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ANIMAL RESEARCH PAIN MANAGEMENT

Increase in the time withdrawal latency and pNR1 after wet cupping therapy (WCT) in rats with neuropathic pain induced with chronic constriction injury

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ABSTRACT

Objective & Objective: Neuropathic pain (NP) is induced by a lesion or disease of the somatosensory system. A lot of individuals seek an alternative form of treatment for their pain because it is typically chronic, severe, and worsens with the pharmacological therapy. Both acute and chronic pain have been successfully treated using wet cupping therapy (WCT). WCT is helpful in reducing many types of pain, although the mechanism by which it does so is still unclear. Recent research has demonstrated that NP and NMDA receptor NR1 (pNR1) change are related. The most popular of NP's animal models, chronic constriction injury (CCI) models, were used in our study to apply in WCT. By observing the rise in pNR1 and time withdrawal latency (TWL) in Rattus norvegicus with CCI, we looked at the association between WCT and pain relief.

Methodology: Our study used a post-test only, randomized controlled trial design, overall with 21 male Rattus Norvegicus CCI models, who were 4 months old and weighed 220-250 g. Three groups, G1 as a sham CCI group, G2 as a CCI group, and G3 as a CCI group plus WCT, were created at random from these rats. In three weeks, each group received two times a week application of WCT to the paralumbar area (on both the left and right sides). Rats' TWL was measured and evaluated using a hot plate to determine their pain threshold and the number of pNR1-expressing glial cells in the spinal cord was computed.

Results: According to this research, the mean and standard deviation of the TWL values for G1, G2, and G3 were: 8.8 ± 0.28, 1.44 ± 0.10, and 14.57 ± 0.45 respectively. While the minimum, maximum and median of pNR1 values for G1, G2, and G3 were, 4.2, 4.2, 4.2; 0.0, 0.9, 0.0; and 5.20, 8.00, 8.00 respectively. With P = 0.0001 and P = 0.0001, respectively, there were significant differences in the TWL by ANOVA test, and pNR1 by Kruskall Wallis test among the groups. P1-P2, P1-P3, and P2-P3 had significantly dissimilar TWLs, e.g., P = 0.0001, P = 0.0001, and P = 0.0001; while P1-P2, P1-P3, and P2-P3 had significantly dissimilar pNR1 increases (P = 0.0001, P = 0.0001, and P = 0.0001).

Conclusion: We can draw the conclusion that WCT reduces pain in CCI rats by raising pNR1 and TWL (NP models). We suggest WCT as a technique that shows promise for reducing pain in peripheral NP models, although additional research is required to validate its mode of action.

Abbreviations: pNR1- phosphorylated NMDA receptor 1;

Keywords: CCI; Chronic constriction injury; pNR2; Neuropathic pain; WCT; Wet cupping therapy

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1. INTRODUCTION

Neuropathic pain (NP) is the pain brought on by a sensorimotor system injury or disease.[1–4] Peripheral and central NP are the two categories in NP.[1] Peripheral neuropathy (PDN), neuropathies linked to viral infections (such as post-herpetic neuralgia, leprosy), carpal tunnel syndrome (CTS), amputation, breast surgery, thoracotomy, chemotherapy-induced peripheral neuropathies, and back surgery are the main causes of peripheral NP.[1,4,5] Stroke ('thalamic pain syndrome' as post-stroke pain), spinal cord injury, and neuropathic soreness linked to autoimmune illnesses (multiple sclerosis) can all result as central NP.[1,4] 20% of people with chronic pain experience NP.[6] It still remains a significant public health issue because it results in a condition with crippling chronic pain that has a negative impact on patients' quality of life and general functioning (hobbies, work, health, social relationships, cognitive function, and sleep), as well as a significant financial burden on both the patient and society.[1,6,7]

The most popular treatments for individuals with chronic pain include both pharmacological and non-pharmacological medications. There are three categories of pharmacological treatments for NP patients: serotonin-noradrenaline reuptake inhibitors (SNRI), anticonvulsants (gabapentin and pregabalin), and tricyclic antidepressants (TCA) are among the first-line treatments for NP patients. Topical opioids and lidocaine are among the second- and third-line medications. Physical therapy, psychotherapy, and surgical treatment are examples of non-pharmacological managements for NP patients.[1]

