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PAIN REDUCTION AFTER WET AND DRY CUPPING THERAPIES: ROLES OF α2β1 INTEGRIN AND μ-OPIOID RECEPTOR IN ANIMAL MODELS

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ABSTRACT---Background: *Wet cupping therapy (WCT) and dry cupping therapy (DCT) are complementary therapies in pain management. Stretching keratinocyte by cupping seems to stimulate the expression of α2β1-integrin. Its benefit in pain reduction could be mediated by the expression of μ-opioid receptor (MOR).*

Objective: *This study aims to determine pain thresholds, α2β1-integrin and MOR after WCT and DCT.*

Method: *Thirty-two adult male Wistar rats were divided into negative control, positive control, DCT, and WCT groups. In DCT group and WCT group, cupping was conducted at left and right paralumbar region 48h after CFA injection. Hot plate test was conducted 24h after therapy to assess pain threshold and immunohistochemistry were conducted from the skin for α2β1-integrin and the spinal cord for MOR.*

Result: *WCG showed the highest expression of α2β1-integrin in the keratinocytes and MOR in the lamina II spinal cord than the other groups. On immunohistochemical examination, the expression of α2β1 integrin showed positive cells (keratinocytes) with brown stain on dry cupping and wet cupping. Significant differences were shown between negative control group showed significant differences with positive control group, dry cupping therapy group, and wet cupping therapy group.*

Conclusion: *WCT is more effective than DCT in reducing pain. The benefit of both therapies in reducing pain in rats could be mediated by the expression of α2β1-integrin and MOR.*

Keywords---*Dry cupping, pain reduction, wet cupping,*

I. INTRODUCTION

Cupping therapy is one of the complementary and alternative therapies that is widely used to reduce pain(Kim *et al.*, 2011). Previous study showed that cupping therapy can be used for acute and chronic pain(Cao *et al.*, 2004). In Indonesia there are two methods of cupping therapy, the dry cupping therapy and wet cupping therapy. Dry cupping therapy cups the skin without bleeding. In wet cupping therapy, the skin is injured and cupped, resulting in blood loss(Arslan, Gökgöz and Dane, 2016). The difference in the effectiveness of each cupping therapy method is not

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widely known.In both cupping methods negative pressure is applied which causes the skin to stretch. Stretched cells cause stimulation of mechanoreceptors of degenerin/epithelial sodium channel (DEG/ENaC), acid-sensing ion channelsASICs, transient receptor potential vanilloid 4TRPV4 and integrins(Delmas, Hao and Rodat-Despoix, 2011).

Integrins are transmembrane receptors that connect the extracellular matrix with the cytoskeleton(Hynes, 2004). Integrins function as mechanoreceptors which transmit forces between the extracellular matrix and the cytoskeleton to maintain tissue structure integrity(Baker and Zaman, 2010). Cell strain causes activation of signal pathways which include mitogen-activated protein (MAP) kinase, Rho guanosine triphosphate (GTP) ase, increased cytoplasmic calcium, and reactive oxygen. These events cause changes in gene expression(Schwartz, 2010). Puncture on the skin on wet cupping is a stressor that can stimulate the expression of endorphin(Subadi *et al.*, 2017). The hypothesis of this study was that wet cupping is more effective than dry cupping because there is an addition of puncture which causes endorphin expression.This study aims to determine pain thresholds, α2β1-integrin and MOR after WCT and DCT.

II. METHODS

This study used post-test control group design. Thirty-two Wistar, 3 months, 250±50 grams, male rats were randomized into 4 groups: negative control (n=8); positive control (n=8); dry cupping (n=8); wet cupping (n=8). In the negative control group, the rats had no exposure at all. In the positive control group, the rats were given complete Freund's adjuvant (CFA) (Sigma-Aldrich, Saint Louis, Missouri, USA). In the dry cupping group, the rats received CFA and dry cupping. In the wet cupping therapy group, the rats received CFA and wet cupping therapy.

Adult Wistar rats were put into plastic cage (4-5 rats per cage). They were acclimatized for 7 days with 12:12 h (dark/light; the light was turned off every 19:30) with temperature maintained at 24^0C. CFA (100 µL) was injected in the center of the right plantar footpad in the three groups of the rats(Fehrenbacher, Vasko and Duarate, 2012). In the groups of dry cupping and wet cupping, 48 hours after the injection, the rats were treated with dry cupping or wet cupping therapy(Subadi *et al.*, 2017). In the dry cupping group, the skin of the right and left paralumbal areas was cupped (2 cm in diameter) with a negative pressure of 200 mmHg for 5 minutes (Subadi *et al.*, 2017). In the wet cupping group, the rats were pricked with lancet 28G before being cupped. Twenty-four hours after wet and dry cupping, all rats were examined for pain thresholdusing a hot plate (Hot/Cold Plate Cat 35100, Ugo Basile, Varese, Italy). The temperature of the hot plate was 51^0C [15]. After examination of the pain threshold, the rats were terminated by cervical dislocation and the paralumbal skin was examined with immunohistochemistry.

