

Curcumin attenuates the expression of NMDAR-NR1 in Chronic Constructive Injury model of neuropathic pain

Xiangdi Yu*¹ and Hong Cao²

¹Department of Anesthesiology, The First College of Clinical Medical Science, China Three Gorges University, Yichang Central People's Hospital, Yichang, China

²Department of Anesthesiology, Second Affiliated Hospital, Wenzhou Medical College; Pain Institute of Wenzhou Medical College, Wenzhou, China

Corresponding author*

Dr. Xiangdi Yu MD

Department of Anesthesiology,

The First College of Clinical Medical Science,

China Three Gorges University, Yichang Central People's Hospital, Yichang, China

E-mail: yuxiangdidream@163.com

Abstract

Objective: Neuropathic pain is a prevalent disease that greatly impairs the patients' quality of life. A lack of the understanding of its aetiology, inadequate relief, and development of tolerance and potential toxicity of classical antinociceptives warrant the investigation of the newer agents to relieve this pain. The aim of the present study was to explore the antinociceptive effect of curcumin and its effect on expression of N-methyl-D-aspartate (NMDA) receptor in spinal dorsal horn and dorsal root ganglion in chronic constriction injury (CCI) mode of neuropathic pain of rats.

Methods: Paw withdrawal mechanical threshold (PWMT) and paw withdrawal thermal latency (PWTL) of rats were measured on 2th pre-operative and 1, 3, 5, 7, 10, 14 post-operative days, and the expression of NMDAR NR-1 in spinal dorsal horn and DRG was measured by Immunohistochemical staining and western-blot.

Results: CCI rats exhibited significant hyperalgesia after operation as compared with control rats. Chronic treatment with curcumin 100mg/kg/day for 14days starting from the 1th day after CCI operation significantly attenuated PWMT and PWTL. Curcumin also inhibited the expression of NMDAR NR-1 in spinal dorsal horn and DRG.

Conclusion: These results indicate an antinociceptive activity of curcumin possibly through its inhibitory action on expression of NMDAR NR-1 in spinal dorsal horn and DRG and point towards its potential to attenuate neuropathic pain.

Keywords: Neuropathic pain, Curcumin, NMDAR NR-1, Dorsal root ganglion, Spinal dorsal horn

1. Introduction

Neuropathic pain is a devastating consequence of nerve injury that is characterized by spontaneous, often burning, pain, an exaggerated response to painful stimuli (hyperalgesia), and pain in response to normally innocuous, for example touch, stimuli (allodynia). Neuropathic pain syndromes are among the most difficult to manage. Although the pain produced by tissue injury can usually be controlled by anti-inflammatory drugs and opioids, neuropathic pains such as postherpetic neuralgia, reflex sympathetic dystrophy, and phantom limb pain are often refractory to these treatments[1].

Curcumin, an extract from the plant *Curcuma longa* was considered as a therapeutic or preventive agent for several major human diseases. So it has attracted many investigations on the biological activity of curcumin, demonstrating a plethora of pharmacological properties, including anticarcinogenic[2], anti-inflammatory[3] and antioxidant[4] effects. It was reported that chronic treatment with curcumin significantly attenuated thermal hyperalgesia and the hot-plate latencies in a diabetic mouse model of neuropathic pain[5]. Moreover, a 24 h-treatment with curcumin reduced N-methyl-D-aspartate (NMDA)-mediated excitotoxic cell damage, estimated as decrease of cell viability and increase in apoptosis. The protection was associated with decrease of NMDA receptor-mediated Ca²⁺ rise and reduction in the level of phosphorylated NR1 subunit of the NMDA receptor[6]. Some studies also suggest that nerve injury leads to neuropathic pain because it triggers an N-methyl-D-aspartate (NMDA) receptor-mediated hyperexcitability of dorsal horn neurons in the spinal cord. Events downstream of the NMDA receptor, including activation of various protein kinases, have also been implicated; these are presumed to underlie the persistence of the pain[7][8][9].

The *N*-methyl-*D*-aspartate receptor (NMDAR) is composed of hetero-oligomers of GluR ζ (NR1), GluR ϵ , and occasionally GluR χ (NR3) subunits[10][11]. The NR1 subunit is ubiquitously distributed in the brain and spinal cord whereas the four ϵ subunits differ in distribution depending on the brain region and developmental stage. The NMDAR is an ion channel and cannot function in the absence of the NR1 subunit. Activation of NMDARs is important for initiating long-lasting changes in synapses and has been implicated in persistent pain by reinforcing glutamate sensory transmission. It is believed that prolonged and repeated stimulation of peripheral nerves activates NMDARs. This results in an increase in Ca²⁺ influx through *N*-methyl-*D*-aspartate (NMDA) channels and activation of various protein kinases[12][13].

Since curcumin is effective to neuropathic pain and has been found to interact with NMDA receptor, so the present study was designed to evaluate the effect of curcumin in neuropathic pain and an attempt was made to look for the participation of NMDAR NR-1 in spinal dorsal horn and DRG in curcumin's antinociceptive effect.

