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Clavulanic acid exhibits anti-inflammatory effects on carrageenan-induced paw edema model of inflammation in rats

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ABSTRACT

The present study was designed to evaluate the anti-inflammatory effects of clavulanic acid, as a GCP-II inhibitor on carrageenan-induced paw edema in rats. Clavulanic acid was administered intraperitoneally (100, 200 or 300 mg/kg) 20 min before the subplantar injection of the carrageenan. Neutrophil infiltration (MPO activity), lipid peroxidation (MDA assay), IL1β, TNF-α and PGE_2 levels were assessed in the paw tissue of clavulanic acid treated rats compared with the control rats. Results showed that intraperitoneal administration of clavulanic acid (200 or 300 mg/kg) significantly reduced maximum paw volume by 54.51% and 59.81% and total inflammatory response (AUC) by 53.17% and 59.13% in comparison with the control group, four hours after induction of inflammation ($p<0.001$). Intraperitoneal injection of clavulanic acid (100 or 200 mg/kg) resulted in a marked reduction of MPO activity in the inflamed paw by 79% and 82% respectively in comparison with the control group ($p<0.01$, $p<0.001$). Moreover, intraperitoneal clavulanic acid (200 mg/kg) reduced the MDA levels by 53.22% in comparison with the control group ($p<0.001$). The levels of IL-1β and PGE_2 in the intraperitoneal clavulanic acid (300 mg/kg) treated rats, were reduced by 61% and 13% respectively in comparison with the control group ($p<0.05$). Overall we suggest that the anti-inflammatory properties of intraperitoneal clavulanic acid could be related to its inhibitory effect on GCP-II activity results in elevating NAAG level and reducing glutamate level increase.

Key words: glutamate, NAAG, inflammation, carrageenan, cytokines

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INTRODUCTION

Glutamate is an important excitatory neurotransmitter in central and peripheral nervous systems of mammals [1]. In addition to using glutamate as a neurotransmitter at central synapses, many primary sensory neurons release glutamate from peripheral terminals. Vesicular glutamate transporters fill neurotransmitter vesicles with glutamate and they are transferred to peripheral terminals. Intense tissue damage or pernicious stimuli cause glutamate release from the peripheral afferent nerve terminals and excessive glutamate release occurs during acute and chronic inflammation [2,3]. The peripheral nerve terminals contain both ionotropic and metabotropic glutamate receptors and activation of these receptors can increase the excitability of primary afferents. Physical trauma or anoxia

increases glutamate levels in peripheral sites of the affected tissues [3]. Interaction of glutamate with G-protein-coupled metabotropic glutamate receptors and NMDA-gated Ca^{2+} channels results in activation of COX-2 and generation of prostaglandin E_2 [4]. *N*-acetylaspartylglutamate (NAAG), which is widely distributed in the central and peripheral nervous systems of mammals, is co-released with glutamate or other small amine transmitters and prevents excessive glutamate release by activating presynaptic mGlu3 receptors [5,6]. These receptors are located on astrocytes and presynaptic axons in the peripheral nerve terminals [6]. NAAG activation of group II mGluRs reduces cyclic-adenosine monophosphate (cAMP) and cyclic-guanosine monophosphate (cGMP) levels in neurons and glia. Conversely, prostaglandin E_2, a peripheral mediator of inflammation, increases sensory neuron response by elevating cAMP levels. Consistent with this, group II mGluR agonists reduce cAMP and prostaglandin E_2-mediated sensitization of sensory neurons [6-9].

NAAG has been cleaved by GCP-II (NAALADase) to release N-acetyl-aspartate and glutamate both *in vitro* and *in vivo* [10]. Inhibition of GCP-II by some novel compounds has been shown to protect NAAG from breakdown, increase the level of NAAG and reduce glutamate level increase. These events cause considerable therapeutic effects in animal inflammatory pain and nervous disorder models [10-13]. The first potent GCP-II inhibitor, 2-(phosphonomethyl) pentanedioic acid (2-PMPA), has been shown to increase NAAG and attenuate glutamate increase [10]. These results suggest the potential for development of GCP-II inhibition as a novel pharmacological approach to the treatment of inflammatory disorders.