Pain was examined using hot plate in bright conditions. The pain threshold was calculated from the moment the rats were placed on the hot plate surface until the nociceptive response occurred. Response showing discomfort (Nocifensive) behaviors include forepaw withdrawal or licking, hind paw withdrawal or licking, stamping, leaning posture and jumping(Deuis, Dvorakova and Vetter, 2017), with a maximum time limit of 30 seconds. One measurement used a stopwatch for each rats to obtain the average value. All the nocifensive behavior responses were conducted by a trained and experienced person in order to have homogenous results.

Expression of α2β1 integrin in keratinocytes and µ-opioid receptors in nerve cells in the spinal cord lamina II was immunohistochemically examined using anti-integrin monoclonal antibody α2β1 (Cat. No MAB2141Z, Chemicon, Merck Millipore co., Darmstadt, Germany) and µ-opioid anti-receptor monoclonal antibody (ab64746, Abcam,

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Cambride, USA). Cells that were positive for α2β1 integrins and μ-opioid receptors were calculated using a microchemical light (Olympus CX21, New York, USA).

III. RESULT

Pain threshold was determined 24 hours after dry cupping therapy and wet cupping therapy, while in positive control group it was performed 72 hours after CFA injection. Figure 1 demonstrates the results of pain threshold experiment. Significant differences were observed between negative control and dry cupping therapy; and negative control and wet cupping therapy. Similar significant differences were also observed between positive control and dry cupping therapy; and positive control and wet cupping therapy. Interestingly, wet cupping therapy and dry cupping therapy showed significant difference. In this study there were two samples in wet cupping group which had to be stopped because it exceeded 30 seconds.

On immunohistochemical examination, the expression of α2β1 integrin showed positive cells (keratinocytes) with brown stain on dry cupping and wet cupping (Figure 2). Negative reactions of α2β1 integrin expression did not show brown stain.Number of keratinocytes expressing α2β1integrin is demonstrated on Figure 3. Significant differences were shown between negative control group and dry cupping therapy group, negative control group and wet cupping therapy group, and positive control group and wet cupping therapy.

On immunohistochemical examination, the expression of μ-opioid receptors showed positive cells (neurons) with brown stain on dry cupping and wet cupping (Figure 4). Negative reactions of μ-opioid receptors did not show brown stain.Number of nerve cells in the spinal cord lamina II expressing μ-opioid receptors is demonstrated on Figure 4. Significant differences were shown between negative control group showed significant differences with positive control group, dry cupping therapy group, and wet cupping therapy group. Similar trend was also observed in positive control group. Interestingly, dry cupping therapy group showed significant difference with wet cupping therapy group.

IV. DISCUSSION

In this study, dry cupping therapy and wet cupping therapy increased the expression of α2β1 integrins in keratinocytes and μ-opioid receptors in spinal cord nerve cells.It seems that the negative pressure applied in dry cupping therapy and wet cupping therapy triggers the expression of α2β1 integrins. In this study, α2β1 integrin expression in wet cupping therapy was higher than that in dry cupping therapy. The difference between wet and dry cupping therapy methods is the puncture. The puncture causes damage to the keratinocyte membrane. Cell damage causes inflammatory reactions and secretion of inflammatory mediators such as bradykinin, prostaglandin, leukotriene, serotonin, histamine, and substance P (Amaya *et al.*, 2013). The integrins which contained subunit β1 play a role in nociceptor sensitization by inflammatory mediators(Dina *et al.*, 2004, 2005). Additionally, they also modulate neuronal excitability and injury after mechanical stimulation to the surrounding extra cellular matrix(Wang *et al.*, 2006; Hemphill *et al.*, 2011). Mechanical stimulation is resulted by both dry and wet cupping therapy.

This study shows that dry cupping therapy and wet cupping therapy can reduce pain. These findings were in accordance with those of other researchers that dry cupping therapy and wet cupping therapy reduced pain(Dalton and BJ, 2017).Pain threshold test in this study was limited to 30 seconds. This study was conducted to prevent tissue

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damage to the rat's paws. There were 25% (2 out of 8) samples in wet cupping therapy group exceeded 30 seconds. It could be caused by effectiveness of wet cupping therapy in increasing pain thresholds.

