Relationship between Mast Cells and Hepatic Myofibroblasts Induced Cirrhosis Rats

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Objective: *The hepatic myofibroblasts (hepatic MFs) can be divided into 3 groups: (a) portal/septal MFs; (b) activated hepatic stellate cell myofibroblasts (HSC/MFs); and (c) interface myofibroblasts (IF/MFs). The portal/septal MFs situated in portal triad, HSC/MFs located in space of Disse and IF/MFs lay in the rim of hepatic nodule. This study was aimed to elucidate the relationship between mast cells and hepatic MFs in cirrhotic rats induced by thioacetamide (TAA).*
Material and Method: *The rats were divided into two groups: control group, TAA-induced. The rats were treated with TAA administration (200 mg/kg) 3 times per week to induce cirrhosis (TAA-induced cirrhosis group). The hepatic MFs and mast cells were demonstrated by light and transmission electron microscopy.*
Results: *The hepatic MFs were often seen to locate in the connective tissue of cirrhotic liver. HSC/MF localized in space of Disse adjacent to mast cells. IF/MFs were also present at the margin of hepatic nodules closed to mast cell. Mast cells were predominantly found together with plasma cells in portal areas in proximity to lymphatic capillary.*
Conclusion: *The present study demonstrated that mast cells were usually situated in close vicinity to the hepatic MFs and lymphatic capillary. It is probable that mast cells directly or indirectly involved in fibrogenesis and lymphangiogenesis in cirrhotic liver.*

Keywords: *Mast cell, Hepatic myofibroblast, Induced cirrhosis*

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Mast cells (MCs) were first discovered by Paul Ehrlich in 1878. Mast cells contain proteoglycan and protease-rich cytoplasmic granules[1]. They show metachromatic with toluidine bule staining[2]. The cell performs multifunctions of immune system[3]. After originating from hematopoietic stem cells in bone marrow[4] they are released into the blood stream and entering the hepatic tissue. Hepatic mast cells have been demonstrated in connective tissue adjacent to the hepatic arteries both in rat and human livers[5,6]. They produce mediators such as histamine, heparin, tryptase, transforming growth factor-beta 1 (TGF-β1), tumor necrosis factor alpha (TNG-α), interleukins, cytokines and basic fibroblast growth factor (bFGF)[7].

The hepatic stellate cells (HSC) (or perisinusoidal cells or vitamin A-storing cells, or lipocytes, or interstitial cells, or fat-storing cells, or Ito cells) store fat-soluble vitamin A in cytoplasm and synthesize collagen fibers into space of Disse. In pathological conditions, HSCs transform into hepatic myofibroblast, which could then produce a large amount of extracellular matrix and collagen fibers, but lose function of vitamin A storing[8-10]. The hepatic myofibroblasts (MFs) are separated into 3 groups: (a) portal/septal MFs; (b) activated hepatic stellate cell myofibroblasts (HSC/MFs); and (c) interface myofibroblasts (IF/MFs)[11]. Each subpopulation of hepatic MFs shows characteristic morphology and localization, which correlates with localization of type I and III collagen in bridging, biliary, perisinusoidall/ pericellular and centrilobular fibrosis[12]. It has documented that the hepatic myofibroblasts and mast cells are frequently found to be in close proximity in the hepatic fibrous septa of an animal infected with *C. hepatica*[13]. Furthermore, many reports have demonstrated that macrophages, hepatic myofibroblasts and mast cells are closely related to the liver fibrosis induced by CCl_4[14] and porcine serum[15]. Our previous report has demonstrated that the number of macrophages, hepatic myofibroblasts and mast cells were increased in animals with hepatic fibrosis and these cells might play a role in the formation

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of hepatic fibrosis[16].

However, no previous studies have reported on the relationship and role of differential types of hepatic myofibroblasts and mast cells in cirrhotic rat induced by thioacetamide (TAA). We hypothesized that the hepatic myofibroblasts and mast cells might be associated with the formation of hepatic nodules in cirrhotic rat, if these two cell types lie in close vicinity.