2. Materials and methods

2.1 Animals and procedures for chronic constriction injury (CCI)

Male Sprague-Dawley rats obtained from Animal Center of Wenzhou Medical College(Zhejiang, China) were housed in groups of two to four in 40 × 60 × 30cm plastic cages with soft bedding under a 12/12h day/night cycle; water and food pellets were available *ad libitum*. Experiments were conducted with the approval of the Animal Care Committee of Wenzhou Medical College and according to the guidelines for investigations of experimental pain in animals published by the International Association for the Study of Pain[14]. Sprague-Dawley rats weighing 200–250 g at the time of surgery were anesthetized with pentobarbital (50 mg/kg, intraperitoneal). CCI rats were produced by loosely ligating a common sciatic nerve according to the method of Bennett and Xie[15]. Sham rats were made following the same surgical procedure except for nerve ligation.

2.2 Grouping and treatment schedule

Sham and CCI rats were randomly selected and divided in four groups of 6 animals each. First group consists of sham rats, second group is the CCI control rats, third group is the solvent control(SC) group, the fourth group consisted of the CCI rats(Cur100) which were treated with curcumin 100mg/kg/day by peritoneal injection for 14days starting from the 1th day after CCI operation. The sham and solvent control groups received vehicle of curcumin (dimethyl sulfoxide[DMSO]), the CCI group was treated nothing.

2.3 Behavioral studies

Paw withdrawal thermal latency (PWTL) and paw withdrawal mechanical threshold (PWMT) of rats were measured on 2days pre-operative and 1, 3, 5, 7, 10, 14 post-operative. Mechanical allodynia was assessed by use of von Frey filaments. Rats were placed in individual plastic boxes (20 × 25 × 15 cm) on a metal mesh floor and allowed to acclimatize for 30 min. The filaments were presented, in ascending order of strength, perpendicular to the plantar surface with sufficient force to cause slight bending against the paw and held for 6 to 8 sec. Brisk withdrawal or paw flinching were considered as positive responses. The paw withdrawal mechanical threshold (PWMT) was determined by sequentially increasing and decreasing the stimulus strength (the “up-and-down” method)[16], and the data were analyzed using the nonparametric method of Dixon, as described by Chaplan et al[16].

Thermal hyperalgesia was assessed with the paw withdrawal thermal latency (PWTL) to radiant heat according to the protocol of Hargreaves et al[17]. Rats were placed in clear plastic cages on an elevated glass plate and allowed to acclimatize for 30 min before testing. A radiant thermal stimulator was focused onto the plantar surface of the hindpaw through the glass plate. The nociceptive endpoints in the radiant heat test were the characteristic lifting or licking of the hindpaw, and the time to the endpoint was considered the PWTL[18]. To avoid tissue damage, a cut-off time of 30 sec was used[19]. There were 5 trials per rat with 5 min intervals between trials. The mean PWTL was obtained from the final 3 stimuli[20].

2.4 Immunohistochemical staining

Immunohistochemistry was used to detect NMDAR NR-1 immunoreactivities in spinal dorsal horn and DRG. Rats anesthetized with pentobarbital sodium (50 mg/kg, intraperitoneal) were perfused through the ascending aorta with 0.9% NaCl, followed by freshly prepared 4% paraformaldehyde in 0.1 M phosphate-buffered saline (PBS, pH 7.4). The lumbosacral spinal cords and L4~5 dorsal root ganglion which were ipsilateral to the operation were carefully dissected out, embedded in paraffin. Five-micron thick cross-sections of paraffin-embedded tissue were cut and mounted on Vectabond adhesive-coated slides. Sections were dewaxed in xylene and rehydrated in ethanol to water, then washed in PBS, sections were incubated with the primary antibody (NMDAR NR-1, 1:100, Cell Signaling, USA) dilution at 4°C for

24 hr. Sections were washed twice with PBS and incubated in goat anti-rabbit serum (1:200) (Chemicon Group, USA) for 1 hr. This step was followed by three washes in PBS and staining using the ABC kit. The DAB chromogenic reaction was monitored carefully for about 5 min and PBS was used to stop the reaction in time.

2.5 Western blot analysis

Western blot was used to detect NMDAR NR-1 expression in spinal dorsal horn and dorsal root ganglion. Rats were rapidly (<1 min) killed through decapitation after being anesthetized with pentobarbital(50 mg/kg, intraperitoneal). The lumbosacral spinal cords and L4, 5 dorsal root ganglion which were ipsilateral to the operation were carefully dissected out and homogenized in SDS sample buffer containing a mixture of proteinase inhibitors. The lumbar segments and corresponding dorsal root ganglion were harvested because CCI has the major impact at these spinal segments. Protein samples were separated via 8% gel electrophoresis and electro blotted onto polyvinylidene fluoride (PVDF) membranes. Nonspecific binding sites were blocked by incubating PVDF membranes for 1h in phosphate-buffered saline containing 5% low-fat dry milk. Membranes were incubated overnight at 4°C with primary antibodies (MDAR NR-1, 1:1000, rabbit polyclonal, Cell Signaling, USA) and for 1 h at room temperature with anti-rabbit secondary antibodies (1:1000). Blots were developed using an enhanced chemiluminescence detection system (ECL, Amersham Pharmacia Biotech, Piscataway, NJ, USA) according to the manufacture's instruction. The Western analysis was made in triplicates. The density of specific bands was measured with a computer-assisted imaging analysis system and normalized against loading controls. Differences were compared using repeated measure one-way ANOVA followed by *post hoc* Newman-Keuls tests.

