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DISTRIBUTION AND DENSITY OF MAST CELLS IN THE HEART OF BACTRIAN CAMEL EMBRYO (*Camelus bactrianus*)

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ABSTRACT

The present study was carried out to gain species-specific data on density, distribution of cardiac mast cell and the relationship between mast cells density (MCD) and microvessels density (MVD) in the heart of camel embryo. Specimens were stained with either toluidine blue or immunohistochemically to identify mast cells.

Rare mast cells were observed in the heart of camel embryo in the present study (ranging from 16.98±6.86 to 25.45±11.49 cells /cm^2) . The most characteristic feature of these cells was that they located predominantly in the perivascular region and rarely scattered among the cardiac myocytes. The highest MCD was found in the right ventricle. Significant differences of MCD were not found between the right and left ventricle. There was a positive correlation between MCD and MVD in ventricle. Determination of the mast cells density, distribution and the spatial relationship with microvessels throughout the heart may contribute to improve our understanding of the pathogenesis of the various cardiovascular diseases of camel embryo.

Key words: Bactrian, camel embryo, heart, mast cells

Mast cells (MCs) participate actively in the pathogenesis of heart failures by secreting their proinflammatory mediators including histamine, tryptase, chymase, heparin and some cytokines (Walls *et al,* 2001). Furthermore, cardiac mast cells degranulation is able to mediate the activation of matrix metalloproteinases activity, collagen degradation, and altered ventricular diastolic function (Chancey *et al,* 2002). MCs can lead to apoptosis of cardiac myocytes and proliferation of nonmyocytes in vitro (Hara *et al,* 1999). The loss of cardiac myocytes and proliferation of nonmyocytes both result in cardiac dysfunction (Diez *et al,* 1998). In guinea pig heart, mast cell degranulation can alter cardiac function by stimulating the intracardiac neurons through the release of histamine and activation of neuronal H1 receptors (Powers *et al,* 2001). Mast cell tumour also was found in the heart of cow (Hill *et al,* 1991).

It was demonstrated that the stabilisation of MCs improves cardiac contractile function following haemorrhagic shock and resuscitation (Santone *et al,* 2008). It has been reported that the absence of MCs accelerates the development of functional and structural changes in the irradiated heart. The role of MCs in radiation-induced heart disease is predominantly protective in contrast to what has been the prevailing assumption (Boerma *et al,* 2005). MCs are predominantly located in the tissues close to vascular structures. Many components contained in their granules can potentially contribute to the formation of new vessels (Rakusan *et al,* 1990).

MCs exhibit a diversity of histological, biochemical, and functional properties, depending on the species and their anatomical location with the same species (Heavey *et al*. 1988). In contrast to other species, data regarding mast cells in the heart of camel is not available. Furthermore, the higher incidence of early embryonic death is one of factors that affect the reproduction and fertility in camel (Mukasa-Mugerwa, 1981). Considering this, the present study was carried out to reveal the information on normal distribution, density and subtypes of MCs in the heart of the camel embryo. Besides, we also studied whether there is a spatial relationship between cardiac mast cells density (MCD) and microvessels density (MVD) during the development of camel embryo.

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Materials and Methods

Animals and specimens

Two hearts, from healthy bactrian camel embryo (2-3 months old), were obtained from a slaughter house of the Right Alasan Banner Food Company in Inner Mongolia Autonomous Region, China. Specimens were fixed in 4% phosphate-buffered formaldehyde overnight. After fixation, the tissues were dehydrated and embedded in paraffin.

Metachromatic staining

For evaluation of MC distribution, sections of 5µm thickness were stained with toluidine blue. Briefly, the sections were deparaffinised in xylol (2 changes,×10 min), and then were hydrated through 100,95,80,70 and 50 % ethanol/water (v/v), 5 min, respectively. At last, the sections were rinsed distilled water for 5 min and stained with 1% toluidine blue for 2 min. Finally, the sections were rinsed distilled water and dehydrated by 2 min changes in 50, 70, 80, 95 and 100% ethanol/water (v/v), followed by 2 min in xylol and cover-slipped using neutral balsam (Country medicine group limited company, China). Mast cells were identified by the characteristic metachromatic staining of secretory granules by toluidine blue.