Material and Method

Animal

Ten male Wistar rats weighing between 150 to 200 g were used in this study. They were obtained from The National Laboratory Animal Centre (NLAC, Salaya, Thailand). The animals were kept in the room maintained at 25°C on a 12-hour light/dark cycle and *ad libitum* to water and food. The rats were divided into two groups: control group, TAA-induced, each containing 5 animals. The TAA-induced rats administered intraperitoneally 200 mg/kg of thioacetamide (TAA) for induction of cirrhosis for 3 times per week of consecutive 12 weeks. After that, the rats were euthanized by 0.3 to 0.5 of Nembutal cc and killed rapidly by decapitation. The rat livers were immediately dissected and removed through a midline abdominal wall incision. This study was conducted according to the National Research Council (NRC) guide for care and use of laboratory animals and was approved by the Faculty of Medicine, Srinakharinwirot University Institutional Animal Care and Use Committee (IACUC) (Animal ethic number 11/2558).

Tissue preparation for semithin and TEM

The specimens were fixed with 2.5% glutaraldehyde in 0.1 M PBS. The specimens were postfixed in 1% osmium tetroxide in 0.1 M PBS. The standard approaches for plastic embedding were used. Semi-thin sections (1 to 1.5 μm) were obtained by an ultramicrotome and stained with toluidine blue. The pictures were taken under the light microscope, connected to a digital camera. Then, the embedded specimens were selected, sectioned at 80 to 85 nm thick by ultramicrotome. The tissue sections were, thereafter, picked up on the grids, stained with 1% uranyl acetate and lead citrate. Areas of interest presented in specimen blocks were then processed for studying their ultrastructures with transmission electron microscope (Hitachi H-7000).

Tissue preparation for immunohistochemistry

After decapitation, the livers were immediately removed and fixed in 2.5% buffered formaldehyde, dehydrated in graded series of ethanol and embedded in paraplast. Serial sections of 5-7 μm were prepared and mounted on poly-L-lysine-coated slides. The slides were stored at 4°C for immunohistochemical staining.

Horseradish Peroxidase Immunohistochemistry

Liver sections were put into autonomic machine for horseradish peroxidase immuno-histochemical staining (BenchMark XT, USA) and were incubated for 30 minutes with goat antimouse mast cells antibody (Abcam, USA), diluted 1: 150 in PBS. Rabbit antigoat IgG, diluted 1: 200 in PBS, was used to incubate as secondary antibody for 30 minutes. Finally, the sections were mounted in Permount (Fischer Scientific Co., Fairlawn, NJ).

Results

Light microscopy

The relationship between mast cells and hepatic myofibroblast (MFs) in cirrhotic rat-induced by thioacetamide (TAA) were explored with semi-thin sections stained with toluidine blue. The hepatic stellate cell located in space of Disse whereas the mast cell could not be seen in control group (Fig.1A). The fibroblast in portal triad could be clearly demonstrated (Fig.1B). In cirrhosis, the mast cell was stained with metachromasia granules and the hepatic MF was contained in a little greenish cytoplasmic fat vacuole. The mast cell was situated close to the portal/septal MF in portal area (Fig. 1C). It could be demonstrated close to the activated hepatic myofibroblast in space of Disse and lay at the border of broad fibrous septa closed to interface MFs (Fig. 1D, E). Furthermore, it was neighboring with plasma cell in the portal triad (Fig. 1F).

Electron microscopy

The mast cell located in space of Disse (Fig. 2A, B). They have many granules size 0.83 μm in diameter. In the portal area, it was usually situated close to connective tissue and lymphatic capillary (Fig. 2C).

Horseradish Peroxidase Immunohistochemtry

The mast cells resided in connective tissue in the portal area of normal rats (Fig. 3A). In cirrhosis, the mast cells were frequently found at broad fibrous septa around the rim of hepatic nodules (Fig. 3B).

