Life Sciences 170 (2017) 64–71

Contents lists available at ScienceDirect

Life Sciences

journal homepage: www.elsevier.com/locate/lifescie

Astrocyte contributes to pain development via MMP2-JNK1/2 signaling in a mouse model of complex regional pain syndrome

Guogang Tian [a,b], Xin Luo [c,f], Chaoliang Tang [c,f], Xiang Cheng [b], Sookja Kim Chung [d,e,f], Zhengyuan Xia [c], Chi Wai Cheung [c,e,f,*], Qulian Guo [a,**]

[a] Department of Anesthesiology, Xiangya Hospital of Central South University, Changsha, China
[b] Department of Anesthesiology and Pain Medicine, Affiliated Haikou Hospital of Xiangya Medical School, Central South University, Haikou, China
[c] Department of Anaesthesiology, The University of Hong Kong, HKSAR, China
[d] Department of Anatomy, The University of Hong Kong, HKSAR, China
[e] Research Center of Heart, Brain, Hormone and Healthy Aging, The University of Hong Kong, HKSAR, China
[f] Laboratory and Clinical Research Institute for Pain, The University of Hong Kong, HKSAR, China

ARTICLE INFO

Article history:
Received 18 October 2016
Received in revised form 23 November 2016
Accepted 30 November 2016
Available online 3 December 2016

Keywords:
Chronic post-ischemia pain
Spinal cord
Astrocyte
c-jun N-terminal kinase
Matrix metalloproteinase-2

ABSTRACT

Background: The activation of spinal glial cells (astrocyte and microglia) is reported in patient with complex regional pain syndrome (CRPS). However, the roles of spinal glial activities in the pathophysiology of CRPS are unclear. Here, we explored the roles of spinal astrocyte and microglia and the molecular mechanisms underlying CRPS using a mouse model of chronic post-ischemia pain (CPIP).

Results: CPIP injury increased the level of glial fibrillary acidic protein (GFAP, reactive astrocyte biomarker), but had no significant impact on ionized calcium binding adaptor molecule 1 (IBA1, reactive microglia biomarker), in the ipsilateral dorsal horn on post-injury day (PID) 3 when the pain threshold started to reduce significantly. Astrocytic inhibition with fluorocitrate but not microglial inhibition with minocycline attenuated the development of allodynia in CPIP-injured mice, which was concomitant with increased spinal levels of phosphorylated c-jun N-terminal kinase 1/2 (pJNK1/2) on PID 3. Furthermore, the intrathecal administration of SP600125 (JNK inhibitor) prevented the development of allodynia in CPIP-injured mice. Double immunofluorescence staining showed that pJNK1/2 was mainly co-localized with GFAP. Subsequently, increased levels of pJNK1/2 were reversed by intrathecal fluorocitrate. Furthermore, the level of spinal matrix metalloproteinase-2 (MMP2) was increased and mainly expressed in NeuN (neuron biomarker) on PID 3 in the CPIP-injured mice, while intrathecal APR 100 (MMP2 inhibitor) delayed the development of allodynia and decreased spinal levels of GFAP and pJNK1/2 on PID 3.

Conclusion: This study shows that activation of astrocyte MMP2/JNK1/2 signaling pathway contributes to the pathogenesis of pain hypersensitivity in the CPIP model.

1. Introduction

Complex regional pain syndrome (CRPS) is a multifactorial pain disorder, with syndromes similar to the abnormalities in the somatosensory, autonomy and motor system, and the aetiology and pathogenesis of CRPS remains largely unknown. CRPS are classified into two subtypes: type-I without a loss in the major nerve and type-II with nerve lesion [5]. The chronic post-ischemia pain (CPIP) model is an animal model developed for the need of CRPS study [7]. In this model, the injury caused early hyperemia and edema followed by chronic pain, which mimicked two essential features of CRPS-I in human. Therefore, the CPIP model has now been used as a valuable approach to explore the pathophysiology of CRPS [7]. Using this model, it is suggested that the central mechanisms might contribute to the pathogenesis of CPIP [8,20,31].