Clavulanic acid is a β-lactamase inhibitor isolated from *Streptomyces Clavuligerus* [14-16].
In spite of possessing β-lactam ring in the structure of clavulanic acid that is a characteristic of beta-lactam antibiotics, it has no significant intrinsic antimicrobial activity. The resemblance of chemical structure in clavulanic acid and beta-lactam antibiotics allows the interaction of clavulanic acid with the enzyme β-lactamase that is secreted by certain bacteria [14,15,17].

Previous studies confirm that there are tertiary structural similarities between β-lactamase and D-alanyl-D-alanin carboxypeptidase-transpeptidase [18-22]. Because of such structural similarity, β-lactamase inhibitors can inhibit carboxypeptidase in that manner in mammals. As previous studies confirmed contribution of glutamate in inflammatory responses, the present investigation was based on the assumption that GCP-II could be inhibited by β-lactamase inhibitors to exhibit anti-inflammatory effects by reducing the level of glutamate in peripheral and central nervous systems [3,4,8,23,24]. We suggested that clavulanic acid known for its activity as a β-lactamase inhibitor was also an inhibitor of mammalian GCP-II (NAALADase) activity and reduced the level of glutamate in peripheral and central nervous systems.

Since glutamate contributes to the inflammatory processes and NAAG reduces the level of glutamate in the peripheral and central nervous systems by acting at mGluR3, we aimed at evaluating the protective effects of clavulanic acid in a rat model of acute local inflammation (carrageenan-induced paw edema). If GCP-II is involved in regulating inflammatory mediator transmission in the peripheral afferent nerve fibers, intraperitoneal injection of clavulanic acid may have some effects on the carrageenan-induced inflammatory responses. We measured the levels of the inflammatory and biochemical factors including myeloperoxidase (MPO), malondialdehyde (MDA), cytokines (IL-1β and TNF-α) and PGE_2 in paw tissue of the rats. NAAG was used as a reference drug and the effect of clavulanic acid on the inflammatory and biochemichal factors in the carrageenan induced rat paw edema model was compared to that of NAAG in the paw tissue of rats.

MATERIALS AND METHODS

Animals

Male Wistar rats (180-200 g) were used in this study. The animals were given food and water *ad libitum*. They were housed in the Animal House of the Tabriz University of Medical Sciences at a controlled ambient temperature of 25±2°C and a 12-h light/12-h dark cycle. The present study was performed in accordance with the Guide for the Care and Use of Laboratory Animals of Tabriz University of Medical Sciences, Tabriz-Iran.

Carrageenan-induced paw edema

Rats were randomized into 5 groups of control and treatment consisting of 6 rats each. The animals in the control group received an intraperitoneal injection of 500 µl normal saline and the animals in the treatment groups received an intraperitoneal injection of 500 µl NAAG solution in saline (10mg/kg) or 500 µl potassium clavulanate solution

in saline (100, 200 or 300 mg/kg) 20 minutes before subplantar injection of 100 µl carrageenan 1% (w/v) in the right hind paw [25]. The volume of the paw was measured by plethysmometer immediately prior to the carrageenan injection and then at hourly intervals from 1 to 4 hours afterward. Data were expressed as a percentage of increase in the paw volume and compared with those of pre-injection values. After measurement of the paw edema, the rats were sacrificed by an overdose of pentobarbital and then the inflamed hind paws were excised with a guillotine.

Myeloperoxidase (MPO) activity

Carrageenan edema was induced as described before. The rats were sacrificed 4 hours later by an overdose of pentobarbital and the inflamed hind paws were excised with a guillotine. Myeloperoxidase (MPO) activity was assayed according to the method of Bradley et al. [26]. The inflamed paw tissues were finely chopped in 1 ml of 50 mM potassium phosphate buffer (pH=6), containing 0.5% hexa-decyl-trimethyl-ammonium-bromide (HTAB). The chopped tissues were homogenized (50 mg/ml) in phosphate buffer (50 mM) (pH=6) containing 0.5% hexa-decyl-trimethyl-ammonium-bromide (HTAB) for 5×45 s at 1 min intervals at 8500 rpm. The homogenates were sonicated for 10 seconds, frozen and thawed 3 times, then sonicated for further 10 seconds and centrifuged at 3000 rpm, in 5°C for 30 min. The supernatant (100 µl) was added to 2.9 ml of phosphate buffer (50 mM; pH=6) containing 0.167 mg/ml of O-dianisidine dihydrochloride and 0.0005% hydrogen peroxide. Five minutes later the reaction was stopped by adding 0.1 ml of 1.2 M hydrochloric acid. The absorbance was measured spectrophotometrically at 400 nm. The concentrations were calculated by using calibration curve and expressed as miliunits of MPO in 100 mg weight of wet tissue (mU/100 mg).

