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Attenuation of inflammatory pain by puerarin in animal model of inflammation through inhibition of pro-inflammatory mediators

Muhammad Zia Ullah[a,1], Ashraf Ullah Khan[a,1], Ruqayya Afridi[a], Hina Rasheed[a], Sidra Khalid[a], Muhammad Naveed[a], Hussain Ali[a], Yeong Shik Kim[b,*], Salman Khan[a,b,*]

[a] Department of Pharmacy, Faculty of Biological Sciences, Quaid-i-Azam University, Islamabad, Pakistan
[b] College of Pharmacy, Natural Products Research Institute, Seoul National University, Seoul, Republic of Korea

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ABSTRACT

In the current study, the puerarin was investigated for both acute Carrageenan and chronic CFA-induced inflammatory pain models. The Puerarin treatment significantly attenuated ($P < 0.001$) the mechanical hyperalgesia and mechanical allodynia in both Carrageenan and CFA-induced hyperalgesia. The Puerarin treatment also remarkably reduced ($p < 0.001$) the thermal hyperalgesic responses in both acute Carrageenan as well as chronic CFA-induced models. Furthermore, the Puerarin administration was also associated with significant inhibition of ($p < 0.001$) paw edema in both Carrageenan and CFA-induced models. The inflammatory mediators such as IL-1β, IL-6, TNF-α and vascular endothelial growth factor (VEGF) are significantly enhanced during inflammatory conditions, however, the Puerarin administration significantly altered ($P < 0.001$) the mRNA expression levels of these mediators. Additionally, the Puerarin treatment also significantly enhanced ($P < 0.001$) the mRNA expressions levels of the anti-oxidant enzymes such as Nrf2, HO-1 and SOD2. The Puerarin treatment is associated with significant ($P < 0.001$) inhibition of the acetic acid-induced Evans blue vascular permeability. Moreover, the concentration of Puerarin in various tissues was analyzed using High-performance liquid chromatography (HPLC) and the results showed that the Puerarin was significantly distributed towards the peripheral tissues such as liver and kidney and less distributed towards the brain.

1. Introduction

Inflammation is the critical function of the innate immune system that serves to initiate specific protective response against the invading pathogens and confer immunity against the offending agent [1, 2]. The process of inflammation is triggered by physical, chemical, microbial agents, antigen-antibody reactions and characterized by tissue swelling, redness and pain [1, 3–5]. Pain serves to alert an individual against the actual or potential danger. Inflammatory pain is triggered by variety of mediators released during the process of inflammation causing the sensitization of nociceptors [3–5]. The release of anti-inflammatory and pro-inflammatory mediators regulate the intensity of hyperalgesic response [3]. Primary nociceptors activation is the common manifestation of all types of inflammatory pain leading to the state of increased responsiveness to a stimuli that is noxious in nature called as hyperalgesia and increased sensitivity to stimuli that is not noxious in nature called as allodynia [3, 4, 6].

The inflammatory pain is directly related with the level of pro-inflammatory cytokines. The inflammatory cytokines expression is induced by the interaction of LPS with TLR, following this interaction TLR trigger the activation of MyD88 dependent pathway and MyD88 independent pathway to induce the activation of NF-κB, MAPKs, PI3K/Akt signaling cascades [7, 8]. The most important inflammatory cytokines implicated in inflammatory pain are TNF-α, IL-1β and IL-6 causing the sensitization of nociceptors [4, 7–9]. Vascular endothelial growth factor (VEGF) have been implicated in cell migration, vascular permeability and several painful conditions such as rheumatoid arthritis and neuropathy [10]. Similarly, the vascular permeability is an important manifestation of inflammatory process associated with tissue swelling, redness and pain [10].