2.6 Statistical analyses

Results were expressed as the mean±SE of at least 3 separate experiments. Results were analyzed by one-way analysis of variance (ANOVA) followed by the Fisher's Least Significant Difference (LSD) test. Differences with *P* values of <0.05 were considered significant.

3. Results

3.1 The change of PWMT and PWTL baseline in each group

There was no statistical difference of PWMT and PWTL baseline among groups. After operation, sham group exhibited no significant difference of PWMT and PWTL at different time points. PWMT and PWTL of CCI control group began to decline from the first day after operation and reached to the minimum value(52.6% of basal value) on the tenth day, then recovered slowly, but it is still lower than the basic value on fourteenth day. CCI control group exhibited a significant decrease of PWMT and PWTL compared with sham group ($p < 0.01$) (Figure1). There was no difference between CCI group and solvent control group ($p > 0.05$) (Figure1).

3.2 Effect of chronic curcumin treatment on nociceptive threshold of PWMT and PWTL

When curcumin treatment started from the first day after operation for 14 days, no statistic difference of PWMT and PWTL exhibited between curcumin group and CCI group on the first, third and fifth day after operation. But PWMT and PWTL of curcumin treatment group was improved as compared to CCI group on the seventh day ($p < 0.05$) (Figure1). On tenth day after operation, PWMT and PWTL of Cur100 was improved as compared to CCI group ($p < 0.05$) (Figure1). On fourteenth day, PWMT and PWTL of curcumin group was significantly improved as compared to CCI group ($p < 0.05$) (Figure1, Figure2).

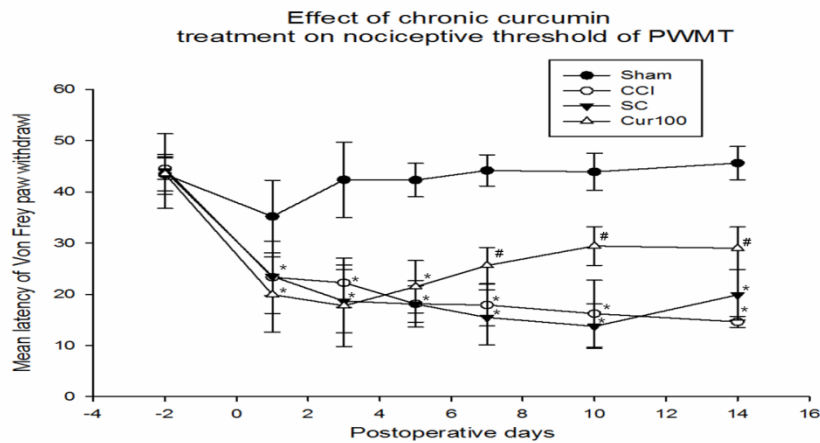
3.3 Effect of neuropathic pain of CCI mode on expression of NMDAR NR-1 in spinal dorsal horn and DRG

The effect of neuropathic pain on NMDAR NR-1 expression was determined in the superficial dorsal horn laminae I-III of the lumbar enlargement and DRG, which are areas that involved in the transmission of nociceptive inputs and sympathetic outflow[21]. In agreement with previous reports, constitutive NMDAR NR-1 expression was weak to absent in the sham group. In association with the development of neuropathic pain in CCI group, there was upregulation of NMDAR NR-1 within the superficial dorsal horn laminae I-III of the lumbar enlargement and DRG compared with the sham group, as revealed by both Immunohistochemical staining and western-blot($p < 0.01$) (Figure 2B,D,F,H). Spinal NMDAR NR-1 expression began to increase on day 3 and continue to rise on days 7 and 14 of the experimental period as compared with the sham group. ($p < 0.01$) (Figure3, Figure4, Figure5, Figure6.)

3.4 Effect of chronic curcumin treatment on expression of NMDAR NR-1 in spinal dorsal horn and DRG

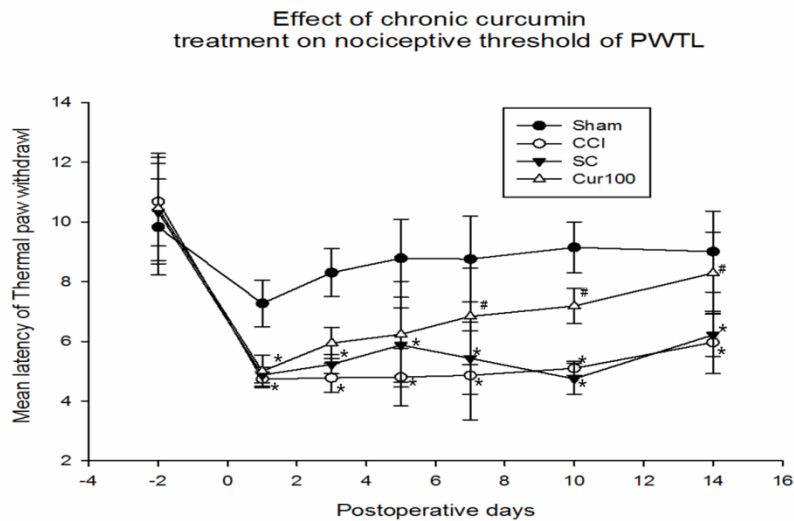
The result from this experiment showed that upregulation of NMDAR NR-1 expression within the spinal dorsal horn and DRG was blocked by the administration of curcumin on day3, 7 and 14 by both Immunohistochemical staining and western-blot compared with the CCI control group. ($p < 0.05$) (Figure7, Figure8, Figure9, Figure10).