Malondialdehyde (MDA) assay

Carrageenan edema was induced as described before. The rats were sacrificed 4 hours later by an overdose of pentobarbital and the inflamed hind paws were excised with a guillotine. Lipid peroxidation in the rat paw tissues was quantified by determination of the MDA levels according to the method of Olgen et al. [27]. The tissues were homogenized in 1.15% KCl to achieve a 10% (W/V) homogenate. The homogenates were centrifuged and 1 ml of each supernatant was added to a mixture containing 3 ml of O-phosphorous acid (1%) and 1 ml of thiobarbituric acid (TBA; 0.67%) in an aqueous solution. The reaction mixture was heated for 60 min up to 95°C, and then was cooled in a room temperature. Then, 3 ml of n-butanol was added to each test tube, and the tubes were shaken vigorously and then centrifuged. The absorbance of n-butanol phase was measured spectrophotometrically at 532 nm and the amount of thiobarbituric acid reactant substances (TBARS) was calculated from a calibration curve and reported as nmol MDA/100 mg tissue.

Determination of IL-1β and TNF-*α* and PGE_2 levels in rat paw tissue

The rat paw tissues were collected 4 hours after inducing the inflammation by carrageenan and homogenized (1g/4ml) in extraction buffer containing 1mM of phenylmethylsulfonyl fluoride, 1 µg/ml of aprotinin and 0.05% Tween-20 in phosphate-buffered saline (PBS). For measurement of PGE_2, the paw tissues were added to a lysis solution (1g/4ml) containing 80% methanol, 20% saline and 1mM indomethacin. Tissues were homogenized on ice with a polytron and centrifuged at 5000 x g for 15 min. The supernatants were stored at –80°C until analysis. IL-1β and TNF-α level in the supernatants was determined [28], using ELISA kits specific for rat IL-1β and TNF-α (eBioscience, USA). PGE_2 level was determined using a PGE_2 EIA kit (Cayman Chemical Company, USA). The sensitivities of the assays for IL-1β, TNF-α and PGE_2 were 31, 39.1 and 7.8 pg/ml, respectively.

Statistical Evaluation

Data were presented as mean± standard error of the mean (SEM). Statistical comparisons were carried out using a one-way analysis of variance (ANOVA) followed by the Tukey post-hoc test for multiple comparisons in order to determine statistical significance ($p < 0.05$) between treatments and control groups.

RESULTS

Effects of clavulanic acid on carrageenan-induced paw edema

The protocol involved intraperitoneal injection of 10 mg/kg of NAAG and 100, 200 or 300 mg/kg of clavulanic acid, 20 minutes before induction of the inflammation by subplantar injection of 100 µl carrageenan 1%. Induction of acute inflammation in control rats resulted in a significant increase in the paw volume, reaching a peak of inflammation after 4 hours. In the control group, four hours after induction of the inflammation, the paw volume increased by 99.99±6.62% in comparison with pre-carrageenan control value. The inhibition of edema formation by NAAG (10 mg/kg) and clavulanic acid (200 or 300 mg/kg) was prominent at all hours (p<0.001) after the

carrageenan injection (Fig.1a). NAAG (10 mg/kg) resulted in a significant reduction in the paw volume by 52.5% after 4 hours in comparison with the control group (p<0.001) (Fig.1a) and reduced total inflammatory response measured as area under the curve (AUC) significantly by 56.9% compared to the control group (p<0.001) (Fig.1b). Intraperitoneal injection of the rats with 200 or 300 mg/kg clavulanic acid 20 minutes before induction of the inflammation resulted in a significant reduction of the paw volume respectively by 54.51% and 59.81% after 4 hours in comparison with the control group (p<0.001) (Fig.1a). Also AUC was significantly reduced in clavulanic acid (100, 200 or 300 mg/kg) treated rats respectively by 23.39% (p<0.01), 53.17% and 59.13% (p<0.001) compared to the control group (Fig.1b).

