretic red blood cells were seen in the cardiac fluid, as referred to the fluid inside the heart sac, whereas the mast cell was also observed in the heart wall especially myocardium.

Keywords: Blood cell, Estuarine snake, Heart structure, Histology, Myocardium

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INTRODUCTION

Extensive reviews showed that vertebrate heart, including its sac-like structure, is an involuntary striated muscle and is thus considered as an extraordinary organ (Olson 2000; Farrell et al., 1998; Roberts 2012). This organ is involved in supplying the blood pressure and oxygen transport efficiency to all part of the body (Olson 2000; Farrell et al., 1998; Roberts 2012). Regarding the reptilian heart, it is one of the most intriguing groups due to its complex internal structure (Jensen et al., 2014; Jensen et al., 2013). Previous observations underscored that the localization of the heart varied in individual snakes and their species; however, their basic structures are similar. A combination of both anatomy and histological techniques revealed that there had been five or six chambers, including the sinus venosus, the left and right atria, and two or three sub-chambers of the ventricle of the heart. This feature has provided clear evidence in most reptiles such as lizards and snakes (Farrell et al., 1998; Webb et al., 1974; Wyneken, 2009). Due to the unique morphology, the heart structure of the reptile differed from that of mammals (Qayyum, 1972) and fish (Senarat et al., 2016). All of these chambers are enclosed by the heart wall, which is typically classified into three layers (the epicardium, the myocardium, and the endocardium). The immensely important component of the cardiac muscle fibers in the heart wall was that they predominantly regulated to allow for the synchronization of the heartbeat (Farrell et al., 1998; Webb et al., 1974). Furthermore, the cardiac muscular tissue/fiber are also considered to be electrically coupled, referring to pass action potential (depolarization and repolarization) (Perni et al., 2012), which was observed in *Python molurus bivittatus* (Snyder et al., 1999) and *Pantherophis guttatus* (Jensen et al., 2013).

Following the literature reviews, they extensively reported that snake heart structure had been found and present in the terrestrial snakes and sea snakes (Farrell et al., 1998; Burggren, 1987; White, 1968; Jensen et al., 2014). However, little is known about the availability of the estuarine snake. It is not clear to us that what the heart structure of the estuarine snake is, including the dog-faced water snake *Cerberus rynchops*? This species is widely found along the coastal South Asia, Sri Lanka, Bangladesh, Myanmar, the Andaman Islands, Malaysian Peninsulas and Thailand (Murphy, 2010) and is listed on Appendix III of CITES and conservative species (UNEP-WCMC, 2013). Hence, we initiated to examine the structure of the heart wall together with the existence of the blood cells in the heart of *C. rynchops*. Our observation aims to bring about the additional information about the structure of estuarine heart snakes, and we expected that the robust findings of this study will be of benefits and might be of use in the further studies on the cardiac evolution and reptilian lineages.

MATERIALS and METHODS

Snake samples and study area

Carcasses of five dead female, *Cerberus rynchops* during adult stage with the snout-vent length 74.00±4.06 cm were generously donated from local fisheries at two stations (N 12°24’15.8”/E 99°58’25.6” 2 and N 12°24’21.6”/E 99°58’37.1”) in the Paknam Pranburi Estuary, Thailand.

Histochemical observations

All samples were dissected, and they were then fixed in Davidson's fixative (about 24 - 36 h) (Dietrich and Krieger, 2009), dehydrated through a series of increasing concentrations of ethanol and infiltrated with liquid paraffin (Paraplast) at 58-60 ºC, as previously followed a standard histological technique (Presnell and Schreibman, 1997; Suvarna et al., 2013). To examine the heart wall and composition of blood cells, the heart tissue blocks were to be trimmed, sectioned at 4 µm thickness, and subsequently stained with Masson,s trichrome (MT) and periodic acid-schiff (PAS) (Presnell and Schreibman, 1997; Suvarna et al., 2013). Then, they, the heart wall and blood cell composition, were observed and brought to photograph with a light microscope (Leica DM750).