Pain, including neuropathic pain, often remains as an underappreciated symptom and the main reason behind patients seeking for medical advice.[2,3,8–11] Compared to other types of pain (visceral and somatic pain), NP has a worst response to analgesics. Complications with opioids could include abuse, addiction, and diversion.[1] Because of its ineffective treatment and side effects, the pharmaceutical treatments of NP are still unsatisfactory and far from optimal.[1,3,10] The most frequent cause of seeking alternative treatment is unsatisfactory medical care for pain management.[3] The frequency of using such therapies increases with how bad the pain is.[1]

The World Health Organization (WHO) decided to enhance conventional medicine more than 30 years ago. The WHO made this decision in light of two things: first, the fact that many people—up to 80% of the population in some countries—do not have access to primary healthcare; and second, the fact that modern therapy frequently yields unsatisfactory results, particularly when it comes to chronic illness and the adverse effects of chemical medications.[1,10]

Cupping therapy (CT) is one of the non-pharmacological methods for treating pain.[3,10] CT is the oldest branch of medicine and is practiced all around the world.[1,3,10] CT was also known as ventusynge in central England and ventoza in France. It was recognized in Indonesia as bekam and in Egypt and other states of Arab as Al-Hijama.[10] Many Asian, African, and European countries have used this therapy to treat a number of illnesses, including pain alleviation, hypertension, stroke rehabilitation, harmonizing immunological, hormonal, and neurological systems, escalating blood flow to the joints, dyslipidemia allergy, and asthma. [1,10,12]

Many people have used CT for thousands of years. [3,10] It has recently gained popularity and acceptance as a way to treat pain brought on by sports injuries as well as other medical conditions like low back pain, such as lumbar disc herniation or radiculopathy, and many of the chronic pains. [1,3,10,12] Dry cupping therapy (DCT) as well as wet cupping therapy (WCT) are the two different forms of CT.[3,10,12]

WCT has been around for a while and is effective for treating many kinds of chronic pain, its exact mechanism of action is still unknown.[1] The spinal cord's level of phosphorylation of the NMDA receptor NR1 (pNR1) subunit revealed that it plays a crucial role in pain.[13,14] The central sensitization process is driven by an increase in neuronal responsiveness, which is correlated with

pNR1 in the spinal cord.[14] Given the significance of pNR1 in central sensitization, the purpose of our study was to analyze the relationship between WCT and pain relief by examining the rise in pNR1 expression in glial cells in the spinal cord and TWL in a CCI as a well-established NP model.

2. METHODOLOGY

This study was carried out in the Veterinary Medicine Department's animal research laboratory at UNAIR, Surabaya City, East Java Province, Indonesia. The Ethics Team of Faculty of Veterinary Medicine at UNAIR, Surabaya, Province of East Java, Indonesia, approved our study with the ethical number 2.KE.015.01.2018.

2.1. Animals

Twenty-one male Rattus Norvegicus, weighing on average 220-250 g, aged 4 months, acted as the test subjects for this experiment. These rats underwent a seven-day acclimatization period at a constant temperature of 26 °C with 12-hour light/dark rotations. These rats were given exempt access to nourishment that included Pelet BR 511, Indonesia, Comfeed, and unlimited amounts of water. Three groups (n = 7) of these rats were created: G1, G2, and G3. G1 was sham chronic constriction injury (CCI) group; G2 was CCI group; and group G3 was made up of CCI and WCT. With a frequency of twice weekly, the WCT phase lasted for 3 weeks. The TWL was measured after three weeks (6 WCTs). The spinal cords were cut off one day following TWL, and immunohistochemistry was used to determine the number of pNR1 expresses.

2.2. Chronic constriction injury (CCI) procedure

CCI is a classic representation of NP.[13] It was first presented by Bennett and Xie in 1988, and Sommer et al. later updated it.[1,3,6] During anesthesia with ketamine and xylazine, and acepromazine, the right part of sciatic nerves (SN) was surgically revealed at mid-thigh degree following skin incision and then released from the adherent tissue just proximal side to the trifurcation of SN. The right side of a rat's SN was wrapped in 4 loosely-tied (1 mm spacing) ligations made of (5-0) chromic catgut until the nerve's diameter was somewhat reduced and just sufficiently tight to contact the SN without interfering the epineural flow.

2.3. Sham CCI procedure

The SN of the rats of sham CCI category were disclosed without chromic catgut ligation.