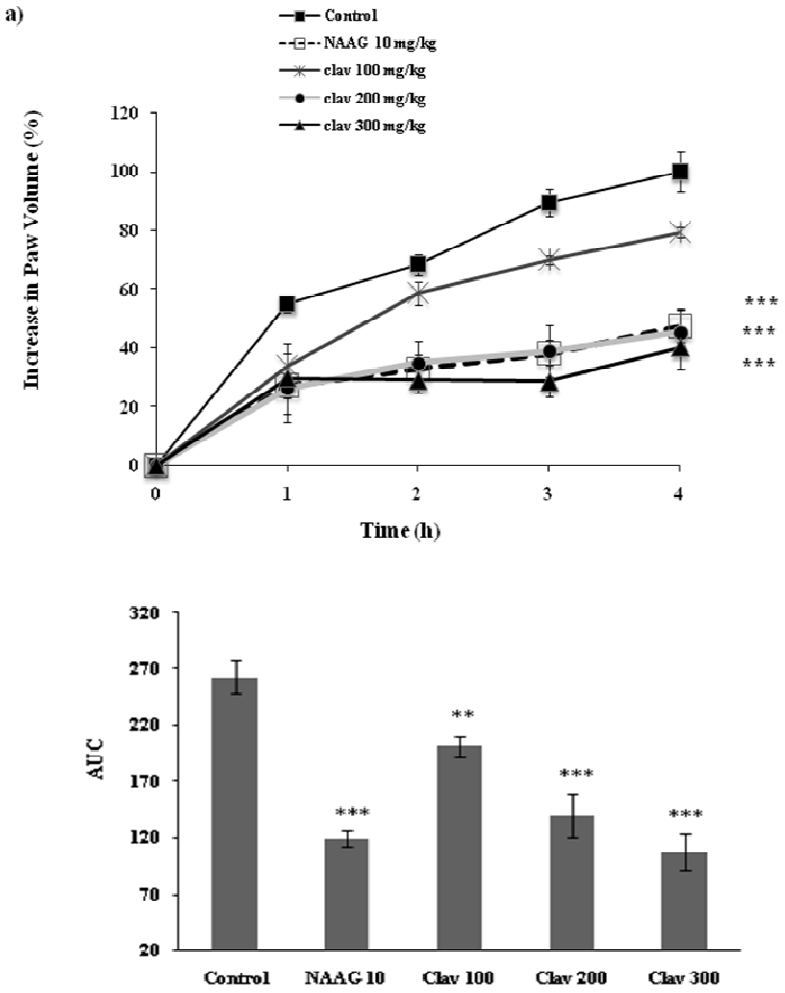

Figure 1: The Effect of intraperitoneal injection of NAAG (10 mg/kg) and clavulanic acid (100, 200 or 300 mg/kg) on carrageenan-induced paw edema in rats. NAAG or clavulanic acid was injected 20 min before induction of inflammation by carrageenan. (a) Results are expressed as percentage of increase in paw volume from control (pre-drug) values; (b) Total edema responses, measured as area under the curve (AUC) of the treated rats compared to control. Each point represents mean±SEM of six rats per group. Asterisks indicate significant changes from control value (*p<0.001), (**p<0.01).**

Effect of clavulanic acid on MPO activity

The subplantar injection of carrageenan into the rat hind paw induced an inflammation (swelling and erythema) that was maximal by 4th hour following the carrageenan administration and produced a time-dependent PMN accumulation in the paw tissue. As shown in Figure 2, the MPO activity was as high as 146.46±16.37 mU/100mg

wet tissue in the control group (carrageenan). Intraperitoneal injection of a single dose of NAAG (10 mg/kg) or clavulanic acid (100 or 200 mg/kg) significantly reduced the enzyme activity by 80.19% (29±6 mU/100mg tissue), 79% (30.8±0.7 mU/100mg tissue) and 82% (26.4±4.4 mU/100mg tissue), respectively in comparison with that in the control group (p<0.01, p<0.001) (Fig.2).