Discussion

HSC/MFs contained greenish cytoplasmic fat

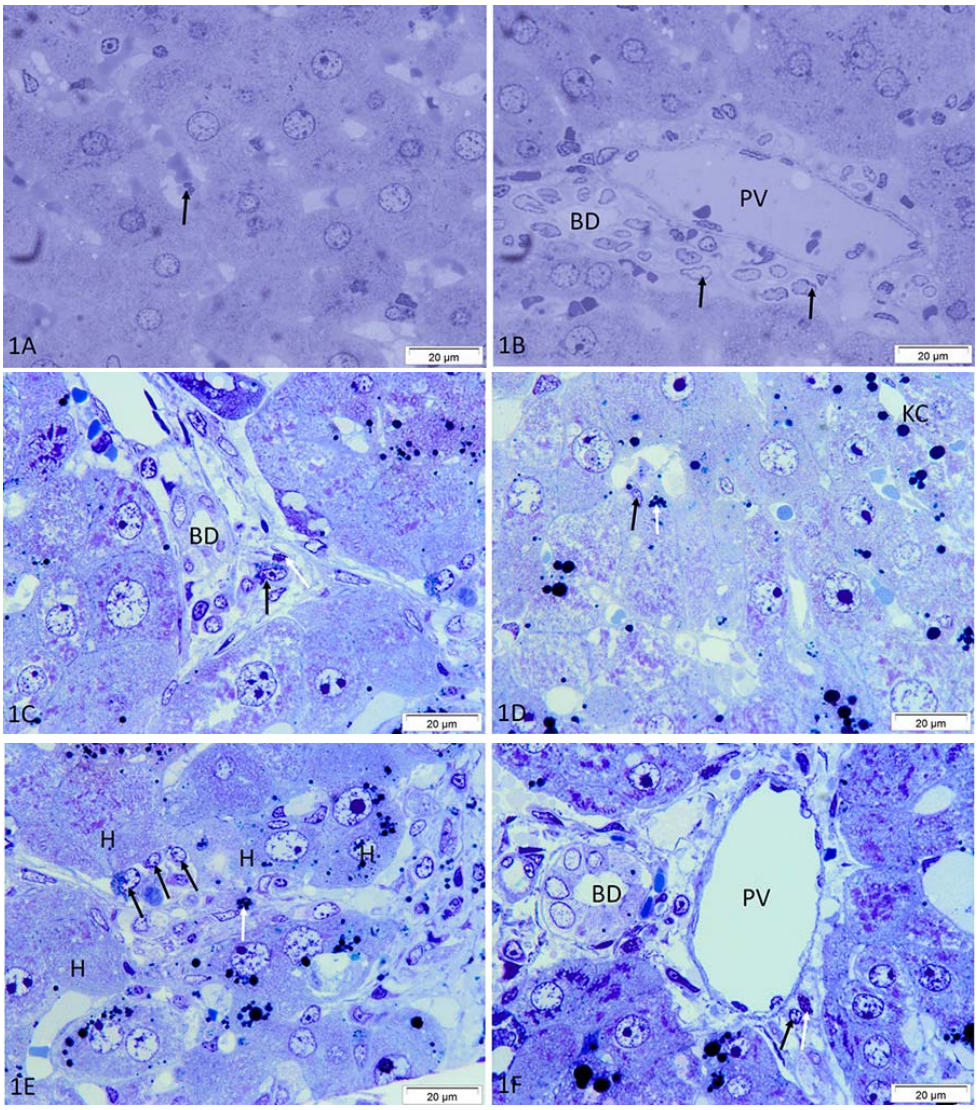

Fig. 1 (A) The hepatic stellate cell (black arrow) situated in space of Disse in normal group; (B) the fibroblast (black arrow) located in portal triad in normal group. BD, bile duct; PV, portal vein; (C) in cirrhosis rat, the mast cell (white arrow) situates closely to theportal/septal MF (black arrow) in portal area. BD, bile duct; (D) in cirrhosis rat, the mast cell (white arrow) lies closed to the activated hepatic stellate cell MF (black arrow) in the space of Disse; (E) in cirrhosis rat, the mast cell (white arrow) lies at the border of Broad fibrous septa closed to interface MFs (black arrows). H, hepatocyte; (F) in cirrhosis rat, the mast cell (white arrow) is neighboring with plasma cell (black arrow head) in portal triad. BD, bile duct; PV, portal vein.

