THE OVERVIEW OF MACROPHAGE, MAST CELL, AND LYMPHOCYTE ACTIVITY IN THE EFFICACY DISTINCTION OF PAIN REDUCTION FOR DRY AND WET CUPPING THERAPIES

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ABSTRACT

There are two methods in cupping therapy, they are dry cupping and wet cupping. Both methods are used to reduce pain; however the effectiveness of each method has not known yet. The aims of this research were to compare the effectiveness in pain relief between dry cupping therapy and wet cupping therapy and to find out the overview of macrophage, mast cell and lymphocyte activity. This study was a randomized controlled trial post test only design. Thirty-two white rats obtained, Wistar type, weight 300 ± 50 grams, age 3 months, divided into 4 groups. Group 1 (n = 8) consists normal rats, group 2 (n = 8) contains rats with induced pain, group 3 (n = 8) consists rats with induced pain and dry cupping therapy while group 4 (n = 8) consists rats with induced pain and wet cupping therapy. Pain variable was obtained by measuring pain threshold reaction time using hot plate. The amount of macrophage, mast cell and lymphocyte were measured with HE stain under the light microscope. Statistical analysis was using oneway Anova and SPSS 17 device. Pain threshold reaction time in negative control group is 10.66 ± 2.71 detik, kelompok kontrol positif 11.78 ± 3.58 detik, kelompok terapi bekam kering 16.93 ± 3.63 detik dan kelompok terapi bekam basah 22.81 ± 6.34 detik. Terapi bekam basah dapat lebih efektif mengurangi rasa sakit dibandingkan dengan terapi bekam kering (p < 0.05) secara signifikan. Makrofag adalah sel komponen imunitas tubuh yang paling dominan dalam terapi bekam basah. (FMI 2014:50:148-152)

Keywords: cupping therapy, pain, macrophage

INTRODUCTION

Chronic pain is the most frequent cause which evokes misery, disability and financial disturbance in community (Smith et al 1999). According to WHO, prevalence of chronic pain in developing countries is 41% (Croft et al 2010). Considering the impact developed by chronic pain, overcome it as soon as possible is an important thing to do. Nowadays, NSAIDs are the medicine most people use to reduce pain worldwide (Buvanendran & Lipman 2010). Prostaglandin that could be inhibited by NSAIDs has an important role in the escalation of inflammatory process (Ricciotti & Fitzgerald 2011). Cell membrane breakdown causes phospholipid leakage which turned into arachydonic acid by the help of A2 phospholipase enzyme. NSAIDs stick on the outer side so they prevent arachydonic acid reaches the active side to produce Prostaglandin (Pountos et al 2011). Long-term treatment using NSAIDs may create complications (Brattwall et al 2010) such as dyspepsia, peptic ulcer and gastric bleeding (Fujimori et al 2010).
Indonesia has many alternative treatments against chronic pain, one of them is cupping therapy (Kasmui 2012). There were two methods of cupping therapy, those are dry cupping therapy and wet cupping therapy. Dry cupping therapy is a method of treatment where skin is being applied for negative pressure against, whereas in wet cupping therapy in addition, skin is also being pricked. Even though cupping therapy has been known for thousands years but experiment just conducted in recent years. Experiments show that both wet and dry cupping therapies are effective to reduce the pain in Brachialgia paraesthetica nocturna (Lüdtke et al 2006), headache (Ahmadi et al 2008), Carpal Tunnel Syndrome (Michalsen et al 2009) and neck pain (Lauche et al 2012). However, still the efficacy distinction of both therapies haven’t been known yet.

There are two responses of acute inflammation: vascular response and cellular response. Cellulare response is being marked by neutrophil activity in early phase of inflammation and macrophage in late phase of inflammation. Cupping therapy increases both innate and acquired immunities (El Sayed et al 2013). Macrophage, mast cell and lymphocyte are cells which playing role in innate immunity. Inflammation induces the uplift of opioid receptor in dorsal cornu of medulla spinalis while cytokines (IL-1, IL-6 and TNF-α) (Garcia et al 2012) that provoked the uplift of the transfer in opioid receptor peripherally. Opioid is known as a potent analgesic. Immune cells which are playing role the most in endorphin expression of cupping therapy haven’t been known yet. The aims of this research were to compare the effectiveness in pain relief between dry cupping therapy and wet cupping therapy and to find out the overview of macrophage, mast cell and lymphocyte activity.