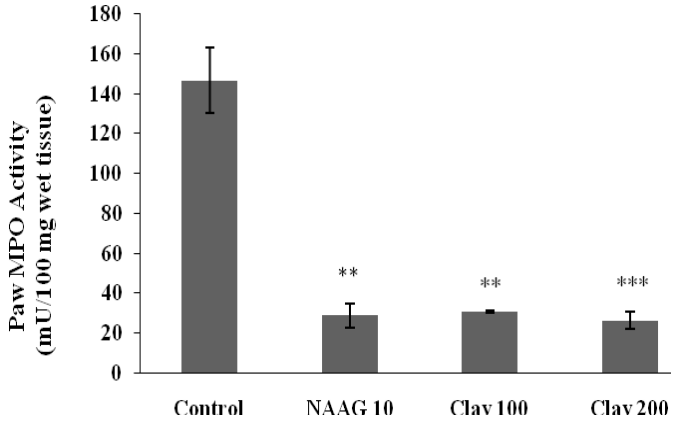

Figure 2: The effect of intraperitoneal injection of NAAG (10 mg/kg) and clavulanic acid (100 or 200 mg/kg) on myeloperoxidase (MPO) activity in the paw of rats. Inflammation was induced in the paw by subplantar injection of carrageenan 20 min after intraperitoneal injection of NAAG or clavulanic acid. Data represented as mean±SEM of six rats per group. Asterisks indicate significant change from the control value ($^{***}p$<0.001), ($^{**}p$<0.01).

Effect of clavulanic acid on MDA level

To determine the lipid peroxidation, MDA level was measured in the hind paw tissue homogenates. The subplantar injection of carrageenan into the rat hind paw induced the inflammatory response that was maximal by 4 hours following carrageenan administration. At this time point the level of MDA in the control group (carrageenan) was 13.32±1.15 nmol/100mg tissue. Intraperitoneal administration of NAAG (10mg/kg) as the reference drug and clavulanic acid (200 mg/kg), diminished MDA level from 13.32±0.17 nmol/100mg tissue in the control group to 6.08±0.35 and 6.23±0.6 nmol/100mg tissue in NAAG (10 mg/kg) and clavulanic acid (200 mg/kg) treated groups, respectively. MDA level in the NAAG (10 mg/kg) and clavulanic acid (200 mg/kg) treated groups was reduced respectively by 54% and 53% in comparison with the control group (p<0.001, p<0.01) (Fig.3).

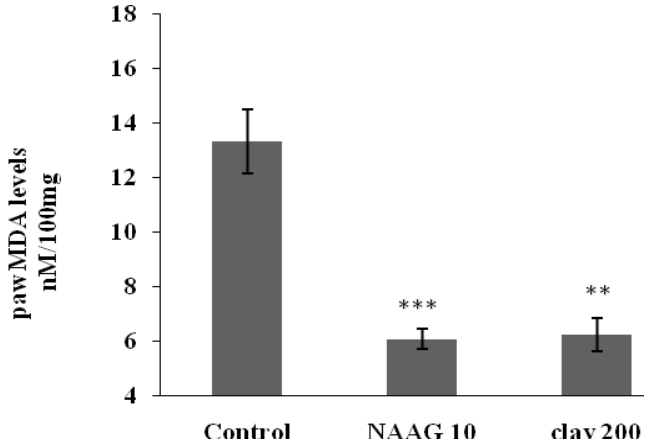

Figure 3: The effect of intraperitoneal injection of NAAG (10 mg/kg) or clavulanic acid (200 mg/kg) on malondialdehyde (MDA) level in the paw of rats. Inflammation was induced in the paw by subplantar injection of carrageenan 20 min after intraperitoneal injection of NAAG or clavulanic acid. Data represented as mean±SEM of six rats per group. Asterisks indicate significant changes from the control value ($^{***}p$<0.001), ($^{**}p$<0.01).

Effects of NAAG and clavulanic acid on IL-1β level in the inflamed paw

The effects of NAAG (10 mg/kg) and clavulanic acid (200 or 300 mg/kg) on the levels of IL-1β in the inflamed paws were examined 4 hours after the induction of inflammation. As shown in Figure 4, IL-1β level in control (carrageenan) rats was 5446.4±957.6 which was reduced in NAAG (10 mg/kg) or clavulanic acid (300 mg/kg) treated rats to 1506.2±137.5 and 3429.15±618.23, respectively. The reduction of IL-1β level following treatment by the NAAG (10 mg/kg) or clavulanic acid (300 mg/kg) were respectively 72% and 61% in comparison with that in the control group (p<0.01) (Fig.4). Results showed that intraperitoneal administration of clavulanic acid (200mg/kg) reduced IL-1β level slightly but not significantly in comparison with that in the control group.

