Mast cells play an important regulators of acupoint sensitization via the secretion of tryptase, 5-hydroxytryptamine, and histamine

Ning Ding

Abstract

Forty SD male rats were divided into five groups randomly (n = 8 per group): the normal control (N) group, the normal saline group (NS), the light osteoarthritis group (A), the mild osteoarthritis group (B) and the heavy osteoarthritis group (C). On day 0, the rats in the A, B and C group were injected with 0.3, 1, 3 mg MIA respectively, and rats in the NS group were injected with 50 µl of saline solution.

The tissues at the acupoints Yanglingquan (GB34), Heding (EX-LE2) and Weizhong (BL40) were dissected. The dimensions of each tissue fragment were 1.5 × 1.5 × 1.5 mm. The tissues were fixed in 10% neutral formalin, dehydrated, embedded in paraffin and sectioned with a 4-µm slicer. Subsequently, the sections were dewaxed, dehydrated and stained in 0.5% toluidine blue for 30 min and then washed with tap-water.

the knee joints of 6 rats in each group were fixed in 10% neutral formalin after samples of GB34, EX-LE2 and BL40 were dissected. Then, the knee joints were placed in 5% formic acid for demineralization, dehydrated, embedded in paraffin and sectioned with a 5-µm slicer. Subsequently, the sections were dewaxed, dehydrated, and stained in 1% fast green for 1.5 min and then differentiated by acetic acid. Next, the sections were stained by 0.5% safranine-O for 1.5 min, differentiated by ethanol, and washed with tap-water.

The tissues at GB34, EX-LE2, and BL40 were dissected, fixed in 4% paraformaldehyde for 2.5 h, and dehydrated by 25% sucrose for 2 days. A frozen 10-µm section was sliced by a freezing microtome. For staining, the sections were washed in 0.1 M PB (pH=7.4) and blocked in 0.1 M PB (pH=7.4) containing 3% normal donkey serum and 0.5% Triton X-100 for 30 min. Next, the sections were treated with mouse monoclonal mast cell tryptase antibody and goat polyclonal serotonin antibody and incubated overnight at 4℃. After PB washes, the sections were exposed to Donkey Anti-Mouse IgG Alexa Fluor 488 and Donkey Anti-Goat IgG Alexa Fluor 594 for 2 hours. Subsequently, the sections were washed with PB and stained with DAPI for 6 minutes. After washing with PB, the sections were observed under a confocal laser scanning microscope. Double immunohistochemical staining using the same staining protocol was undertaken, to examine the co-expression of mast cell tryptase and HA.

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Materials

- Normal Donkey Serum 017-000-121 by Contributed by users
  Triton X-100 T8787-50ML by Sigma Aldrich
- 4% paraformaldehyde/1XPBS solution by Contributed by users
  500ml Neutral Buffered Formalin 10% 786-1056 by G-Biosciences
  Fast Green FCF FB0452.SIZE.5g by Bio Basic Inc.
  Formic acid, 88% FC3840.SIZE.500ml by Bio Basic Inc.
  Safranin O SB0815.SIZE.25g by Bio Basic Inc.
  Toluidine blue O TB0962.SIZE.5g by Bio Basic Inc.
  Mono-iodoacetate 14386 by Sigma
  Anti-Mast Cell Tryptase antibody ab2378 by Abcam
  Anti-Serotonin antibody ab66047 by Abcam
- Anti-histamine antibody C67884 by Contributed by users
  Donkey Anti-Goat IgG H&L (Alexa Fluor® 594) ab150136 by Abcam
  Donkey Anti-Rabbit IgG H&L (Alexa Fluor® 594) ab150076 by Abcam
  Donkey Anti-Mouse IgG H&L (Alexa Fluor® 488) ab150105 by Abcam

Protocol

Animal Groups and Interventions

Step 1.

Forty SD male rats were divided into five groups randomly (n = 8 per group): the normal control (N) group, the normal saline group (NS), the light osteoarthritis group (A), the mild osteoarthritis group (B) and the heavy osteoarthritis group (C).

