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N-(2-hydroxy phenyl) acetamide produces profound inhibition of c-Fos protein and mRNA expression in the brain of adjuvant-induced arthritic rats

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Abstract Chronic pain and cognitive decline are characteristic symptoms of rheumatoid arthritis. One of the immediate early gene c-*fos* is overexpressed during peripheral and central noxious conditions and can be used as a marker for neuronal activity/excitability. In the adjuvant-induced arthritis Sprague–Dawley rat model, we examined the dynamics of c-Fos protein and mRNA expression in the amygdala, cortex, hippocampus, and thalamus and evaluated the effects of *N*-(2-hydroxy phenyl) acetamide (NA-2), a derivative of salicylic acid. The paw volume was assessed as an indicator of peripheral edema and the hyperalgesia associated with arthritis was monitored by gait analysis. The region of interests of the brain from arthritic and non-arthritic animals were used to isolate the RNA and were then reverse transcribed into cDNA. The PCR products were electrophoresed on 1 % agarose gel and the gels were visualized in gel-doc system. The frozen brain sections were stained for c-Fos using immunohistochemistry. Negative control experiments were performed without the primary and secondary antibodies to rule out the non-specific tissue binding of antibodies. We report a significant increase in the c-Fos expression in the arthritic control animals. In comparison to the control group, the treatment of NA-2 treatment was found to block the development of the arthritis-induced c-Fos protein and mRNA expression and peripheral edema. It also significantly reduces the gait deficits which were otherwise observed in the arthritic control group. Both the immunohistochemistry and PCR analysis revealed NA-2 to be more potent in comparison to member of non-steroidal anti-inflammatory drug.

Keywords Anti-inflammatory · Anti-arthritic activity · Gait behaviors · Immunohistochemistry

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Introduction

Rheumatoid arthritis (RA) is an autoimmune disease characterized by the inflammation, tissue injury, pain hypersensitivity, and proliferation of synovial cells causing bone and cartilage destruction [1, 2]. In extreme conditions it also results in a progressive disability due to inflamed joints [3]. During tissue inflammation, central and peripheral sensitizations lead to pain hypersensitivity [4, 5]. It is believe that in the process of central sensitization any noxious stimuli that provokes the Ca^{2+} influx can trigger the expression of immediate early genes (IEGs) in neuronal cells [6, 7] and regulates the long-term changes in cellular phenotype and function including adaptation, plasticity, learning, and memory [8–10]. It has been reported that during peripheral and central noxious conditions, c-*fos* which is one of the IEG is overexpressed and can be used as a marker for neuronal activity/excitability [11–13]. Furthermore, product of c-*fos* gene i.e., c-Fos along with c-Jun protein act as a transcriptional factor activating protein-1 (AP-1) to regulate the expression of downstream genes possessing AP-1-binding sites such as BDNF, kainic

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