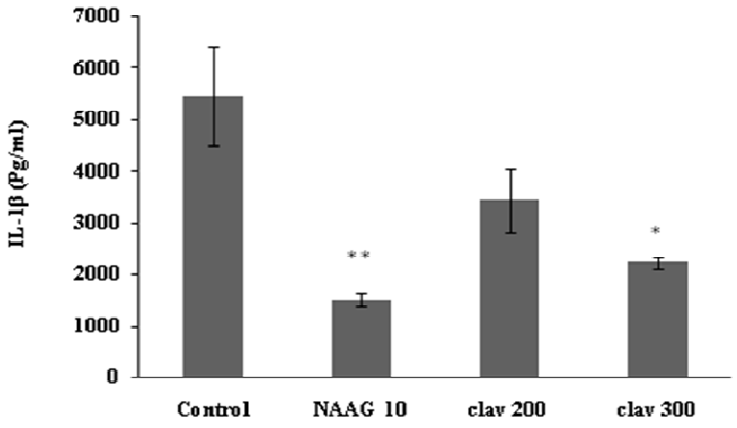

Figure 4: The effect of intraperitoneal injection of NAAG (10 mg/kg) or clavulanic acid (200 or 300 mg/kg) on IL-1β level in inflamed paw of rats. Inflammation was induced in the paw by subplantar injection of the carrageenan 20 min after intraperitoneal injection of the NAAG or clavulanic acid. Data represented as mean±SEM of six rats per group. Asterisks indicate significant changes from the control value ($^{**}p \leq 0.01$), ($^{*}p<0.05$).

Effects of clavulanic acid on TNF-α level in the inflamed paw

The effects of NAAG (10 mg/kg) or clavulanic acid (200 or 300 mg/kg) on the levels of TNF-α in the inflamed paw were examined 4 hours after the induction of inflammation. As shown in Figure 5, TNF-α level in the control (carrageenan), NAAG (10 mg/kg) or clavulanic acid (200 or 300 mg/kg) treated rats was 240.4±11.13, 183.2±10, 200.78±22.8 and 155.62±13.6 pg/ml, respectively. TNF-α level in the clavulanic acid (300 mg/kg) treated rats was reduced by 35.15% in comparison with that in the control group (p<0.05) (Fig.5). Results showed that administration of NAAG (10 mg/kg) or clavulanic acid (200mg/kg) reduced TNF-α level slightly but not significantly in comparison with that in the control group.

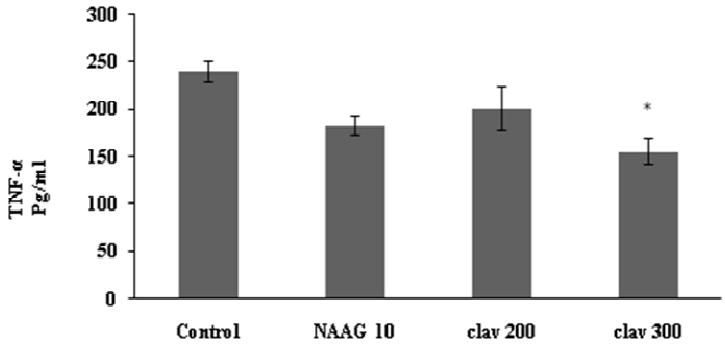

Figure 5: The effect of intraperitoneal injection of NAAG (10 mg/kg) or clavulanic acid (200 or 300 mg/kg) on TNF-α level in inflamed paw of rats. Inflammation was induced in the paw by subplantar injection of the carrageenan 20 min after intraperitoneal injection of the NAAG or clavulanic acid. Data represented as mean±SEM of six rats per group. Asterisks indicate significant changes from the control value ($^{*}p \leq 0.05$).