MIA was dissolved in 50μl of sterile saline water. MIA solution was injected into the right knee joint through the infrapatellar ligament. On day 0, the rats in the A, B and C group were injected with 0.3, 1, 3 mg MIA respectively, and rats in the NS group were injected with 50 μl of saline solution. No intervention was carried out in the N group.

Toluidine blue staining

Step 2.

At day 14, 6 rats in each group were killed by an intraperitoneal injection of pentobarbital (150 mg/kg body weight). The tissues at the acupoints Yanglingquan (GB34), Heding (EX-LE2) and Weizhong (BL40) were dissected. The dimensions of each tissue fragment were 1.5 × 1.5 × 1.5 mm.

The tissues were fixed in 10% neutral formalin, dehydrated, embedded in paraffin and sectioned with a 4-μm slicer. Subsequently, the sections were dewaxed, dehydrated and stained in 0.5% toluidine blue for 30 min and then washed with tap-water. After vitrification with dimethylbenzene and neutral balata fixation, the sections were observed under a light microscope (BX53, Olympus

Corporation, Japan), at a 40 × magnification. Six visual fields were randomly collated for each section.

The number of MCs and their degree of degranulation at the subcutaneous connective tissue were recorded. MCs were classified as 'extensively degranulated' (>50% of the cytoplasmic granules exhibiting fusion, staining alterations, and extrusion from the cell), 'moderately degranulated' (10-50% of the granules exhibiting fusion or discharge), or 'normal'.

Safranine-O and Fast Green staining

Step 3.

At day 14, the knee joints of 6 rats in each group were fixed in 10% neutral formalin. Then, the knee joints were placed in 5% formic acid for demineralization, dehydrated, embedded in paraffin and sectioned with a 5-μm slicer. Subsequently, the sections were dewaxed, dehydrated, and stained in 1% fast green for 1.5 min and then differentiated by acetic acid. Next, the sections were stained by 0.5% safranine-O for 1.5 min, differentiated by ethanol, and washed with tap-water. After vitrification with dimethylbenzene and neutral balata fixation, the sections were observed under a light microscope (BX53, Olympus Corporation, Japan), and subjected to a quantitative osteoarthritis cartilage histological analysis based on the method described by Pritzker.

Immunofluorescence staining

Step 4.

At day 14, 2 rats in each group were anesthetized and perfused with 4% paraformaldehyde. The tissues at GB34, EX-LE2, and BL40 were dissected, fixed in 4% paraformaldehyde for 2.5 h, and dehydrated by 25% sucrose for 2 days. A frozen 10-μm section was sliced by a freezing microtome (Leica Corporation, Germany) at -25℃. The primary antibodies included the mouse monoclonal mast cell tryptase antibody (1:100, Abcam, USA), the goat polyclonal 5-hydroxytryptamine antibody (5-HT) (1:100, Abcam, USA), and the rabbit polyclonal histamine (HA) antibody (1:200, LSBio, USA). Donkey Anti-Mouse IgG Alexa Fluor 488 (1:200, Abcam, USA), Donkey Anti-Goat IgG Alexa Fluor 594 (1:200, Abcam, USA), and Donkey Anti-rabbit IgG Alexa Fluor 594 (1:200, Abcam, USA) were used as corresponding secondary antibodies. The solution 4’,6-diamidino-2-phenylindole dihydrochloride (DAPI, Solarbio, Beijing, China) was applied for counterstaining. For staining, the sections were washed in 0.1 M PB (pH=7.4) and blocked in 0.1 M PB (pH=7.4) containing 3% normal donkey serum and 0.5% Triton X-100 for 30 min. Next, the sections were treated with mouse monoclonal mast cell tryptase antibody and goat polyclonal serotonin antibody and incubated overnight at 4℃. After PB washes, the sections were exposed to Donkey Anti-Mouse IgG Alexa Fluor 488 and Donkey Anti-Goat IgG Alexa Fluor 594 for 2 hours. Subsequently, the sections were washed with PB and stained with DAPI for 6 minutes.

After washing with PB, the sections were observed under a confocal laser scanning microscope (FV1000, Olympus Corporation, Japan). Double immunohistochemical staining using the same staining protocol was undertaken, to examine the co-expression of mast cell tryptase and HA.