2.4. Wet cupping therapy (WCT)

Following a week of adaptation, all groups underwent WCT utilizing cups employing the cupping, puncture and cupping (CPC) approach. On the rat's skin, two 2 cm-diameter cups were applied to the left and right paralumbar areas. The cups were removed after the -200 mmHg negative pressure had been applied for 5 min. Ten needles were applied during the puncture step in each cup area. Repeated cupping in the same manner led to a minor amount of blood removal.

2.5. Time withdrawal latency (TWL)

Rats' pain thresholds were measured using a stopwatch and a hot plate using Cold/ Hot Plate Cat #35100, Italy, Varese, Ugo Basile on all three categories (G1, G2, and G3). TWL was calculated from the moment the rat was placed on the heated superficies (51 °C) until it showed signs of pain, such as stroking, standing, jumping off, and licking the hot plate. A 20 second time limit was applied when the rat was placed on the heated surface (51 °C) to prevent tissue injury. This TWL was calculated following six rounds of WCT.

2.6. Determination of pNR1 expression

After therapy, animals were killed by dislocation of cervical and spinal cords of these rats were then taken, split, and cultivated to count the expression of pNR1 using an immunohistochemical approach. Using the immunohistochemical approach and the monoclonal anti-pNR1 antibody using sc-365634 Santa Cruz Biotechnology from Dallas, Texas, USA, the expressions of pNR1 positive glial cells were counted. Utilizing an Olympus CX21 light microscope, the positive glial cells for pNR1 expression were computed.

2.7. Statistical analysis

Post-test-only control group design was used in this study. ANOVA was used to examine data with a normal distribution, followed by Least Significant Difference (LSD), while Kruskal Wallis test was used to investigate data with an abnormal distribution, followed by Mann-Whitney U test. P = 0.05 was regarded as statistically significant result. SPSS 23 was used to analyze the data.

3. RESULTS

TWL measurements were taken in each of the three groups (G1, G2, and G3) in the third week, with results of 8.8 ± 0.28, 1.44 ± 0.10, and 14.57 ± 0.45, respectively. TWL computed among categories (P1-P2 group, P1-P3 group, and P2-P3 group) showed necessary distinctions with P = 0.0001 (P = 0.05) in ANOVA test, and these differences were then confirmed by the LSD test with P = 0.0001, P = 0.0001, and P = 0.0001 results, respectively.

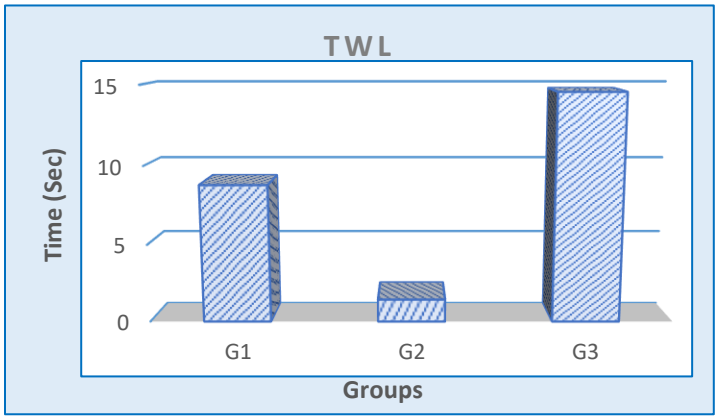

Figure 1: TWL measurements in each of the three groups

Table 1: Time withdrawal latency

Groups	Time withdrawal latency	ANOVA
G1	8.8 ± 0.28	P = 0.0001*
G2	1.44 ± 0.10	
G3	14.57 ± 0.45	