Effect of clavulanic acid on PGE_2 level in the inflamed paw

The effects of NAAG (10 mg/kg) or clavulanic acid (200 or 300 mg/kg) on the levels of PGE_2 in the inflamed paws were examined 4 hours after the induction of inflammation. As shown in Figure 6, PGE_2 level in control (carrageenan), NAAG (10 mg/kg) or clavulanic acid (200 or 300 mg/kg) treated rats was 5.88±0.07, 5.11±0.148, 5.125±0.2 and 5.096±0.13 pg/ml, respectively. PGE_2 level in the NAAG (10 mg/kg) or clavulanic acid (200 or 300 mg/kg) treated rats was reduced by 13.9%, 12.77% and 13.28%, respectively (p<0.05). Results showed that administration of NAAG (10 mg/kg) or clavulanic acid (200 or 300 mg/kg) significantly reduced the PGE_2 level in comparison with that in the control group (Fig.6).

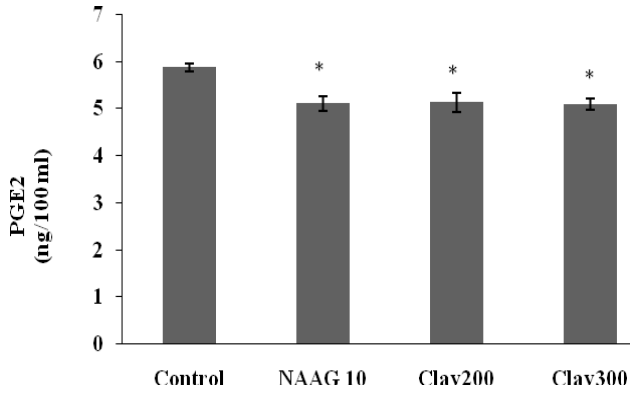

Figure 6: The effect of intraperitoneal injection of NAAG (10 mg/kg) or clavulanic acid (200 or 300 mg/kg) on PGE_2 level in inflamed paw of rats. Inflammation was induced in the paw by subplantar injection of the carrageenan 20 min after intraperitoneal injection of the NAAG or clavulanic acid. Data represented as mean±SEM of six rats per group. Asterisks indicate significant changes from the control value ($^*p$ <0.05).

DISCUSSION

As mentioned in the previous studies, effective bacterial peptidase or β-lactamase inhibitors inhibiting mammalian peptidase or protease include particularly β-lactam containing compounds. According to the structural similarity and perfect overlap between the active sites of serin β-lactamase (Class A, C and D) and the key sequence of amino acids in the rat and human GCP-II [19,29], such bacterial β-lactamase inhibitors appear to inhibit GCP-II (NAALADase) activity that allows modulation of the concentration or function of some neurotransmitters [3].

Glutamate carboxypeptidase II (GCPII; N-acetylated α-linked acidic dipeptidase: NAALADase), a peptidase hydrolyzing NAAG to glutamate and N-acetylaspartate, is found primarily in the peripheral nerves [3].

Blocking GCP-II reduces glutamate level elevation protecting carrageenan injected paw tissue from significant inflammatory responses that is occurred by high levels of glutamate [11]. It has been previously mentioned that clavulanic acid, a recognized β-lactamase inhibitor, is also a potent inhibitor of GCP-II [21,22].

Here we report new insights about the functions and possible mechanisms of clavulanic acid as a GCP-II inhibitor. In accordance with the hypothesis of the present study, clavulanic acid, a β-lactam containing compound which is an effective β-lactamase and peptidase inhibitor, can function in neurochemical mediation of glutamatergic pathways and has the ability of reducing inflammatory responses. In the present study, the authors clearly demonstrated that the intraperitoneal injection of clavulanic acid (200 or 300 mg/kg) attenuated the peripheral inflammatory response induced by carrageenan injection.

NAAG and NAAG-peptidase (GCP-II) are widely located in the peripheral nerves of mammals [3]. Inhibition of the NAAG-peptidase (GCP-II) reduces inactivation of NAAG, increases the levels of NAAG and decreases the levels of

glutamate. Activation of NAAG on the presynaptic mGlu3 receptors reduces the levels of cAMP and Ca^{2+} influx into the nerve endings and reduces the activity of postsynaptic potentials resulting in lower amounts of glutamate release per action potential [11].

It is known that the elevated glutamate release from the peripheral afferent nerve fibers caused by inflammation stimulates the excitatory amino acid receptors in the peripheral tissues followed by sensitization of the afferents by inflammatory mediators. Glutamate activates phospholipase A_2 and phospholipase C, platelet-activating factor, arachidonic acid and diacylglycerol, lipids which are responsible for the inflammatory responses [3,4,30].