The expression of μ-opioid receptors in wet cupping therapy was higher than that in dry cupping therapy. μ-opioid receptor activation inhibits pain transmission in the spinal cord(Al-Hasani and Bruchas, 2011). In a previous study, β-endorphin expression was found after wet cupping therapy(Subadi *et al.*, 2017). β-endorphin causes analgesia through bonding with μ-opioid receptors. In central nervous system, β-endorphin binds with μ-opioid receptor, causing a cascade of interactions that inhibits the expression of substance P and GABA that contribute to pain transmission(Sprouse-Blum *et al.*, 2010).

In this study, the expression of opioid receptor in wet cupping therapy was higher than that in dry cupping therapy. It was allegedly because puncture was applied in wet cupping therapy, which caused cell injury. Cells affected by lesion will express inflammatory cytokines that can trigger endorphins through the POMC in the hypothalamus(Slominski *et al.*, 2000).

V. CONCLUSION

This study provided evidence-based data that wet cupping therapy is more effective in reducing pain as compared to dry cupping therapy. Pain reduction mechanism in both therapies was mediated by the expression of α2β1 integrin and μ-opioid receptorswhich are higher in wet cupping.

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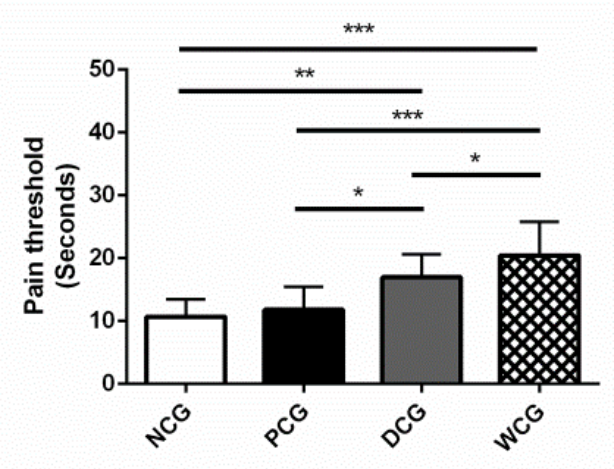

Figure 1: Results of Pain Threshold Experiment

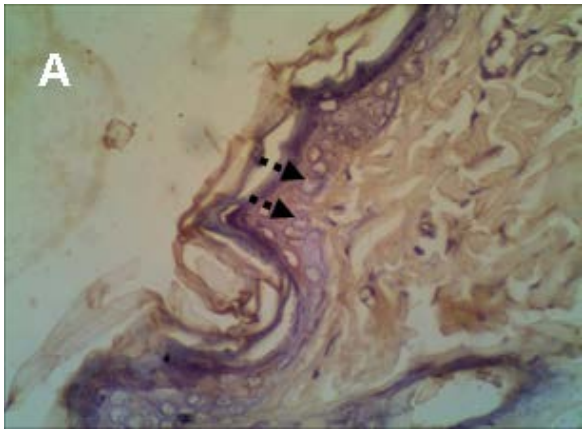

Figure 2: Expression of α2β1 Integrin withKeratinocytes

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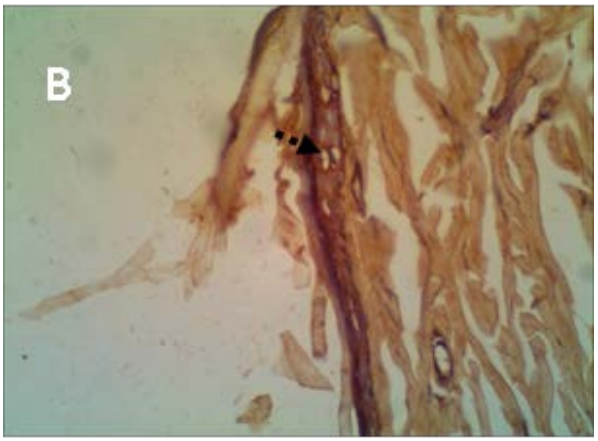

Figure 3: Number of Keratinocytes Expressing α2β1 Integrin

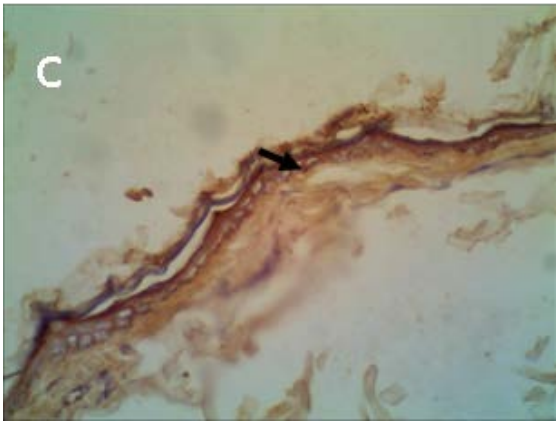

Figure 4: Result of Immunohistochemical Examination TheExpression of μ-opioid Receptors with Neurons

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