Figure 1: Effect of curcumin treatment on the paw withdrawal thermal latency in CCI rats



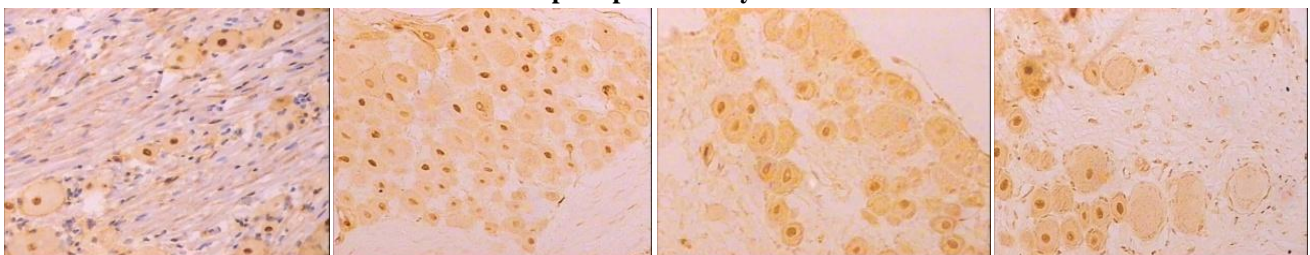
(n = 6 rats/group; mean ± SD; *p < 0.01 vs sham group; #p < 0.05 vs CCI group). (Sham operation group (Sham), chronic constrictive injury group (CCI), solvent contrast group (SC), and curcumin treated group (Cur100))

Figure 2: Effect of curcumin treatment on the paw withdrawal mechanical threshold with Von Frey filaments in CCI rats



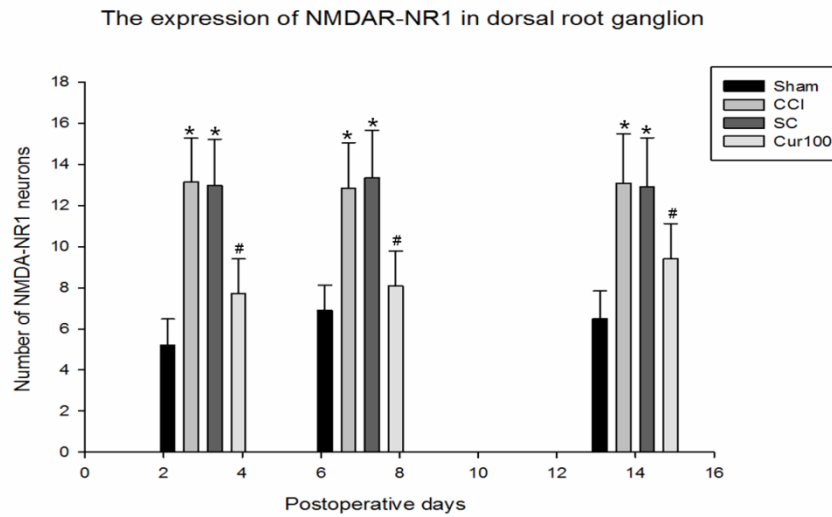
(n = 6rats/group; mean ± SD; *p < 0.01 vs sham group; #p < 0.05 vs CCI group). (Sham operation group (Sham), chronic constrictive injury group (CCI), solvent contrast group (SC), and curcumin treated group (Cur100)).

Figure 3: The expression of NMDAR-NR1 neurons within the dorsal root ganglion after CCI on the 7th postoperative days



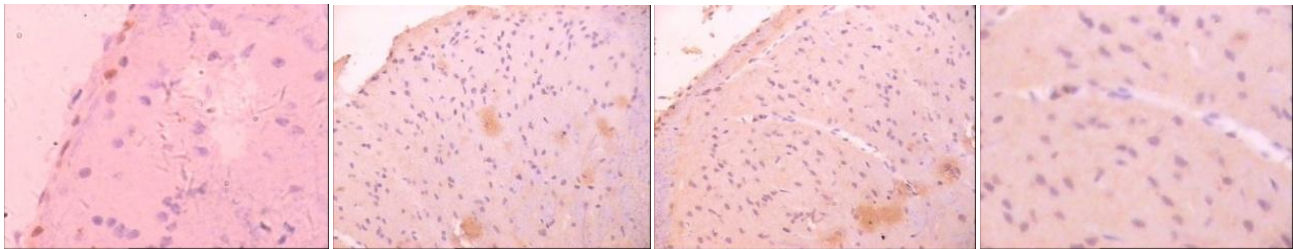
From right to left: Sham group, CCI group, SC group, Cur100 group. (Sham operation group (Sham), chronic constrictive injury group (CCI), solvent contrast group (SC), and curcumin treated group (Cur100))