The various strategies employed to deal with inflammatory pain conditions such as non-steroidal inflammatory drugs (NSAIDs) (Aspirin,

Abbreviations: CFA, Complete Freud's adjuvant; DMSO, Dimethyl Sulfoxide; NF-κB, Nuclear factor-κB; IL, Interleukins; SOD2, Sulphur oxide dismutase; Nrf2, Nuclear factor erythroid 2 (NF-E2); TNF-α, Tumor necrosis factor alpha; NO, Nitric oxide; VEGF, Vascular endothelial growth factor; NSAID's, Non-steroidal anti-inflammatory drugs; CNS, Central nervous system; HPLC, High Performance Liquid Chromatography

* Corresponding authors.
E-mail addresses: kims@snu.ac.kr (Y.S. Kim), skhan@qau.edu.pk (S. Khan).
[1] These authors contributed equally to this work.

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Diclofenac sodium, and Piroxicam), Opioids (morphine) and analgesic adjuvants (Gabapentin) [4, 6]. However, these therapeutic strategies are associated with various intractable side effects such as prolonged use of NSAIDs causes the GIT (gastrointestinal tract) irritation, while chronic Opioids use are associated with the tolerance and dependence [4, 6]. Thus, to develop a new and novel anti-inflammatory drug associated with less side effects and more effective in nature, the researchers have turn their attention towards the natural products [4, 6].

Natural products offer important source of new drug development against various pathological conditions. Puerarin is a natural compound isolated from *Pueraria lobata* (Fabaceae) and have been tried for the treatment of ischemic condition, hyperglycemia, apoptosis, anti-oxidant, anti-platelets aggregation and to dilate blood vessels [11]. In the current study the potential analgesic role of Puerarin was explored in mice model of inflammatory pain.

2. Material and methods

2.1. Chemicals and reagents

The various chemicals used in this study includes complete Freud's adjuvant (CFA), Dexamethasone (Shaigan pharmaceuticals, Pakistan), acetic acid (Shaigan pharmaceuticals, Pakistan), Evans blue dye (Shaigan Pharmaceuticals, Pakistan) and Puerarin. All the drugs were dissolved in 2% DMSO and 0.9% sodium chloride solution. The Puerarin is an isoflavonoid in isolated from the roots of *Pueraria lobata* and belongs to the family of Fabaceae.

2.2. Animals

Male albino mice having age of 4–5 weeks and weight of 25–30 g were used in the current study. All animal activities were carried out in pathogen free zone of Quaid-i-Azam University, Islamabad, following procedures outlined in guideline for the care and use of laboratory animal Quaid-i-Azam University under the approval of Bio-Ethical Committee Code (BEC-FBS-QAU2017-5). Animals were housed in stainless steel cages at standard conditions of 23 ± 0.5 °C and humidity of 10% in 12 h light-dark cycle. Animals were acclimated for one week before study and fed with standard diet and water. All animal activities were performed following procedures of “Principles of Lab Animal Care” from NIH publications and previously specified guidelines for investigation of pain in conscious animals [12]. All the experimental animals were used once and all the activities were performed from 8:00 am to 6:00 pm. During this study animals were divided into four groups such as Vehicle control treated with normal saline dissolved in 2% DMSO, Negative control treated only with CFA, Positive control group was subjected to the treatment with Dexamethasone dissolved in 2% DMSO (5 mg/kg) and the treatment group received Puerarin dissolved in 2% DMSO (50 mg/kg). All the groups except Vehicle control was subjected to intraplantar (i.pl) CFA injection, while, normal saline, dexamethasone and Puerarin was administered intraperitoneal (i.p).

2.3. Behavioral experiments

2.3.1. Measurement of mechanical hyperalgesia induced by carrageenan and CFA

The Puerarin was investigated against both acute Carrageenan and chronic CFA-induced mechanical hyperalgesia using Randall Selitto (Digital paw pressure Randall-Selitto meter, IITC Life Science Inc., Woodland Hills, CA) according to the previously reported methods [13]. In acute Carrageenan-induced hyperalgesia all the animals were divided into six groups such as normal control (2% DMSO), negative control was administered with Carrageenan only (100 µg/paw), positive control (dexamethasone 5 mg/kg) and the Puerarin was administered at the dose of 5 mg/kg, 25 mg/kg, and 50 mg/kg. The Carrageenan (100 µg/paw) was administered into the right hind paw 1 h before the administration of drugs. The mechanical hyperalgesia was evaluated 4 h after the administration of Carrageenan.