RESULTS and DISSCUSION

There have been some previous studies on heart histology in the snakes. Unfortunately, the number of these studies was relatively low, and they are discontinuously published (Farrell et al., 1998; Jensen et al., 2014; Jensen et al., 2013; Webb et al., 1974; Wyneken, 2009). Hence, this study was certainly the first study and had thus the most up-to-date information on histological property of the *C. rynchops* heart in Thailand.

Histology of the heart wall

The heart in C. rynchops was located within the pericardium. Normally, it was composed of three basic chambers: the sinus venosus, two atria and the ventricle, as previously seen in *P. molurus bivittatus* (Snyder et al., 1999) and *Pa. guttatus* (Jensen et al., 2013). According to histological techniques, all chambers were enclosed by the heart wall (Figure 1A). A primary observation showed that there are three layers in the heart wall: the epicardium, myocardium and endocardium in longitudinal sections (Figures 1A-1E). Based on observations at high magnification, the characteristics of the epicardium found that it contained a single sub-layer of flattened epithelial cells and a thin layer of the connective tissue (Figure 1C). A good development of the myocardium was the largest layer of the heart wall (Figure 1C). At the high magnification, specialized cardiac muscle containing a great syncytium of anastomosing fiber (cardiomyocytes) was observed (Figures 1E-1F). The structure of cardiomyocytes is considered to play an important role in the bind natriuretic peptides (Cerra et al., 1997) and the anti-freeze mucins (Icardo, 2012). The presence of the endocardium was characterized by a thin layer of loose connective tissue together with epithelial cells (Figures 1C-1F).

In this study, we were firmly interested in the heart wall of the atrium-ventricle structures due to their largest chambers. The atrium-ventricle structures were similarly known and identified in *P. molurus bivittatus* (Snyder et al., 1999) and *Pa. guttatus* (Jensen et al., 2013). The wall of the left and right atria was similar in structure. A thin layer of the epicardium and endocardium was seen (Figure 1C), and a thicker layer of the myocardium contained the cardiac muscular tissue and fiber (Figure 1C). Additionally, our observation showed that the cardiac muscular tissue and cardiac cell also reacted positively with MT and PAS methods (Figures 1C, 1H), indicating the presence of fiber and glycoprotein.

The roles of the atrium were similar to those of other vertebrates which were involved to support the atrial architecture and blood pump (Icardo, 2012; Snyder et al., 1999). The blood from the fish body entered the atrium via the sinus venosus. Like in some vertebrates, the atrium played a significant role in the contractions for pumping the blood into the ventricle (Icardo, 2012). In the ventricle, the results further showed that its heart wall was thicker than that of the atrium (Figures 1D-1E), as likely reported in higher vertebrates (Jensen et al., 2014; Jensen et al., 2013; Wyneken, 2009) and fish (*Rastrelliger brachysoma* (Senarat et al., 2016). In particular, the thickest layer of cardiomyocytes was observed in the myocardium (Figures 1F-1H). Thus the evidence strongly favored the conclusion that cardiomyocytes had the important roles and involved in supporting the pumping action in some vertebrates (Genten et al., 2008; Roberts 2012). Moreover, a report regarding the function of the cardiac cell concerns to electrically coupled, as suggested to pass action potential (depolarization and repolarization) (Perni et al., 2012).

The existence of the blood cells in the heart

The red blood cells were seen in the cardiac fluid (Figure 2A). The size of the mature erythrocyte of *C. chanos* was about 8 μm in diameter and an eclipse shape (Figures 2A-2B). The surface of the normal red blood cell was smooth-shape, whereas the middle of the oval to round nucleus of this cell was shown and surrounded by the red/ pinkness cytoplasm (MT and PAS methods) (Figures 2A-2B). Atretic red blood cells were also observed (Figure 2A). All samples showed that the mast cell (MC) was observed in the heart wall especially myocardium (Figure 2C). Oval-shaped Mc contained the middle nucleus. MC had a distinct feature because of containing the large secretory granules, in which these granules positively reacted to PAS method (Figure 2D). It was possible that their secretory granules deposited of heparin proteoglycan - a weak anticoagulant and histamine, resulting to promote the inflammatory reactions (chronic inflammation and tissue remodeling) (Metz et al., 2007; Galli et al., 2008). Additionally, a few melano-macrophage centers (MMCs) were available in all collected specimens; they were relatively brown color (Figure 2D).