Immunohistochemistry

Tryptase can be used as markers for mast cells. Tissue specimens were embedded in paraffin and serially sectioned (5µm). For immunohisto- chemical staining, paraffin sections were deparaffinised in xylene, hydrated and then placed in phosphate buffered saline (PBS; pH 7.6). Antigen retrieval was performed by boiling for 16 min in citrate buffer (0.01 M). Sections were treated with 3% hydrogen peroxide for 30 min to quench endogenous peroxidase activity, rinsed with deionised water and then washed with PBS. Sections were incubated firstly with 1% goat serum to reduce non-specific staining and then incubated with mouse antibodies to human tryptase at 4°C overnight. The slides were then washed with PBS and goat anti-mouse IgG diluted at 1:200 was incubated at room temperature for 2 h. And then sp-HRP diluted at 1:200 was added. At last, Peroxidase was visualised by applying 0.01% DAB and 0.01% hydrogenperoxide in PBS-buffer. For control sections either primary or secondary antibodies were omitted and replaced by buffer.

MCD and MVD

Mast cells were identified by two methods of staining, metachromatic staining using 0.5% toluidine blue and immunohistochemistry using tryptase. The density of microvessels was evaluated when vessels was observed around the mast cells. All mast cells and microvessels in the whole section were counted under a standard light microscope (Leica, Germany) at a ×200 magnification using a square eyepiece reticule and expressed as cells / cm^2. Ten sections from each part of heart were counted.

Statistical analyses

The non-parametric Kruskal-Wallis test followed by the Dunn's multiple comparison test were carried out to evaluate the mast cells numbers obtained by immunostaining and by toluidine blue. Statistical analyses were carried out with the statistical software SPSS 17.0. Relationships between MCD and MVD were estimated by Spearman' rank correlation coefficient (rs). P-values < 0.05 were considered as statistically significant. All grouped data are expressed as means±SD.

Result

MCs were observed in the heart of camel embryo in the present study. The typical shape of MCs stained in the heart was round or oval. The most characteristic feature of these cells was that they located predominantly in the perivascular region (Fig 1. A, B and C). Few MCs scattered among the cardiac myocytes ware also found (Fig 1 D). MCs containing tryptase were detected in each part of the heart (Fig 2). MCD in the heart of camel embryo is relatively low, ranging from 16.98±6.86 to 25.45±11.49 cells/cm^2. The highest MCD was observed in the right ventricle, whereas that of the right atrium was the lowest. In contrast to the right ventricle, the MCD of the left ventricle and atrium was relatively low (Fig 3). There were no significant differences between the density of the ventricle and that of the atrium (Fig 4).

It appears that large number of the mast cells was in close contact with microvessels. The Spearman's correlation coefficient revealed a significant correlation between MCD and MVD in the ventricle (rs=0.892, p=0.003) (Fig 5). Conversely, no significant associations between MCD and MVD in the atrium were found (rs = -0.472, p=0.285) (Fig 6).

Discussion

The shape of mast cells in the heart of bactrian camel embryo was consistent with that of mast cells in the small intestine of dromedary camels (Al-Zghoul *et al,* 2008). It was demonstrated that the MCD of the right ventricle is relatively higher than that of left ventricle. The results are consistent with the study