Figure 4: Effect of curcumin on the expression of NMDAR-NR1 in dorsal root ganglion after CCI operation



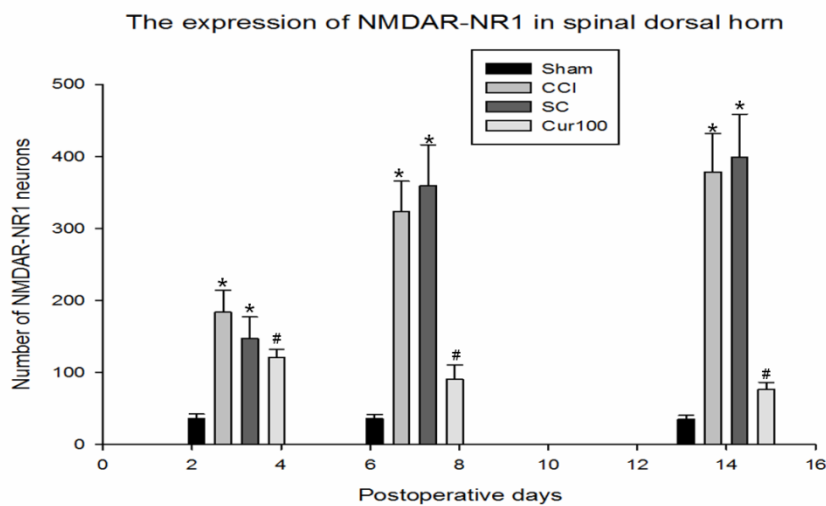
(Mean±SD, n=6)* : $P < 0.01$ vs Sham group; # : $P < 0.05$ vs CCI group. (Sham operation group (Sham), chronic constrictive injury group (CCI), solvent contrast group (SC), and curcumin treated group (Cur100))

Figure 5: The expression of NMDAR-NR1 neurons within the spinal dorsal horn after CCI on the 7th postoperative days



From right to left: Sham group, CCI group, SC group, Cur100 group. (Sham operation group (Sham), chronic constrictive injury group (CCI), solvent contrast group (SC), and curcumin treated group (Cur100))

Figure 6: Effect of curcumin on the expression of NMDAR-NR1 in spinal dorsal horn after CCI operation



(mean±SD; n=6)* : $P < 0.01$ vs Sham group; # : $P < 0.05$ vs CCI group. (Sham operation group (Sham), chronic constrictive injury group (CCI), solvent contrast group (SC), and curcumin treated group (Cur100))

Figure 7: Semi-quantitative value of NMDAR-NR1 expression within the spinal dorsal horn after CCI on 3th, 7th, 14th postoperative days

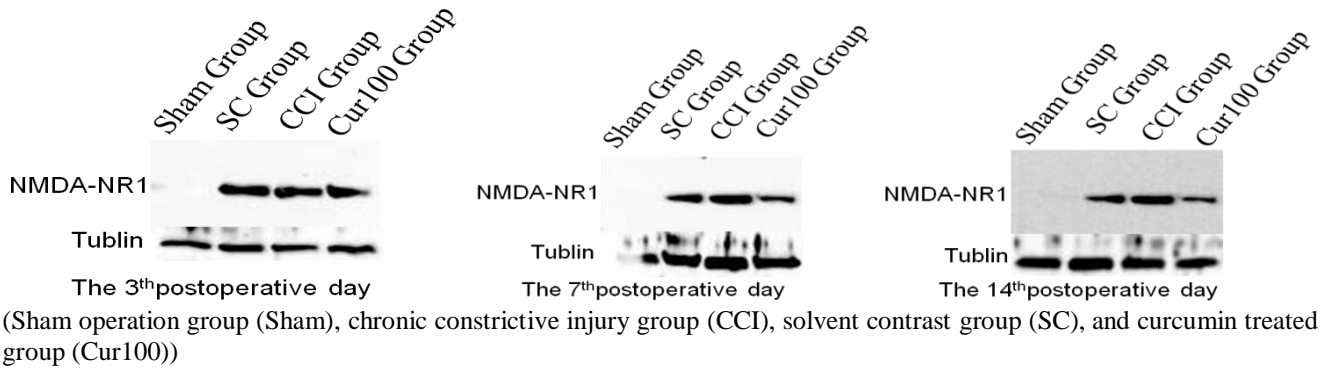
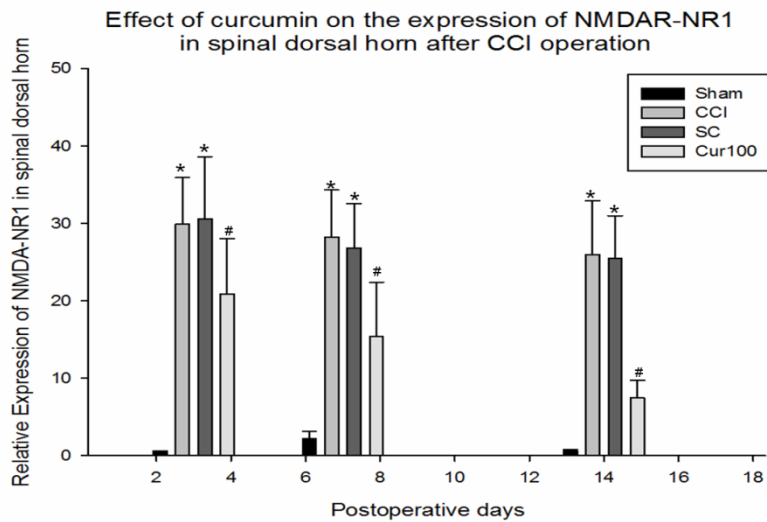