vacuoles in normal human liver[17]. In this study, a little greenish cytoplasmic fat vacuole of hepatic myofibroblast could be observed. It is likely that HSC/MFs in thioacetamide-treated liver transformed into hepatic myofibroblasts which probably loss of some lipid droplets.

Increased numbers of myofibroblasts and mast cells are frequently found together in a wide variety of settings, such as normal wound repair and scleroderma skin, which suggests that mediators produced by the mast cells could play a role in the regulation of myofibroblast differentiation and function[18]. Franceschini, B et al, 2006 suggest that mast cells contribute to capillarisation by recruiting other liver matrix-producing cells, thus increasing the secretion of cytokines and other mediators during the progression of liver fibrosis[5]. Since in this study the mast cells were frequently found to be situated close

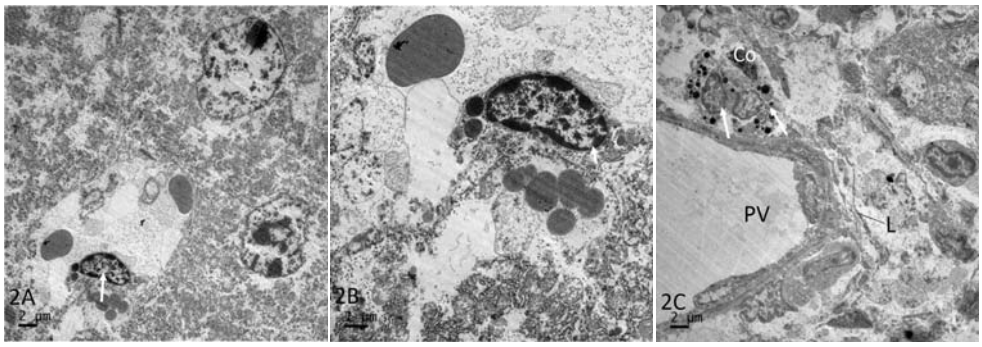

Fig. 2 (A) The mast cell (white arrow) situated in space of Disse.; (B) higher magnification of area in fig 2A. White arrow, mast cell; (C) the mast cells (white arrows) usually situates closed to connective tissue and lymphatic capillary in portal area. (Co, connective tissue; L, lymphatic vessels; PV, portal vein).

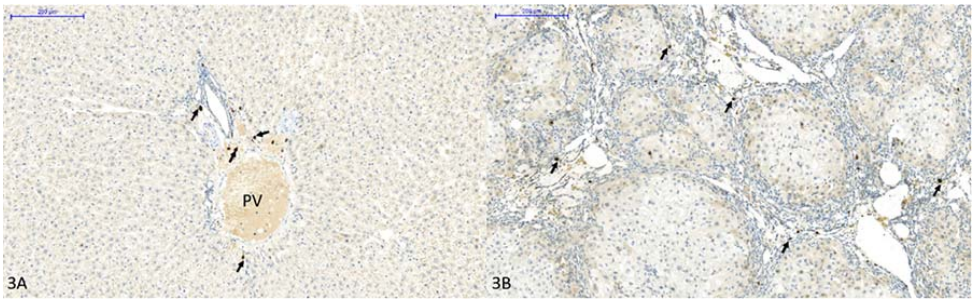

Fig. 3 (A) The mast cells (black arrows) located in portal area of normal rat. PV, portal vein; (B)the mast cells (black arrows) lay in rim of hepatic nodules in cirrhosis rat.

to the IF/MFs which lay at rim of hepatic nodule more than other types of hepatic MF in cirrhosis rat, it is probable that mediators of the mast cells play a role in the development of liver fibrosis to cirrhosis. In addition to mast cells, the complex of myofibroblast and cholinergic nerve terminals might have interplayed for collagen synthesis[15].