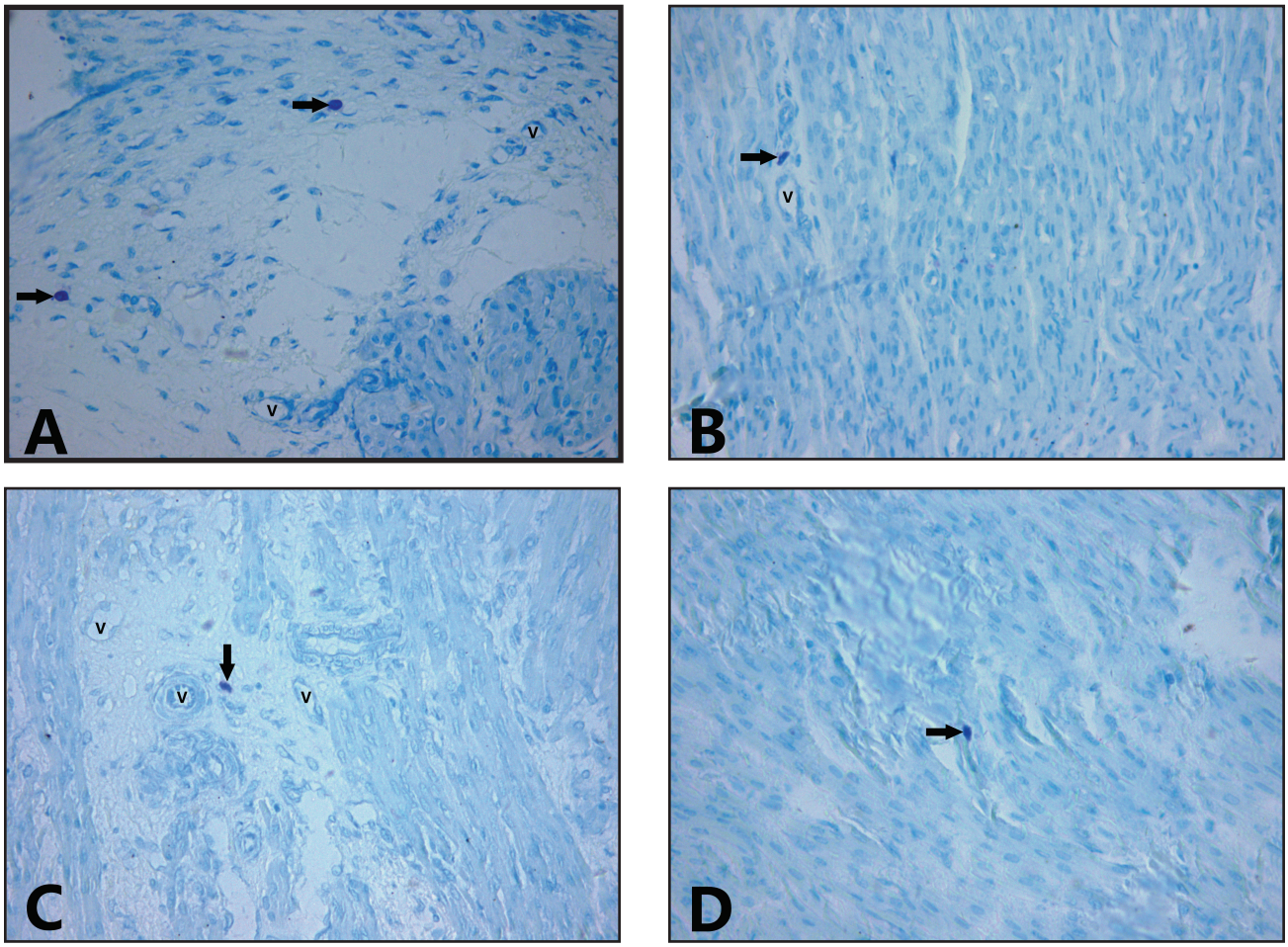

Fig 1. Mast cells distribution in the heart of embryo camel. Sections were stained with 0.5% toluidine blue. (A) The right ventricle; (B) The right atrium; (C) The left ventricle; (D) The left atrium. Arrows in the pictures indicate mast cells, V, vessel. Magnification, × 200.

carried out on adult rats (Rakusan *et al,* 1990). Both left ventricle and left atrium had relatively low MCD in the heart of camel embryo. Although no significant differences were found between MCD of the ventricle and that of the atrium, the number of mast cells in the former is still relatively larger than that in the latter. The results are not consistent with previous report in the guinea pig (Ghanem *et al,* 1988). The distribution characteristics of mast cells may be associated with the function of the camel heart.