MATERIALS AND METHODS

Whole procedures have been tested by Ethical Committee in Faculty of Veterinary Medicine Airlangga University. White rats, Wistar type, weight 300-500 grams originated from Laboratory of Research and Integrated Testing (LPPT) Gajah Mada University. Four to five rats are being stalled with sufficient pellet and water. Complete Freud’s Adjuvant (CFA) is produced by Sigma Chemicals Company (USA). Experimental Pain in Animal Model has been reported by Huang et al (2008) that was 100 L CFA injected on the surface of plantar side of their left foot using 28-gauge needle and after that they are being placed in room temperature and experiments will be performed in the next 48 hours.

In dry cupping group, researchers performed the vacuum technique using -200 mmHg negative pressure for about 5 minutes on left and right back side. Whereas, in wet cupping group researchers performed the prick technique for about 10 pricks onto left and right back side with lancet and after that continue to perform vacuum technique for about 5 minutes. Betadine was applied into the wound and they are returned to the cage after that. Pain Threshold Reaction Time Test measured using hot plate device (Ugo Basile, Italy). First, white rats are being adapted by being placed inside Plexiglas box (23 x 18 x 14 cm). The surface of the hot plate is being heated until reach 51°C temperature using digital thermometer with 0,1°C accuracy. The provision of heat is stopped if there’s no response within 30 seconds to prevent tissue damage.

Forty-eight hours after the injection of CFA, 32 rats are divided randomly into 4 groups: negative control group, positive control group, dry cupping therapy group and wet cupping therapy group. Negative control group is normal white rats group. Positive control group is white rats group which injected with CFA. Dry cupping therapy is white rats group which injected with CFA and applied with dry cupping therapy. Wet cupping therapy group is white rats group which injected with CFA and applied with wet cupping therapy.

Dry cupping therapy started by shaving right and left back fur on lumbar area around 2.5 x 2.5 cm, then it placed a 2.0 cm diameter cup onto the shaved area and after that sucking is applied with -200 mmHg negative pressure for 5 minutes. The last step was removed the cup. Wet cupping therapy first step was also similar with thus on dry cupping therapy. Right afterwards the shaved area sterilized with 10% liquid iodine. Then, shaved area punctured with lancet 10 times. The next step was placed a 2.0 cm diameter cup onto the shaved area and after that sucking is applied with -200 mmHg negative pressure for 5 minutes. After treatment done, cup should be removed, then continued with sterilized punctured area with 10% liquid iodine. Twenty-four hours after the procedure, experimental animals were anesthetized with chloroform and cervical spine dislocation is applied. Skin tissue in cupped area was taken and placed into 10 % formaldehyde fixative buffer solution. Data was presented in the mean ± Standard Deviation formula. The distinction among groups was analyzed using one-way analyses of variance (Anova). Conclusion made after obtained p < 0.05 on statistically distinction.

RESULTS

Pain threshold reaction time in each group which is using hot plate (Ugo basile) on this experiment can be seen in table 1. It was showing that there was no difference of pain threshold reaction time between
negative control group and positive control group significantly. In the other hand, both the pain threshold reaction time between dry cupping therapy group versus positive control group and dry cupping therapy group versus wet cupping therapy show significant difference. Hematoxylin and Eosin (HE) stain in Picture 1 shows the activity of macrophage, mast cell and lymphocyte on each group. Table 2 shows mean and standard deviation of the amount of macrophage, mast cell and lymphocyte on each group. Those data mentioned above show that macrophage cell is the most dominant cell in wet cupping therapy group while mast cell and lymphocyte is the most dominant cell in positive control group.

Table 1. The comparison of pain threshold reaction time among groups.

<table>
<thead>
<tr>
<th>Group</th>
<th>Pain Threshold Reaction Time</th>
<th>Anova one way Significance (p)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>X</td>
<td>SD</td>
</tr>
<tr>
<td>K1</td>
<td>10.6±2.7</td>
<td>6.9</td>
</tr>
<tr>
<td>K2</td>
<td>11.7 ± 3.5</td>
<td>7.5</td>
</tr>
<tr>
<td>P1</td>
<td>16.9±3.6</td>
<td>3.6</td>
</tr>
<tr>
<td>P2</td>
<td>22.8±6.3</td>
<td>6.3</td>
</tr>
</tbody>
</table>

Notes: *Significance when α = 0.05
[a,b,c] Different superscripts showed the difference among groups (based on LSD).