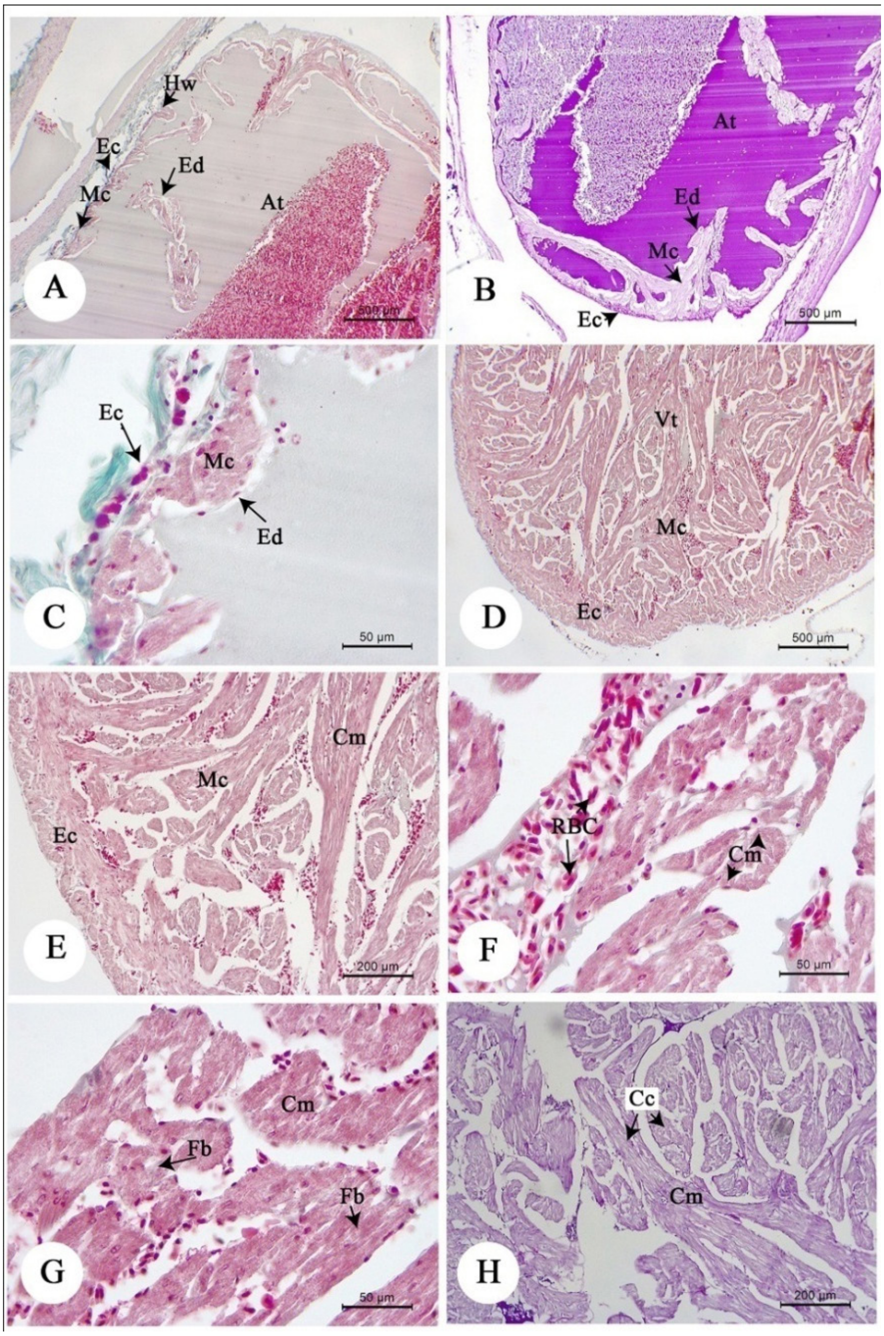

Figure 1 Light micrograph of the heart wall (Hw) in the atrium-ventricle of the heart in *Cerberus rynchops*. A-C. Structure and histochemistry of the atrium (At) was composed of thin layer of the epicardium (Ec) and endocardium (Ed), whereas thicker layer of the myocardium (Mc) was observed. D-H. Structure and histochemistry of the ventricle (Vt) was showed consisting of three layers (epicardium (Ec), myocardium (Mc) and a thick endocardium (Ed) that the MC was thicker than that of the atrium. Note: Cc = cardiac cell, Cm = cardiac muscle, Fb = fibers, RBC = red blood cell. Note: A, C-G = Masson's trichrome (MT) and B, H = periodic acid-Schiff (PAS).