Degranulation of the cardiac mast cells leads to ventricular oedema and increase the coronary blood flow and venous blood histamine levels (Chancey *et al,* 2002). It has been reported that the increase in the number of mast cells and mast cell degranulation of heart are closely associated with cardiovascular pathology, especially unstable angina and silent myocardial ischemia (Deedwania, 1995).

The spatial relationship between cardiac mast cells density (MCD) and MVD during the development of camel embryo were revealed in the present study. The results indicated that mast cells tend to concentrate around blood vessels. There was a positive correlation between MCD and MVD in ventricle. At the peak of mast cell density, the percentage of mast cells close to capillaries is also the highest, which means that mast cells may play a special role in the formation of new vessels. In addition, the localisation of mast cell close to coronary vessels in ventricles could be of pathological significance. It has been demonstrated that mast cells can secrete vascular endothelial growth factor (VEGF) (Boesiger *et al,* 1998), which contributes to the neovascularisation process (Yancopoulos *et al,* 2000). Histamine can also stimulate new vessel growth by acting through H1 and H2 receptors (Sorbo *et al,* 1994). It was also reported that mast cells present to sites of neovscularlisation and stimulate vascular tube formation by secreting tryptase (Blair *et al,* 1997). Conversely, it was not found that MCD was significantly related to MVD in the atrium.