Increasing body of studies indicates that glial cells are involved in the abnormal pain perception in animals under different pathological conditions [6], and in the patients suffering from chronic pain [22]. Damage to the tissue or the nerve causes the change of glial cells from "normal" status to "reactive" status. Subsequently, these reactive glial cells synthesize and release cytokines and chemokines, which contribute to the neuroinflammation and central mechanisms of pathological pain [6,25]. Previously, it has been shown that the levels of proinflammatory cytokines (like IL-1β and IL-6) in the cerebrospinal fluid (CSF) were enhanced in patients with CRPS [1,2], which implicated the involvement of

* Correspondence to: C.W. Cheung, Department of Anaesthesiology, The University of Hong Kong, Room 424, 4/F, Block K, Queen Mary Hospital, 102 Pokfulam, Hong Kong.
** Correspondence to: Q. Guo, Department of Anesthesiology, Xiangya Hospital, Central South University, 87 Xiangya Road, Changsha City, 410008, Hunan Province, China.
E-mail addresses: cheucw@hku.hk (C.W. Cheung), qulianguo2011@163.com (Q. Guo).

http://dx.doi.org/10.1016/j.lfs.2016.11.030
0024-3205/

glia-related neuroinflammation in the pathophysiology of CRPS. Recently, Valle et al. reported that spinal astrocyte and microglia were activated in a patient with CRPS by detecting the glial markers in the autopsy materials [9]. However, the roles of glial activation in the central mechanisms of CRPS are unclear, which might limit the development of the glia-targeted therapy for CRPS in patients.

Recently, we found that chemokine axis CXCL12/CXCR4 axis contributes to the development of pathological pain, including CPIP [24,25]. In mice CPIP model, it was found that spinal levels of CXCL12 and glial fibrillary acidic protein (GFAP) were increased and co-localized on post-operative day (PID) 3, implicating that astrocyte-dependent CXCL12 might play an important role in the early stage of CPIP pathology [23]. Therefore, it is highly possible that astrocyte might contribute to the development of CPIP. As the roles and mechanisms of glial activation in the pathogenesis of CPIP, potentially CRPS in human, remain largely unclear, the goal of this study is to testify this hypothesis using mice CPIP model.

2. Materials and methods

2.1. Animals

Animal experiments were approved by the Committee on the Use of Live Animals in Teaching and Research (CULATR) (permit No. 2610-11) and performed following the guidelines for the care and use of laboratory animals as established by the Laboratory Animal Unit (LAU) at the University of Hong Kong. In this study, adult male C57BL/6 wild-type mice (28–30 g) were used. Mice were housed at 23 ± 3 °C, with a 12-hour light/12-hour dark cycle (lights on at 07:00) and the humidity (25%–45%). Animals were offered free access to water and food (Lab Diet 5012 (0.5% phosphorus, 1.0% calcium and 3.3 IU/g of vitamin D3)).

2.2. CPIP model

The CPIP injury produced swelling and mechanical allodynia in the hindpaw, mimicking the clinical characters in patients with CRPS type-I [7,27]. Animals were anesthetized with gaseous isoflurane and O2. Durometer O-rings (O-rings West) were placed around right hindlimb to produce ischemia in mice. Three hours later, the rings were removed to initiate reperfusion.

2.3. Hindpaw volume

In this study, the volume of mice hindpaw was measured with the U-shaped volume sensor (IITC) following the manufacturer's protocol.

2.4. Study drugs

Fluorocitrate (2 μg per day, Sigma #F9634), minocycline (10 μg per day, Sigma #M9511), SP600125 (5 μg per day, Sigma #S5567) and APR 100 (10 μg per day, Santa Cruz #203522) were freshly prepared in 1% Dimethyl Sulphoxide (DMSO) (diluted in saline solution) on the day of the experiment. We took DMSO (1%, diluted in saline solution) as the vehicle in this study.