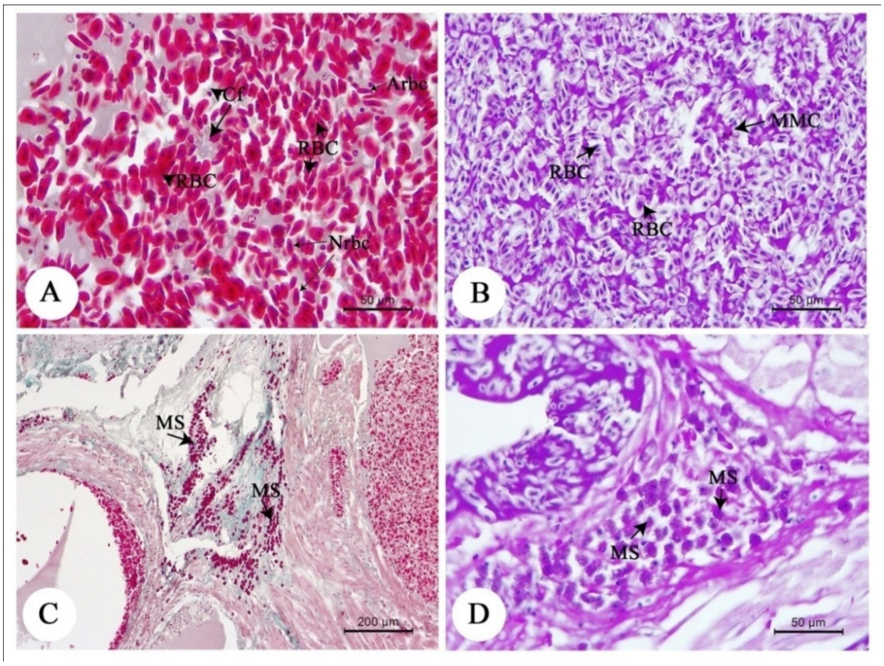

Figure 2 Light micrograph of red blood cells (RBC) and mast cell (MS) in *Cerberus rynchops*. A. Both normal (Nrbc) and atretic (Arbc) red blood cell and melano-macrophage centers (MMC) were found in the cardiac fluid (Cf). C-D. Several mast cells (MS) were detected in the heart wall. Note: A, C = Masson's trichrome (MT) and B, D = periodic acid-Schiff (PAS).

The presence of this phenomenon could have a link with phagocytosis of the parasites because this reaction was the basic response to an infection which suggested host immune responses and chronic inflammatory lesions (Agius and Roberts, 2003). Consequently, we assumed that the presence of MC and MMCs in *C. rynchops* could be of help in the future regarding the responses to the parasite infections, as previously supported by Alvarez-Pellitero et al. (2007) and Sitja-Bobadilla (2008). Prior to the year 2008, there has been little known about the benefits of MC and MMC; they might lead to the answers of a potential health risk, which needs to be investigated in further studies.

CONCLUSION

The present study showed that the heart structure of *C. rynchops* was typically composed of the sinus venosus, atrium, and ventricle. Three layers including the epicardium, the myocardium and the endocardium of the heart wall were observed. Strikingly interesting, major components of the cardiac muscle (or heart muscle) with consisting of the cardiac muscle cells, or fibers in the myocardium were recorded. Several blood cells were also detected with different areas. The red blood cells, the mast cell and a few melano-macrophage centers were observed in the heart. Although this was a characterization of the heart structure in the estuarine snake, as similar to other reptiles, our observation would be applied to further study, such as ultrastructure and physiology as well as reptilian lineages.

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