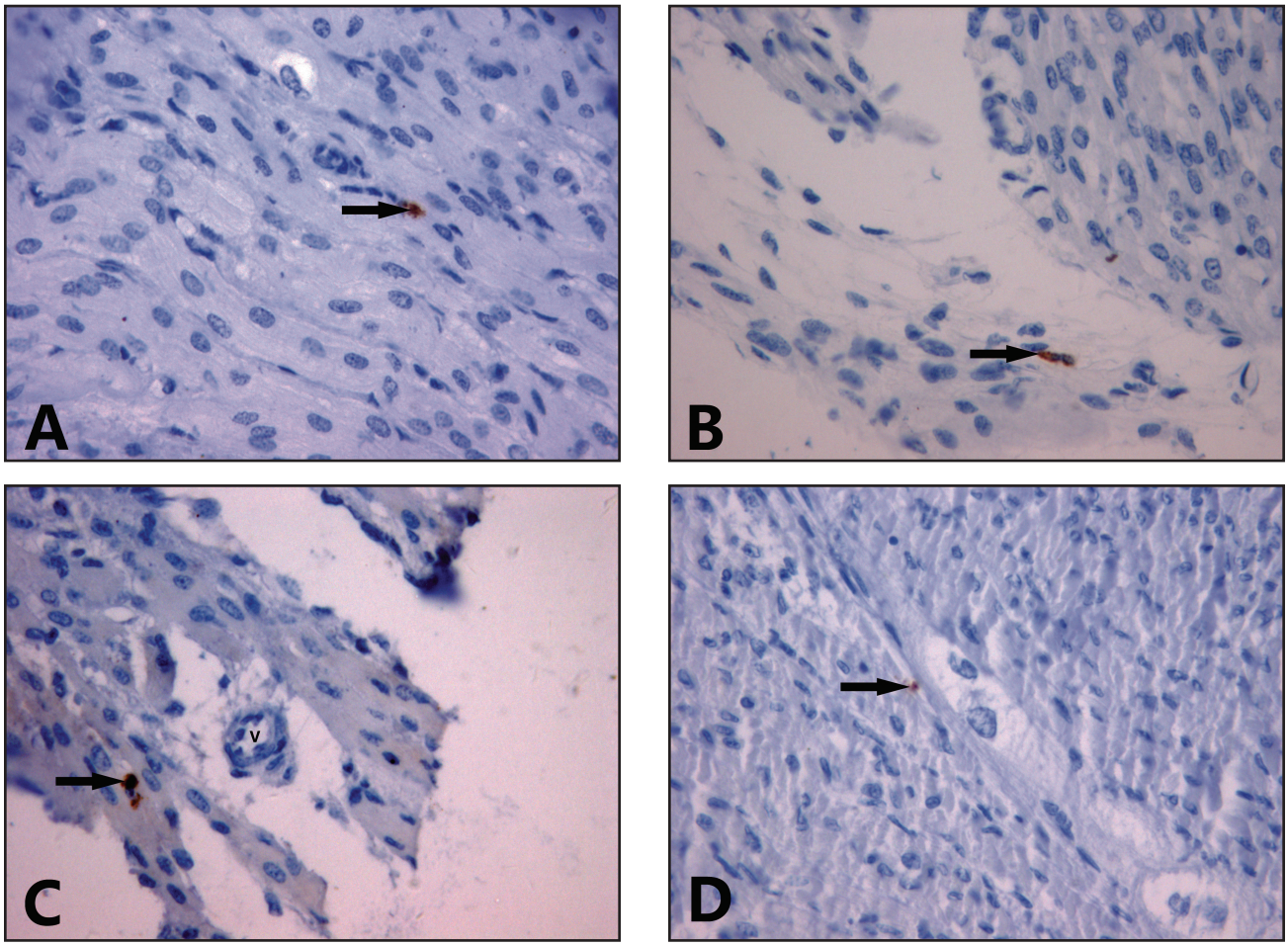

Fig 2. Mast cells distribution in the heart of embryo camel. Sections were immunostained with mouse antihuman skin mast cell tryptase. (A) The right ventricle; (B) The right atrium; (C) The left ventricle; (D) The left atrium. Arrows in the pictures indicate mast cells, V, vessel. Magnification × 400.

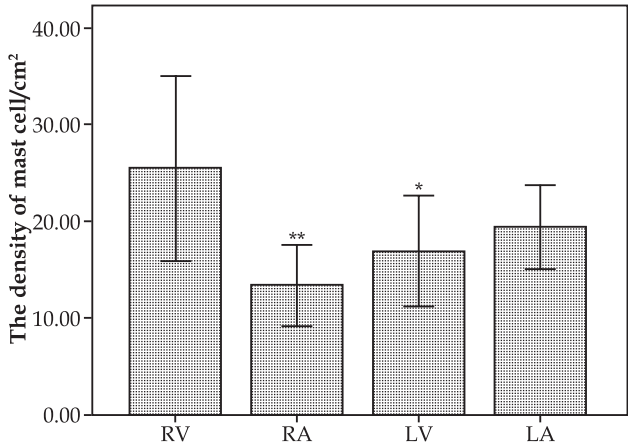

Fig 3. Comparison of MCD in each part of the heart. Values are presented as means±SDs. ** P<0.01 vs. RV; *P<0.05vs. RV.

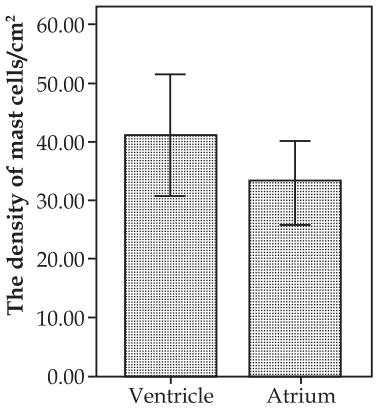

Fig 4. Comparison of MCD between ventricle and atrium. Values are presented as means±SDs.

According to their content of mast cell-specific proteases containing tryptase, chymase and both proteases, mast cells were divided into three subtypes, MCT, MCC and MCTC, respectively. Tryptase seems to participate in proinflammatory, whereas chymase seems to be more involved in inflammatory reaction (Welle, 1997). It was reported that there are functional differences of the various mast cell subtypes (Welle, 1997). Tryptase can promote cytokine to release and collagen to deposit. Moreover, the injection of human