Figure 8: Effect of curcumin on the expression of NMDAR-NR1 in spinal dorsal horn after CCI operation



(mean ±SD, n=6)* : P < 0.01 vs Sham group; #:P < 0.05 vs CCI group (Sham operation group (Sham), chronic constrictive injury group (CCI), solvent contrast group (SC), and curcumin treated group (Cur100))

Figure 9: Semi-quantitative value of NMDAR-NR1 expression within the dorsal root ganglion after CCI on 3th, 7th, 14th postoperative days.

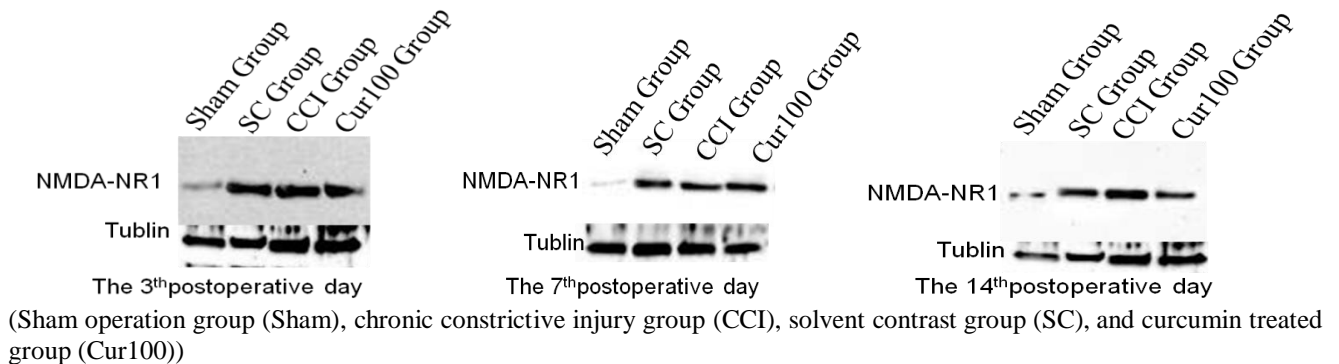
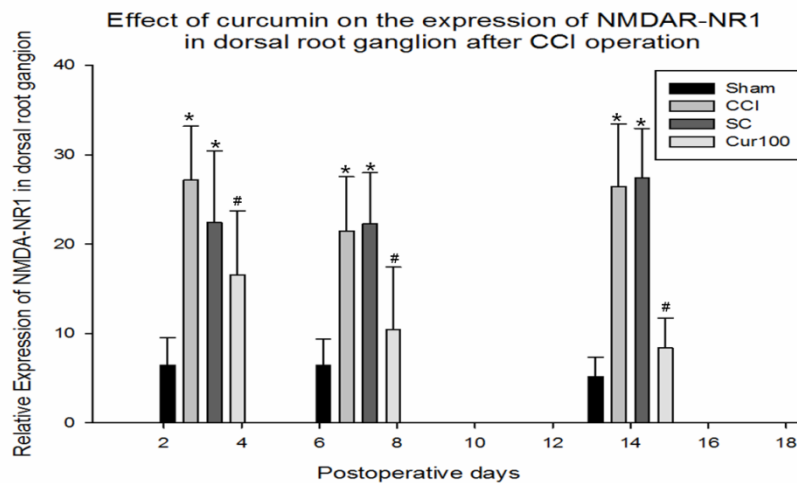


Figure 10: Effect of curcumin on the expression of NMDAR-NR1 in dorsal root ganglion after CCI operation

(mean \pm SD, n=6)*: $P < 0.01$ vs Sham group; #: $P < 0.05$ vs CCI group (Sham operation group (Sham), chronic constrictive injury group (CCI), solvent contrast group (SC), and curcumin treated group (Cur100))

4. Discussion

The present study demonstrates that (1) CCI group exhibited a significant decrease of PWMT and PWTL compared with sham group, this is in line with observation of Bennett GJ and Xie[15], (2) peripheral nerve injury induced a timed-dependent expression of neuronal NMDAR NR-1 within the superficial spinal dorsal horn and DRG that were ipsilateral to the operation, (3) Chronic treatment with curcumin significantly attenuated mechanical and thermal hyperalgesia, (4) curcumin reduces the expression of neuronal NMDAR NR-1 within the superficial spinal dorsal horn and DRG that were ipsilateral to the operation. These findings indicate that curcumin prevents neuropathic pain through reducing the expression of neuronal NMDAR NR-1 within the superficial spinal dorsal horn and DRG.

Many studies indicate that abnormal pain-related activities in different pain models involve the activation of central N-methyl-D-aspartate (NMDA) receptors[22][26]. The role of such receptors in hyperalgesia associated with chronic nerve injury produced by sciatic nerve constriction[23][25] or with peripheral inflammation produced by formalin[27] or by carragenin[28] is well documented in rats.