In agreement with previous report, the present study had confirmed that cytokines and other mediators of mast cell could stimulate HSC/MFs to produce extracellular matrix protein and encourage with portal/septal MFs, IF/MFs to synthesize numerous collagen fibers in the cirrhotic rat[5,19]. Moreover, the histamine granules released from mast cells had vasodilatation effect[6]. The interstitial fluids leaked from blood capillaries and flowed into blind end lymphatic capillary. It could be concluded that mast cells involve directly or indirectly in fibrogenesis and lymphangiogenesis induced cirrhosis liver.

What is already known on this topic?

Presence of mast cells in relation to hepatic myofibroblasts in the CCl_4-induced cirrhotic rat has been reported. However, its relation to different populations of hepatic MFs and lymphatic capillary in thioacetamide-induced rats has not yet been documented.

What this study adds?

The mast cells are found to be situated close to the hepatic myofibroblast for which those cells help to involve directly or indirectly in fibrogenesis and lymphangiogenesis in cirrhotic liver.

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Potential conflicts of interest

None.

References

1. Beaven MA. Our perception of the mast cell from Paul Ehrlich to now. Eur J Immunol 2009; 39: 11-25.

2. Sridharan G, Shankar AA. Toluidine blue: A review of its chemistry and clinical utility. J Oral Maxillofac Pathol 2012; 16: 251-5.
3. Galli SJ. New insights into "the riddle of the mast cells": microenvironmental regulation of mast cell development and phenotypic heterogeneity. Lab Invest 1990; 62: 5-33.
4. Welle M. Development, significance, and heterogeneity of mast cells with particular regard to the mast cell-specific proteases chymase and tryptase. J Leukoc Biol 1997; 61: 233-45.
5. Franceschini B, Ceva-Grimaldi G, Russo C, Dioguardi N, Grizzi F. The complex functions of mast cells in chronic human liver diseases. Dig Dis Sci 2006; 51: 2248-56.
6. Francis H, Meininger CJ. A review of mast cells and liver disease: What have we learned? Dig Liver Dis 2010; 42: 529-36.
7. Levi-Schaffer F, Pe'er J. Mast cells and angiogenesis. Clin Exp Allergy 2001; 31: 521-4.
8. Bloom W, Fawcett DW. Bloom and Fawcett: A textbook of histology. 12th ed. New York: Chapman & Hall; 1994: 652-68.
9. Senoo H. Structure and function of hepatic stellate cells. Med Electron Microsc 2004; 37: 3-15.
10. Poonkhum R, Watanapokasin R, Pradidarcheep W. Protective effect of alpha-mangostin against type-I collagen formation in thioacetamide-induced cirrhotic rat. J Med Assoc Thai 2012; 95 (Suppl 12): S93-8.
11. Ijzer J, Roskams T, Molenbeek RF, Ultee T, Penning LC, Rothuizen J, et al. Morphological characterisation of portal myofibroblasts and hepatic stellate cells in the normal dog liver. Comp Hepatol 2006; 5: 7.
12. Poonkhum R, Pradidarcheep W, Nilbu-Nga S, Chaunchaiyakul S. Distribution of hepatic myofibroblasts and type I and III collagen in rat liver cirrhosis induced by thioacetamide. Int J Morphol 2011; 29: 501-8.
13. Jeong WI, Do SH, Hong IH, Ji AR, Park JK, Ki MR, et al. Macrophages, myofibroblasts and mast cells in a rat liver infected with Capillaria hepatica. J Vet Sci 2008; 9: 211-3.
14. Santos AB, Tolentino MJr, Andrade ZA. Pathogenesis of hepatic septal fibrosis associated with Capillaria hepatica infection of rats. Rev Soc Bras Med Trop 2001; 34: 503-6.
15. Akiyoshi H, Terada T. Mast cell, myofibroblast and nerve terminal complexes in carbon tetrachloride-induced cirrhotic rat livers. J Hepatol 1998; 29: 112-9.
16. Jeong WI, Lee CS, Park SJ, Chung JY, Jeong KS. Kinetics of macrophages, myofibroblasts and mast cells in carbon tetrachloride-induced rat liver cirrhosis. Anticancer Res 2002; 22: 869-77.
17. Dancygier H. Clinical hepatology: Principles and practice of hepatobiliary diseases. New York: Springer-Verlag Berlin Heidelberg; 2010.
18. Gailit J, Marchese MJ, Kew RR, Gruber BL. The differentiation and function of myofibroblasts is regulated by mast cell mediators. J Invest Dermatol 2001; 117: 1113-9.
19. Grizzi F, Franceschini B, Gagliano N, Moscheni C, Annoni G, Vergani C, et al. Mast cell density, hepatic stellate cell activation and TGF-beta1 transcripts in the aging Sprague-Dawley rat during early acute liver injury. Toxicol Pathol 2003; 31: 173-8.