** P < 0.05 considered as significant; Data presented as Mean ± SD*

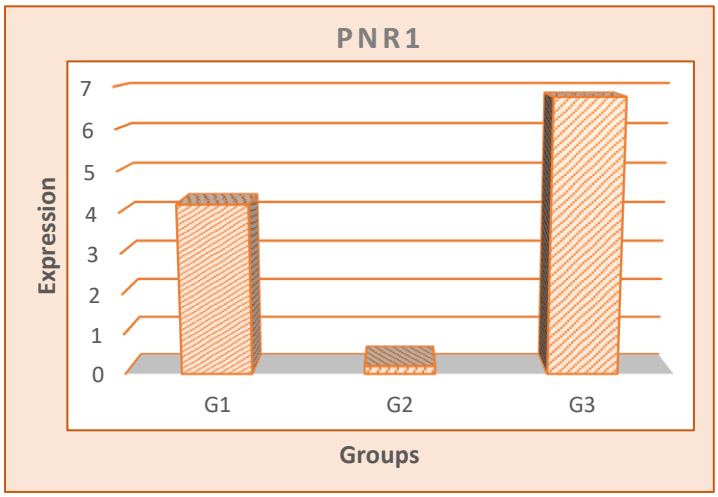

Figure 2: Immunohistochemistry-based expression of glutamate transporter-1 (GLT-1)

Table 2: Expression of pNR1

Groups	Minimum	Maximum	Median	Kruskal Wallis
G1	4.2	4.2	4.2	P = 0.0001*
G2	0.0	0.90	8.0	
G3	5.20	8.00	8.00	

** P < 0.05 considered as significant*

Immunohistochemistry was used to measure the expression of pNR1. In contrast to the negative glial cells of the spinal cord, which responded negatively to the expression of pNR1, the positive glial cells of the spinal cord possessed chromogen brown color. Measurement of pNR1 expression results in each of the three groups (G1, G2, and G3) were 4.2, 0.2 and 6.76, respectively, as shown in Figure 2 and Table 2. Kruskal-Wallis analysis declared a significant difference between groups (P = 0.0001), followed by a Mann-Whitney U test that revealed differences significantly of pNR1 count between G1-G2, G1-G3, and G2-G3 groups (P = 0.0001). Distribution of pNR1 expression from G1 to G3 was abnormal.

4. DISCUSSION

Our current study's objective was to assess WCT's therapeutic benefits on NP. Understanding the mechanism of NP and creating a thorough, ideal, and efficient treatment plan depend heavily on animal models.[6] The widely utilized animal model of peripheral NP, CCI rats, were created by Bennet and Xie. This CCI model has contributed to expand the field of study into NP causes and successful treatment. Comprehending of nociception and the factors providing to pathophysiology of chronic NP states has improved as a result of research using the CCI model.[1,6]

According to several earlier studies, CT is widely acknowledged as an alternative or complementary treatment for pain relief.[1] There are two different kinds of CT: DCT and WCT.[3,12] Because the WCT is better than the DCT, WCT was chosen in our current investigation.[12] In our study, the NP in CCI models was dramatically reversed by WCT after 6 applications. An earlier study found that performing CT twice per week for a total of five sessions greatly reduced fibromyalgia pain. For a general neck ache, one CT was used five times in two weeks.[1] CT can be used with different types of suction pumps (electric or manual), as well as different types of cupping (DCT [12], WCT [12], WCT with CPC [12,15], or WCT with PC techniques [12]) [10]; various type of cups (plastic [10], bamboo [12,16], glass [12], metals,[12] or other objects), various degree of negative emphasis, frequency, total amount of cupping, interval, length of vacuum, total number of punctures throughout, and choice of skin area to be treated with CT. [1] CT for pain in NP in our study used two plastic cups with electric suction applied to the right and left paralumbar regions for five minutes, twice weekly for three weeks (a

total of six cups) in -200mmHg negative pressure. Even though several experiments utilized different parameters and techniques in varied settings, the results were usually the same: CT lessens pain. Our study's findings are consistent with those of earlier research. According to our study, WCT causes an increase in TWL after the operation, which lessens the discomfort experienced by CCI models.

The intraneural edema, localized ischemia, and Wallerian degeneration were associated with the CCI model of constriction of the sciatic nerve. Previous research revealed that the behavioral alterations that occurred after a lesion or injury in CCI rats were caused by the sensitization of C-fibers. Recent studies have shown that a partial injury of this nerve causes both A- and C-fibers to become sensitized, which has the effect of causing and maintaining CCI's pain behavior.[1] Previous research has also identified a number of behavioral indicators of spontaneous pain in CCI rats, including minor guarding, ipsilateral hind paw limping, moderate autotomy, avoidance of putting weight on the injured side, and excessive licking. The largest pain-related behaviors and postural asymmetries were observed in these rats in the second week after the surgery, but behavioral abnormalities like mechanical and thermal hyperalgesia, chemical hyperreactivity, and cold allodynia have been recorded to occur within one week. These NP changes have been shown to last for at least 7 weeks after surgery.[1] Our study came to the same conclusion that the CCI models' NP behavior began to develop in the third week after the surgery.