Also it has been shown that NMDA glutamate receptors modulate COX-2 expression in inflammatory conditions [30]. Our results suggest that the intraperitoneal injection of the clavulanic acid (200 or 300 mg/kg) or NAAG (10mg/kg) attenuates the peripheral inflammatory response induced by subplantar carrageenan injection. We suggest that the anti-inflammatory responses seen by clavulanic acid are related to the action of clavulanic acid on GCP-II inhibition resulting in elevation of NAAG level and reduction of glutamate level increase in the peripheral afferents. Infiltration of leukocytes from the vessels into the inflamed area is one of the various mechanisms that support the inflammatory responses [31]. Carrageenan-induced paw edema is a suitable model for generating free radicals in the paw tissue after inflammatory conditions.

Clavulanic acid was injected 20 minutes prior to the induction of inflammation by carrageenan and its significant anti-inflammatory effect was detectable at both phases of the carrageenan induced inflammation model. It seems that the early phase of the carrageenan induced edema is related to the production of histamine, leukotrienes, platelet-activating factor and cyclooxygenase products, while the delayed phase of response has been related to neutrophil infiltration, production of neutrophil-derived free radicals, such as hydrogen peroxide, superoxide and $OH^{\bullet}$ radicals and release of other neutrophil-derived mediators [25,32].

These free radicals promote lipid peroxidation, increase vascular permeability, elicit cellular recruitment and produce tissue damage [33]. In accordance with our data, we showed for the first time that intraperitoneal clavulanic acid (200 or 300 mg/kg) was an effective *in vivo* inhibitor of the neutrophil infiltration; as determined by the MPO levels in the inflammatory paw tissue. Myeloperoxidase is an enzyme found primarily in azurophilic granules of neutrophils, which is used as a marker for tissue neutrophil content and its inhibition implies the presence of anti-inflammatory activity. MDA is an index of ROS-mediated injury and an end product of lipid peroxidation and free radical formation. We demonstrated that paw tissue concentration of MDA was significantly decreased by intraperitoneal administration of clavulanic acid (200 mg/kg) and the results were similar to that of NAAG as reference drug.

Reduction of MDA level was observed in inflamed paw tissues after intraperitoneal administration of clavulanic acid (200 mg/kg) indicated the ability of clavulanic acid to attenuate the oxidative stress [34,35]. Prostaglandins and interleukins are produced during inflammation and interact with their respective receptors located on the peripheral terminals of primary afferents [36].

Prostaglandin E_2 (PGE_2) is one of the most important metabolites of arachidonic acid generated through an enzymatic cascade controlled by cyclooxgenase (COX) enzymes. PGE_2 elicits a wide range of inflammatory responses including increase of vascular permeability. The significance of this role is emphasized by the broad clinical administration of cyclooxgenase inhibitors to reduce inflammation in a variety of inflammatory disorders. We found that intraperitoneal clavulanic acid (200 or 300 mg/kg) significantly reduced the levels of PGE_2 in inflamed paw tissue and the results were similar to that of NAAG as reference drug.

We measured the release profile of proinflammatory cytokines (TNF-α and IL-1β) at the site of inflammation and compared with equivalent profiles after the carrageenan injection. We assessed whether the cytokines might play a functional role in anti-inflammatory effects of clavulanic acid. Herein, we report that injection of the clavulanic acid reduces IL-1β levels in the paw tissue. Four hours after the challenge, the levels of IL-1β were reduced coincident with the significant reduction of PGE_2 levels. At the 4^{th} hour time point, when IL-1β and PGE_2 were inhibited, MPO activity was also reduced significantly in comparison with that in the control group.

In conclusion, this experiment showed that the carrageenan successfully induced edema in the paw. Clavulanic acid reduced the paw volume; the responses were great at both phases of the inflammation. MDA level was reduced by

clavulanic acid. We found that the pattern of proinflammatory mediator concentration in the paw tissue changed by clavulanic acid was correlated with the number of infiltrating cells. The anti-inflammatory properties of clavulanic acid could be related to its inhibitory effect on GCP-II activity results in elevating NAAG level and reducing glutamate level.

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