2.5. Intrathecal injection

In this study, the single intrathecal injection was performed daily at 1 h before the operation and up to PID 3. Animals were anesthetized by inhalation anesthesia with isoflurane and O_2. A microliter syringe (Hamilton) with a 30-gauge needle (BD) was applied to make a spinal cord puncture. A total volume of 5 μl of drug(s) was delivered to the subarachnoid space between the L3 and L5 lumbar spinal cord. Successful injection was indicated by a tail configuration of the "S" type or tail swinging immediately following the administration.

2.6. von Frey test

The paw withdrawal threshold (PWT) of mice was assessed by von Frey test, and the protocol was mentioned in our previous research [24]. Animals were placed on a metal mesh floor with a transparent plastic dome for nearly 30 min before the experiment. During the experiment, a series of von Frey filaments (IITC) was applied to the plantar surface of mice hindpaws. When the force was sufficient to bend the filaments into an "S" shape and the mouse withdrew the hind paw from the filament, displayed value of this force was taken as the PWT of mice.

2.7. Immunohistochemistry

Mice were deeply anesthetized with sodium pentobarbital and perfused with the phosphate buffered saline (PBS), followed by 4% paraformaldehyde (PFA) in 0.1 M PBS via the cardiovascular system. L3–L5 lumbar spinal cords were collected from these mice and post-fixed in 4% PFA. Samples were dehydrated in 25% sucrose at 4 °C overnight. Frozen samples in tissue freezing medium (Jung) were sliced longitudinally at 15 μm by a cryostat (Leica). Then sections were blocked by 4% goat serum in PBS containing 0.1% Triton X-100 (PBST) at room temperature for 2 h and incubated with antibody(s) against GFAP (1:250, Abcam, #10062), ionized calcium binding adaptor molecule 1 (IBA1, 1:100, Abcam, #15690), NeuN (1:200, Abcam, #177487), phosphorylated c-jun N-terminal kinase (JNK) (1:50, Cell signaling, #4668) and/or matrix metalloproteinase-2 (MMP2, 1:200, Abcam, #37150) at 4 °C overnight. Then, sections were washed by PBS and incubated with secondary antibody conjugated with Goat Anti-Rabbit IgG H&L (Alexa Fluor 488) (1:1000, Abcam, #150077) and/or Donkey Anti-Mouse IgG H&L (Alexa Fluor 568) (1:1000, Abcam, #150077) for 2 h at room temperature. DAPI (Vector) was used to stain nuclear in sample sections. The immunoreactivity in these sections was captured with the confocal scanning microscope LSM 700 and LSM 710 (Zeiss), and the immunofluorescent images were analyzed by Image-Pro Plus (Media Cybernetics).

2.8. Western blotting

Animals were euthanized with pentobarbital before the sample harvest. Ipsilateral L3–L5 lumbar spinal cord was quickly removed and homogenized in ice-cold RIPA lysis buffer. Protein samples were prepared following our previous protocol [24]. These samples were separated in 10% SDS-PAGE and transferred to PVDF membranes (Bio-Rad). The protein samples on the PVDF membrane were incubated with first antibodies against GFAP (1:2500, Abcam, #10062), IBA1 (1:2500, Abcam, #15690), phosphorylated JNK (1:1000, Cell signaling, #4668), total JNK (1:3000, Cell signaling, #9258), and GADPH (1:10,000, Sigma, #G9545) as a loading control. The expression levels of targets were visualized with horseradish peroxidase (HRP)-conjugated secondary antibodies (anti-mouse IgG, HRP-linked (1:2000, Cell signaling, #7076), Anti-rat IgG, HRP-linked (1:2000, Cell signaling, #7074)) and followed by the exposure to X-ray films (Kodak, USA). Gray scale of all bands in the scanned images (films) was determined by ImageJ software (NIH, USA) for the analysis.

2.9. Statistical analysis

The data were expressed as means ± SEM. The results from the immunohistochemical work and Western blotting test were analyzed with *t*-test. The results from the behavioral test were analyzed with two-way ANOVA. In all cases, $p < 0.05$ was considered statistically significant.