Along with NP behaviors, CCI also causes behaviors that resemble depression and anxiety. Chronic neuropsychiatric disorders (NP) are caused by a persistent stressor called chronic neuropsychiatric disorders (CP). Important clinical issues are around the psychological illnesses that chronic NP causes. Anxiety and depression symptoms related to NP have been extensively documented in various clinical trials.[13] N13 As a crucial subunit of the ionotropic glutamate receptor, the N-methyl-D-aspartate (NMDAR) receptor (NR1), plays a crucial role in psychiatric illnesses. [13] Previous research revealed that rats with CCI had lower pNR1 levels in their hippocampi.[13] It is possible that CCI-induced decreases pNR1 in hippocampus and it will inhibit the translocation of NR1 subunits and affect NR1-mediated neuronal activity.[13]

As in earlier studies, one or seven days after the CCI surgery, there was a considerable increasing number of pNR1 in all of I-VI laminae of the L4/ L5 contralateral dorsal horn. Induction with thermal hyperalgesia, but not mechanical allodynia, in NP rats was totally stopped by pretreatment with 0.3 mg/ kg of RTX s.c. in intraplantar or scruff of the neck two days before CCI. Intriguingly, RTX therapy significantly reduced the elevation of pNR1 brought on by CCI in I–II as well as V–VI spinal laminae, but not III–IV laminae, when compared to CCI rats treated with vehicle. These results show that activation of CSPAs is necessary for boosting pNR1 expression in I-II as well as V-VI spinal laminae, which in turn contributes to the emergence of thermal hyperalgesia in NP rats.[14] In the spinal cord's CCI rats, pNR1 was found to be downregulated 21 days after the surgery and to become normal after WCT. The spinal cord pNR1 modulation during the WCT treatment may provide some clues as to how WCT actually works to exert its anxiolytic and antidepressive effects. It was proposed that the therapeutic effects of WCT on emotional disorders brought on by CCI may be linked to the pNR1 regulation. The expression of pNR1 in the spinal cord of CCI rats decreased, and WCT was able to successfully reverse this expression. This finding raises the possibility that a mechanism underlies the effects of WCT treatment on pain relief, antidepressant activity, and anxiolytic activity.

5. STRENGTHS

Our research included both strengths and limitations. The introduction of a sham-CCI group was one of the study's strengths. This sham-CCI organization was used to regulate the CCI group. To demonstrate the effects of nerve damage caused by sciatic nerve exposure without ligation, a sham-CCI procedure was carried out.

6. LIMITATIONS

Along with its advantages, our study has certain limitations, one of which is the absence of WCT negative control. Future research may compare alternative therapies such dry needling, electroacupuncture, and DCT to WCT utilizing an RCT design. Another limitation is that our study only evaluated NP behaviors, not chronic-NP-induced emotional behaviors, which are closely related to pNR1. Our study similarly assessed pNR1 in the spinal cord, but did not specifically count the number of lamina.

7. CONCLUSION

In conclusion, the findings of this study and earlier research have supported the hypothesis that WCT has positive impact on pain management. Although other researches utilized different pain models (rats with inflammatory ache or humans with sundry types of aches), different CT techniques (WCT with puncture-cupping-PC-method), and varied settings, the results were similar: Significantly less discomfort is felt after CT. The NP-affected rats showed symptoms of anxiety and depression. Additionally, WCT has impressive

therapeutic effects on emotional issues linked to CCI. The therapeutic benefits of WCT may be related to its control over pNR1 in the spinal cord. The current study offered an efficient WCT alternative for treating psychological impairments and pain behaviors brought on by chronic NP.

8. Data availability

The numerical data generated during this research is available with the authors.

9. Acknowledgement

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10. Conflict of interest

The study utilized the hospital resources only, and no external or industry funding was involved.

11. Authors' contribution

EQ: Conception of the work, research, manuscript drafting

HBH: Research, performed the analysis, manuscript editing

SK: Conception of the work, performed the analysis, manuscript writing

WW: Literature research, manuscript reviewing and revising

IS, LA: Conception of the work, manuscript reviewing and revising

ANFA, VAP: Literature research, manuscript reviewing and revising

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