The hypothesis has been made for nociceptive behavior that NMDA receptor activation influences hyperalgesia and not spontaneous pain that is thought to be due to other processes like the release of nitric oxide (NO)[29]. Furthermore, it has been suggested that NMDA receptor involvement occurs in stimulated neuronal activity but not in spontaneous activity[29][30]. It has also been reported that the nociceptive reflexes and responses of spinal dorsal horn neurons to noxious stimulus, are largely unaffected by NMDA receptor antagonists that instead block the facilitation of such responses in models of tonic and chronic pain[23][26][31]. In addition the NMDA receptor antagonist effect on nociceptive visceral reflexes, but not on nociceptive somatic reflexes has been described[32]. Thus, there is converging evidence indicating a diverse NMDA receptor involvement in the various abnormal pain states.

Chronic constriction of the sciatic nerve in the rat (CCI rat) produces characteristic symptoms of neuropathic pain [15]and dynamic changes of spinal dorsal horn neuronal spontaneous and stimulated activity[33][34]. The changes involve baseline hyperactivity and increased excitability expressed as abnormal responses to afferent inputs with prolonged after-discharges. Such electrophysiological events are generally thought to represent the correlate of spontaneous pain and hyperalgesia[35].

The CCI rats thus provide a suitable model to examine the involvement of the NMDA receptors on the abnormal pain related activity and testify the treatment of novel drugs on abnormal pain.

Curcumin, an extract from the plant *Curcuma longa* was considered as a therapeutic or preventive agent for several major human diseases. Previous study has shown that chronic treatment with curcumin significantly attenuated thermal hyperalgesia and the hot-plate latencies in a diabetic mouse model of neuropathic pain[5]. It has also been observed in our study that curcumin at varying doses attenuated thermal hyperalgesia and mechanical hyperalgesia in the CCI model of neuropathic pain, at the same time, the expression of NMDAR NR-1 in spinal dorsal horn and DRG was inhibited in the curcumin-treated groups, this is consistent with the known effect of curcumin on NMDAR in excitotoxic

cell damage[6].

However, in our previous study, we found that there were rats dead in the 300mg/kg Curcumin treatment; it may be related to its toxic action at this level of dosage. The results also showed that 30mg/kg Curcumin treatment is not as effective as 100mg/kg Curcumin. So we chose Cur100 in this study.

Based on the present preliminary results, we conclude that curcumin is a novel antinociceptive agent and can be used as a therapeutic option in the treatment of neuropathic pain. Further studies are warranted to explore the exact mechanism of curcumin's antinociceptive effect.

References

- [1] Annika B. Malmberg, Chong Chen, Susumu Tonegawa, Allan I. Basbaum. Preserved Acute Pain and Reduced Neuropathic Pain in Mice Lacking PKC γ . *Science* 1997; 278(5336):279-83.
- [2] Aggarwal BB, Kumar A, Bharti AC. Anticancer potential of curcumin: preclinical and clinical studies. *Anticancer Res* 2003; 23:363–398.
- [3] Abe Y, Hashimoto S, Horie T. Curcumin inhibition of inflammatory cytokine production by human peripheral blood monocytes and alveolar macrophages. *Pharmacol Res* 1999; 39:41–47.
- [4] Das KC, Das CK. Curcumin (diferuloylmethane), a singlet oxygen ((1)O(2)) quencher. *Biochem Biophys Res Commun* 2002; 295:62–66.
- [5] Sharma S, Kulkarni SK, Agrewala JN, Chopra K. Curcumin attenuates thermal hyperalgesia in a diabetic mouse model of neuropathic pain European Journal of Pharmacology. *Eur J of Pharmacology* 2006; 536(3):256-61.
- [6] . Matteucci A, Frank C, Domenici MR. Curcumin treatment protects rat retinal neurons against excitotoxicity: effect on N -methyl-D-aspartate-induced intracellular Ca²⁺ increase. *Exp Brain Res* 2005; 167: 641–648.
- [7] Bursztajn s, Rutkowski MD, Deleo JA. The role of the N-methyl-D-aspartate receptor NR1 subunit in peripheral nerve injury-induced mechanical allodynia, glial activation and chemokine expression in the mouse. *Neuroscience* 2004; 125(1):269-75.
- [8] Sotgiu ML, Biella G. Differential effects of MK-801, a N-methyl-D-aspartate non-competitive antagonist, on the dorsal horn neuron hyperactivity and hyperexcitability in neuropathic rats. *Neurosci Lett*. 2007; 283(2):153-6.
- [9] Tao YX, Rumbaugh G, Wang GD. Impaired NMDA receptor-mediated postsynaptic function and blunted NMDA receptor-dependent persistent pain in mice lacking postsynaptic density-93 protein. *J Neurosci* 2003; 23(17):6703-12.
- [10] Cull-Candy S, Brickley S, Farrant M. NMDA receptor subunits: diversity, development, and disease. *Curr Opin Neurobiol* 2001; 11: 327.
- [11] Hollmann M, Jonas P, Monyer H. Structure of ionotropic glutamate receptors. *Hand Exp Pharmacol* 1999; 141: 3–98.
- [12] Dickenson A, Chapman V, Green G. The pharmacology of excitatory and inhibitory amino acid-mediated events in the transmission and modulation of pain in the spinal cord. *Gen Pharmacol* 1997; 28:633–638.
- [13] Woolf CJ, Costigan M. Transcriptional and post translational plasticity and the generation of inflammatory pain. *Proc Natl Acad Sci USA* 1999; 96:7723–7730.
- [14] Zimmermann, M. Ethical guidelines for the investigations of experimental pain in conscious animals. *Pain* 1983; 16:109–110
- [15] Bennett GJ, Xie YK. A peripheral mononeuropathy in rat that produces disorders of pain sensation like those seen in man. *Pain* 1988; 33(1):87-107.
- [16] Chaplan SR, Bach FW. Quantitative assessment of tactile allodynia in the rat paw. *J Neurosci Meth* 1994; 53:55-63.
- [17] Hargreaves K, Dubner R, Brown F, Flores C, Joris J. Hyperalgesia. *Pain* 1988; 32:77-88.
- [18] Chen J, Luo C, Li H, Chen H. Primary hyperalgesia to mechanical and heat stimuli following subcutaneous bee venom injection into the plantar surface of hindpaw in the conscious rat: a comparative study with the formalin test. *Pain* 1999; 83:67-76.
- [19] Ji GC, Ma F, Zhang YQ, Wu GC. Thermal hyperalgesic effects induced by intracerebroventricular injection of interleukin-1beta in rats. *Sheng Li Xue Bao* 2002; 54:325- 328.
- [20] Sun YY, Luo C, Li Z, Chen J. Differential actions of intrathecal nociceptin on persistent spontaneous nociception, hyperalgesia and inflammation produced by subcutaneous bee venom injection in conscious rats. *Sheng Li Xue Bao* 2004; 56:321-327.
- [21] Furst S. Transmitters involved in antinociception in the spinal cord. *Brain Res Bull* 1999; 48:129-141.
- [22] Coderre TJ. The role of excitatory amino acid receptors and intracellular messengers in persistent nociception after tissue injury in rats. *Mol. Neurobiol*. 1993; 7:229-46.
- [23] Davar G, Hama A, Deykin A, Vos B, Maciewicz R. MK-801 blocks the development of thermal hyperalgesia in a rat