ความสัมพันธ์ระหว่าง mast cells และ hepatic myofibroblasts ในหนูที่ถูกชักนำให้เกิดตับแข็งด้วยสารไธโออะเซตาไมด์

รักษวรรณ พูนคำ, ศิริภา รอดเนียม, ณัฏฐ์ปวิตร แก้วหนูนวล, เฉง นิลบุหงา, วิสุทธิ์ ประดิษฐ์อาชีพ

วัตถุประสงค์: hepatic myofibroblasts (hepatic MFs) แบ่งออกเป็น 3 กลุ่ม คือ portal/septal MFs; activated hepatic stellate cell myofibroblasts (HSC/MFs); และ interface myofibroblasts (IF/MFs) ใน portal/septal MFs อาศัยอยู่ใน portal triad ส่วน HSC/MFs พบใน space of Disse และ IF/MFs นอนอยู่ที่ขอบของ hepatic lobule ในการทดลองครั้งนี้เพื่อศึกษาความสัมพันธ์ของ mast cell และ hepatic MFs ในหนูที่ทำให้เกิดตับแข็งด้วยสารไธโออะเซตาไมด์

วัสดุและวิธีการ: หนูแบ่งออกเป็น 2 กลุ่ม คือ กลุ่มควบคุมและกลุ่มทำให้เกิดภาวะตับแข็งด้วยการฉีดสารไธโออะเซตาไมด์ 200 มิลลิกรัมต่อกิโลกรัม เป็นเวลาสามครั้งต่อสัปดาห์ต่อเนื่อง 16 สัปดาห์ แล้วนำมาศึกษาด้วยกล้องจุลทรรศน์แสงและกล้องอิเลคตรอนแบบส่องผ่าน

ผลการศึกษา: hepatic MFs สามารถสังเกตได้ที่เนื้อเยื่อเกี่ยวพันของตับแข็ง HSC/MF ฝังตัวอยู่ใน space of Disse ใกล้กับ mast cells ส่วน IF/MFs พบที่ขอบของ hepatic nodules ใกล้ชิดกับ mast cell นอกจากนี้ยังพบ mast cells และ plasma cell เด่นชัดใน portal area ชิดกับหลอดน้ำเหลืองเริ่มต้น

สรุป: mast cell วางตัวใกล้ชิดกับ hepatic MFs และหลอดน้ำเหลืองเริ่มต้นส่งผลให้ mast cells เกี่ยวข้องกับการก่อรูปของคอลลาเจนและการงอกของหลอดน้ำเหลืองในตับแข็งทั้งทางตรงและทางอ้อม