Similarly, during chronic CFA-induced model all the animals were divided into four groups such as normal control (2% DMSO), negative control (administered with CFA only 20 µl/paw), positive control (dexamethasone 5 mg/kg), and Puerarin (50 mg/kg). Forty min prior to the administration of different drugs, CFA (20 µl/paw) was injected into right hind paw. Following the administration of CFA, mechanical hyperalgesia was evaluated using digital Randall Sellito. Before measuring the mechanical hyperalgesia individual animals were restrained in restrainer for acclimatization. The tip of the apparatus was applied directly to the middle portion of the hind paw with gradual increase of pressure. Application of pressure was stopped upon paw withdrawal reflex and final reading was recorded which was displayed on the screen of apparatus. The CFA-induced acute effect of Puerarin (50 mg/kg) was evaluated at the interval of 2, 4 and 6 h. however, to explore the chronic effect of Puerarin (50 mg/kg) the data reading were recorded from day 0–6, 4 h after the administration of drugs. Three readings were taken for each animal and average was noted. In order to assess the tolerance effect of Puerarin, the dose was skipped at day five as described previously [4].

2.3.2. Measurement of acute carrageenan and chronic CFA-induced mechanical allodynia

Mechanical allodynia was assessed using Von Frey filaments (Stoelting, USA) according to the previously described method [14]. Mice were placed in clear plastic chamber on a stainless steel mesh floor and allowed to acclimatize. Von Frey filament was applied on the plantar surface of the right hind paw with gradual increase in the applied force. The strength of the filaments in the series that evoked at least three positive responses among the five trials was designated the pain threshold. The Puerarin was evaluated against both acute Carrageenan and chronic CFA-induced mechanical allodynia as described in above. In order to investigate the acute effect of Puerarin against the Carrageenan-induced allodynia all the animals were divided into 6 groups such as normal control (2% DMSO), negative control (100 µg/paw), positive control (dexamethasone 5 mg/kg), Puerarin (5 mg/kg, 25 mg/kg and 50 mg/kg). The Carrageenan was administered into the right hind paw 1 h after the administration of drugs. The mechanical allodynia was investigated 4 h after the administration of Carrageenan. Similarly, Puerarin (50 mg/kg) was also evaluated against the CFA-induced mechanical allodynia. The CFA-induced acute effect was determined 2 h after the administration of CFA for 6 h at the interval of 2 h, while, to explore the chronic effect the mechanical allodynia was measured for 6 days. In order to assess the tolerance effect, the dose was skipped at day 5 as described previously [4].

2.3.3. Evaluation of thermal hyperalgesia

2.3.3.1. Hot plate test. In order to assess the Puerarin (50 mg/kg) mediated analgesic activity against the CFA-induced thermal hyperalgesia using hot plate. The temperature of the hot plate was adjusted at 55–60 °C. Animal were placed on hot plate one by one and, paw withdrawal or paw licking response was observed. The time taken to evoke such reflexes was noted and designated as paw withdrawal latency. The Cut off time for heat stimulation was 60 s [15].

2.3.3.2. Cold acetone test. Puerarin (50 mg/kg) was also investigated against CFA-induced acetone test to assess the cold pain. Animals from each group were placed on metal mesh floor of the small Plexiglas cubicle containers one by one. Acetone was applied drop wise to the plantar surface of the hind paw of mice. The time taken by animal while paw licking or withdrawal, was observed for both acute and chronic pain. The Cut off time for the paw licking response was assigned 60 s [16].