- model of experimental painful neuropathy. *Brain Res.* 1991; 553: 327-30.
- [24] Kawamura, M and Omote K. Involvement of increased excitatory amino acids and intracellular Ca²⁺ concentration in the spinal dorsal horn in an animal model of neuropathic pain. *Pain* 1996; 68: 85-96.
- [25] Mao J, Price DD, Mayer DJ, Yand Hayes RL. Intrathecal MK-801 and local nerve anesthesia synergistically synergistically reduce nociceptive behaviors in rats with experimental peripheral mononeuropathy, *Brain Res.* 1992; 576:254-262.
- [26] Woolf CJ, Thompson SWN. The induction and maintenance of central sensitization is dependent on N-methyl-Daspartic acid receptors activation; implications for the treatment of post-injury pain hypersensitivity. *Pain* 1991; 44 293-299.
- [27] Murray CW, Cowan A, Larson AA. Neurokinin and NMDA antagonists (but not a kainic acid antagonist) are antinociceptive in the mouse formalin model. *Pain* 1991; 44:179-185.
- [28] Hylden RK, Wiliams JLK, Ruda GM, Dubner R. The effects of non-competitive NMDA receptor antagonist MK-801 on behavioral hyperalgesia and dorsal horn neuronal activity in rats with unilateral inflammation. *Pain* 1992; 50:331-344.
- [29] Hoheisel U, Sander B, Mense S. Myositis-induced functional reorganisation of the rat dorsal horn: effects of spinal superfusion with antagonists to neurokinin and glutamate receptors. *Pain* 1997; 69:219-230.
- [30] Hoheisel U, Sander B, Mense S. Blockade of nitric oxide synthase differentially influences background activity and electrical excitability in rat dorsal horn neurons. *Neurosci. Lett* 1995; 188:143-146.
- [31] Haley JE, Sullivan AF, Dickenson AH. Evidence for spinal N-methyl-D-aspartate receptor involvement in prolonged chemical nociception in rat. *Brain Res.* 1990 518:218-226.
- [32] Olivar T, Laird JM. Differential effects of N-Methyl-Daspartate receptor blockade on nociceptive somatic and visceral reflexes. *Pain* 1999 79: 67-73.
- [33] Laird JM, Bennett GJ. An electrophysiological study of dorsal horn neurons in the spinal cord of rats with an experimental peripheral neuropathy. *J. Neurophysiol.* 1993 69:2072-2085.
- [34] Sotgiu ML, Biella G, Riva L. Poststimulus afterdischarges of spinal WDR and NS units in rats with chronic nerve constriction. *Neuro Report* 1995 6: 1021-1024.
- [35] Sotgiu ML, Lacerenza M, Marchettini P. Effect of systemic lidocaine on dorsal horn neuron hyperactivity following chronic peripheral nerve injury in rats, Somatosens. *Motor Res.* 1992; 9:227-233.