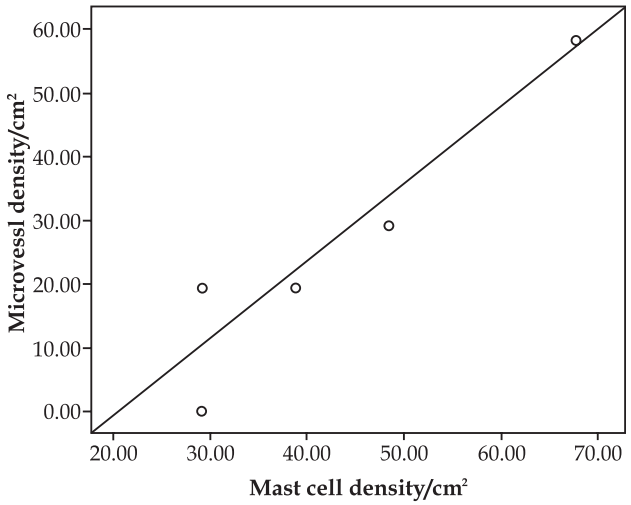

Fig 5. Correlation between MCD and MVD in the ventricle. (rs=0.892, p=0.003).

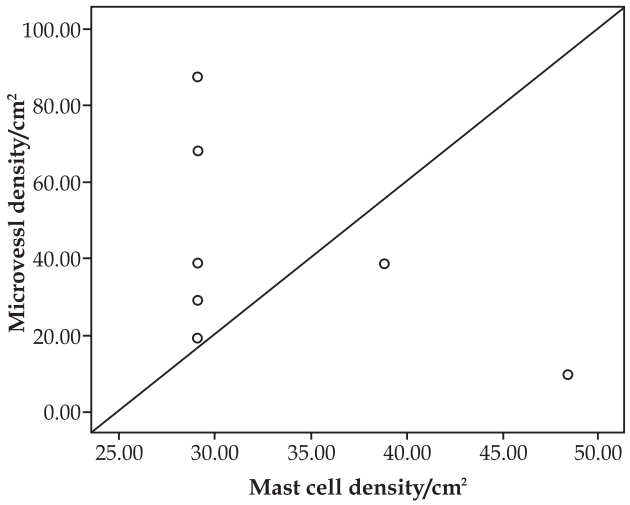

Fig 6. Correlation between MCD and MVD in the atrium. (rs = -0.472, p=0.285).

tryptase into guinea pigs and mice is capable of promoting the accumulation of granulocytes.

The subtype of mast cells in human heart belongs to MCTC according to the principle (Patella *et al,* 1995). The presence of tryptase was demonstrated for the first time in the present study. Although in this study, the MCT density and distribution were only reported. It is necessary to do further work to evaluate and estimate other mast cell subtypes in the camel heart.

Cardiac mast cells could play a role in involving allergic or inflammatory responses and in the regulation of microvascular events (Galli, 1993; Dvorak, 2005). Determination of the mast cells density, distribution and the spatial relationship with microvessels throughout the heart of camel embryo may help to reveal their involvement in certain pathological conditions and may improve our understanding of the pathogenesis of the various cardiovascular disease.

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Reference

Al-Zghoul MB, Al-Rukibat RK, Alghadi M, Caceci T and Bani Ismail Z (2008). Distribution and density of mast cells in camel small intestine and influence of fixation techniques. European Journal of Histochemistry 52:237-242.

Blair RJ, Meng H, Marchese MJ, Ren S, Schwartz LB, Tonnesen MG and Gruber BL (1997). Human Mast Cells Stimulate Vascular Tube Formation Tryptase Is A Novel, Potent Angiogenic Factor. Journal of Clinical Investigation 99:2691-2700.doi: 10.1172/JCI119458.

Boerma M, Wang JR, Wondergem J, Joseph J, Qiu XH, Kennedy RH and Jensenl MH (2005). Influence of Mast Cells on Structural and Functional Manifestations of Radiation-Induced Heart Disease. Cancer Research 65:3100-3107. doi:10.1158/0008-5472.CAN-04-4333.

Boesiger J, Tsai M, Metal M, Yamaguchi M, Brown LF, Claffey KP, Dvorak HF and Galli SJ (1998). Mast cells can secrete vascular permeability factor/vascular endothelial growth factor and exhibit enhanced release after immunoglobulin E-dependent up regulation of Fce receptor I expression. Journal of Experimental Medicine 188:1135-1145. doi: 10.1084/jem.