Table 2. The summary result of the mean and and standard deviation of macrophage, mast cell and lymphocyte on each group

<table>
<thead>
<tr>
<th>Group</th>
<th>Macrophage</th>
<th>Mast Cell</th>
<th>Lymphocyte</th>
</tr>
</thead>
<tbody>
<tr>
<td>K0</td>
<td>2.75 ± 1.28</td>
<td>2.87 ± 1.55</td>
<td>3.62 ± 2.44</td>
</tr>
<tr>
<td>K1</td>
<td>12.50 ± 3.58</td>
<td>13.25 ± 2.76</td>
<td>12.00 ± 2.26</td>
</tr>
<tr>
<td>P1</td>
<td>14.12 ± 4.18</td>
<td>6.50 ± 2.50</td>
<td>3.87 ± 2.58</td>
</tr>
<tr>
<td>P2</td>
<td>15.75 ± 4.52</td>
<td>4.00 ± 2.07</td>
<td>4.12 ± 2.10</td>
</tr>
</tbody>
</table>

Figure 1. Slice of skin tissue with hematoxylin and eosin stain in negative control group (A), positive control group (B), dry cupping control group (C) and wet cupping control group (D). Red arrow points macrophage cell, yellow arrow points macrophage cell, green arrow points lymphocyte and black arrow points keratin cell. Four-hundred-power magnification with light microscope and Nikon E100 camera.
DISCUSSION

Wet and dry cupping therapies have been used by our ancestors for thousands of years (Ahmadi et al 2008). Herodotus, 400 years BC, recorded that wet and dry cupping therapies have been used by Egyptians (Turk & Okifuji 2010). Even though those have been known for thousands of years, the scientific research about cupping therapy just conducted in recent years. Farhadi et al (2009) in Iran conducted the scientific research in 98 non-specific Low Back Pain patient and concluded that wet cupping therapy was effective to reduce the pain. Other researcher also shows that the exposure of both wet and dry cupping therapies are effective to reduce the pain.

In our experiment, we are using white rats as experimental animals which are induced by complete Freund’s adjuvant. We obtain that wet and dry cupping therapies are increasing the pain threshold reaction time longer than control group. The pain threshold reaction time in wet cupping therapy group is longer than dry cupping therapy’s significantly. We receive also that wet cupping therapy is more effective than dry one and macrophage cell is the most dominant one in both dry and wet cupping therapies although in wet cupping therapy the dominancy was shown obviously.

We use inflammatory process paradigm in our experiment which is based on fact that both wet and dry cupping therapies induce redness on applied skin (Figure 1). Inflammation is an early defense mechanism to injury. Main effector cells in this early defense mechanism include macrophage, neutrophil, dendritic cell and NK (Natural Kill) cell. Cell response to the inflammatory process is being marked with the approach of leucocyte into the injury. In early inflammatory process, neutrophil and monocyte are dominant around the injury area. Moment after that, neutrophil and monocyte have migrated to the affected area where neutrophil came earlier on a huge amount. In the end stage of inflammatory process, neutrophil has decreased whereas macrophage became dominant. Macrophage is considered to be the most important regulator in inflammatory reaction and source of growth factor production such as PDGF (Platelet-Derived Growth Factor), Fibroblast Growth Factor, TGF-β and TGF-α (Li et al 2007).

Peripheral inflammation evokes chemical mediators which are originated from tissue damage and inflammatory cells. Chemical mediators such as cytokine, prostaglandin, nitric oxide, etc trigger the peripheral terminal oh sensory nerve. Cytokines such as Interleukin-1 (IL-1), IL-6 and Tumor Necrotic Factor (TNF-α) are being produced around the bruise and induce the sensitization (Pol & Puig 2004). During the inflammatory process, Interleukin-1 can trigger the paraventricular nucleus of hypothalamus and evokes the release of corticotropin-releasing hormone and activates Hypothalamic-Pituitary-Adrenal (HPA) axis. On cellular level, the bond between opioid antagonist and opioid receptor ( , δ and ) will obstruct adenylyl cyclase and lead to the degradation of cAMP, the elevation of potassium conductant, the inhibition of voltage sensitive Ca2+ channel and the degradation of excitation or inhibition transmitter output. Opioid also activate protein kinase C and calcium output from internal storage and activate Extracellular signal Related Kinase/Mitogen-Activated Protein Kinase (ERK/ MAPK) which lead to the phosphorylation of many targets in cytoplasm as well as cell nucleus (Pol & Puig 2004).

CONCLUSION

Both wet and dry cupping therapies can be used as alternative methods to reduce the pain where wet cupping therapy is more effective than dry cupping therapy. The activity of macrophage cell can be used to determine the activity of cell signaling in both wet and dry cupping therapies.

REFERENCES

a randomized controlled trial. Complement Ther Med 17, 9-15