Chancey AL, Browe GL and Janicki JS (2002). Cardiac mast cell mediated activation of gelatinase and alteration of ventricular diastolic function. American Journal of Physiology - Heart and Circulatory Physiology 282:2152-2158. doi:10.1152/ajpheart.00777.

Deedwania PC (1995). Mental stress, pain perception and risk of silent ischemia. Journal of American Colloids Cardiology 25:1504-1506.

Diez J, Fortuno MA and Ravassa S (1998). Apoptosis in hypertensive heart disease. Current Opinion Cardiology 13:317-325.

Dvorak AM (2005). Mast cell-derived mediators of enhanced microvascular permeability, vascular permeability factor/vascular endothelial growth factor, histamine, and serotonin, cause leakage of macromolecules through a new endothelial cell permeability organelle, the vesiculo-vacuolar organelle. Chem. Immunology Allergy 85:185-204.

Galli SJ (1993). New concepts about the mast cell. The New England Journal of Medicine 328:257-265.

Ghanem NS, Assem ESK, Leung KBP and Pearce FL (1988).

Cardiac and renal mast cells: morphology, distribution, fixation and staining properties in the guinea pig and preliminary comparison with human. Agents and Actions 23:3-4.

Hara M, Matsumori A, Ono K, Kido H, Hwang MW, Miyamoto T, Iwasaki A, Okada M, Nakatani K and Sasayama S (1999). Mast cells cause apoptosis of cardiomyocytes and proliferation of other intramyocardial cells *in vitro*. Circulation 100:1443-1449.

Heavey DJ, Ernst PB, Stevens RL, Befus AD, Bienenstock J and Austen KF (1988). Generation of leukotriene C4, leukotriene B4, and prostaglandin D2 by immunologically activated rat intestinal mucosa mast cells. Journal of Immunology 140:1953-1957.

Hill JE, Langheinrich KA and Kelley LC (1991). Prevalence and Location of Mast Cell Tumors in Slaughter Cattle. Veterinary Pathology 28:449-450.

Mukasa-Mugerwa E (1981). The camel (*Camelus dromedarius*): a bibliographical review31–32. http://www.google.com/books?id=mpMSXAHUULoC&printsec=frontcover&dq=The%20camel%20(Camelus%20dromedaries)%3A%20a%20bibliographical%20review&lr&hl=zh-CN&source=gbs_slider_thumb#v=onepage&q&f=false.

Patella V, Marino I, Lamparter B, Arbustini E, Adt M and Marone G (1995). Human heart mast cells: isolation, purification, ultrastructure, and immunologic characterization. Journal of Immunology 154:2855–2865.

Powers MJ, Peterson BA and Hardwick JC (2001). Regulation of parasympathetic neurons by mast cells and histamine in the guinea pig heart. Auton Neurosci-Basic 87:37–45. doi.10.1016/S1566-0702 (00)00260-5.

Rakusan K, Sarkar K, Turek Z and Wicker P (1990). Mast cells in the rat heart during normal growth and in cardiac hypertrophy. Circulation Research 66:511–516.

Santone DJ, Shahani R, Rubin BB, Romaschin AD and Lindsay TF (2008). Mast cell stabilisation improves cardiac contractile function following hemorrhagic shock and resuscitation. American Journal of Physiology - Heart and Circulatory Physiology 294:2456–2464. doi:10.1152/ajpheart.00925.

Sorbo J, Jakobsson A and Norrby K (1994). Mast-cell histamineis angiogenic through receptors for histamine 1 and histamine 2. International Journal of Experimental Pathology 75:43–50.

Walls AF, He SH, Buckley MG and McEuen AR (2001). Roles of the mast cell and basophil in asthma. Clinical & Experimental Allergy Review 1:68-72.

Welle M (1997). Development, significance, and heterogeneity of mast cells with particular regard to the mast cell-specific proteases chymase and tryptase. Journal of Leukocyte Biology 61:233–245.

Yancopoulos GD, Davis S, Gale NW, Rudge JS, Wiegand SJ and Holash J (2000). Vascular specific growth factors and blood vessels formation. Nature 407:242–248. doi: 10.1038/35025215.