Malaysian Bee Venom Abrogates Carrageenan Induced Inflammation in Rats

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Received 13 April 2010, accepted subject to revision 21 May 2010, accepted for publication 08 November 2010.

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Summary

This study was carried out to test the hypothesis that Malaysian bee venom (MBV) has the potential to produce a potent anti-inflammatory effect. MBV was obtained by electrical stimulation technique without causing death to the bees. An animal model with carrageenan (CR) induced inflammation was employed and paw volume was measured at each specific time point during the period of this study. Our findings demonstrate that MBV has an anti-inflammatory effect on 1% CR induced inflammation in the rat paw, making it potentially useful in the development of future anti-inflammatory therapies.

Keywords: Malaysian bee venom, anti-inflammation, carrageenan, rat.

Introduction

Discovery of new anti-inflammatory drugs from natural sources with lower and fewer side effects is demanding. The current anti-inflammatory drugs that have been conventionally used to treat patients with inflammatory associated diseases have various side and adverse effects (Verpoorte, 1999).

Additionally, Pincus et al. (1992) reported that patients were at risk of serious side effects including gastric ulcerogenicity and renal failure from long treatment with non-steroidal anti-inflammatory drugs (NSAIDs). It was found that the application of NSAIDs did not improve the patients’ condition since it was aimed to reduce disease symptoms (Scott et al., 1998).

Bee venom (BV) therapy which utilizes the application of bee venom to treat various diseases has been used since ancient times in traditional medicine (Beck, 1935; De Klobusitzky, 1971; Billingham et al., 1973; Hider, 1988). BV was mainly used to treat many inflammatory disorders such as rheumatoid arthritis (De Klobusitzky, 1971; Yoshimoto, 1985; Beck, 1935; Billingham et al., 1973; Caldwell, 1999; Eiseman et al., 1982; Hadjipetrou & Yangou, 1984; Somerfield et al., 1988), Lyme disease (Lubke et al., 1997), Multiple Sclerosis and osteoarthritis (Somerfield, 1986; Castro et al., 2005).

Furthermore, research in various animal experimental models with inflammatory diseases demonstrated that BV administration was successfully effective in suppressing the inflammation. (Kwon et al., 2001; Eiseman et al., 1982; Hadjipetrou & Yangou, 1984; Kwon et al., 2002; Kang et al., 2002; Kwon et al., 2003). Interestingly, BV administration through acupuncture point (acupoint) was proven successful for producing a strong therapeutic effect as compared injection to non-acupoint area (Kwon et al., 2001).

Modern biochemical analysis has been employed to identify the components in BV. As a result, there are at least 18 different components including a variety of peptides (i.e melittin, apamin, adolapin and mast-cell-degranulating (MCD) peptide), enzymes (i.e., phospholipase [PL] A2, hyaluronidase) and biologically active amines (i.e., histamine and epinephrine). Besides that, BV also contains nonpeptide components such as lipids, carbohydrates and free amino acids. These BV components were reported to have a wide variety of pharmaceutical properties (Lariviere & Melzack, 1996).

On the other hand, research on bee venom fractionation has been established in order to identify those specific components with anti-inflammatory activity. In this regard, adolapin which was discovered as a prostaglandin antagonist, possesses a strong anti-inflammatory effect which is 70 times more powerful than indomethacin (Shkenderov and Koburova, 1982). MCD peptide was
also showed to have a potent anti-inflammatory activity (Martin & Hartter, 1980; Shkenderov & Koburova, 1982).

Until today, the exact mechanism and basis underlying the anti-inflammatory effect of BV is unknown and still remains to be discovered but many theories were suggested to justify and postulate its entire mechanism based on the previous and on-going research findings. Since BV contains a wide number of constituents, attempts to investigate them individually are slightly challenging because synergistic interaction among the BV components may occur.

However, BV administration was reported to stimulate the function of immune system (Vick et al., 1972) and to affect the release of cortisol production which is known as natural anti-inflammatory agent (Vick & Shipman, 1972).

Melittin which is the major component of BV was found to suppress inflammation by inhibiting phospholipase (PLA) enzymatic activity (Saini et al., 1997). This enzyme was abundantly released in severe inflammatory disorders and actively found to cause tissue and organ degradation which will lead to the loss of their functions (Mihelich & Schevitz, 1999). Furthermore, melittin was also found to block the production of neutrophil superoxide (Somerfield et al., 1986).

Based on the wide literature of BV application in traditional medicine and abundance evidence reported on BV anti-inflammatory effect, we hypothesize that MBV has the potential to produce a strong anti-inflammatory effect. In the present study, we investigate the potential of MBV anti-inflammatory effect on carrageenan-induced inflammation in rat paw model.

**Materials and methods**

**Chemicals:**

Carrageenan was purchased from Sigma Chemical Co. (St. Louis, MO, USA)

**MBV extraction:**

Venom from Malaysian bees (*Apis melifera*) was extracted using electrical stimulation device according to the method of Lariviere & Melzack (1996). The samples were then freeze-dried prior stored at cold room until used.

**Animals:**

Male Sprague-Dawley rats approximately the same age and weighing about 200-300 g were used for this study. These animals were obtained from Animal House, University of Malaya (UM). The rats were kept in a quiet and clean experimental room with a temperature of 22°C at least one day prior to the beginning of the experiment. Rats were exposed to a 12 hr alternating light-dark cycle (7:00 a.m. onset). Food and water were available *ad libitum*. Animals were fasted and given only water at least 12 hour prior to and during the experiments. Methods used in the present study was cleared and approved by the Animal Ethics Committee, Faculty of Medicine, UM (PM/12/7/2006/MNAM).

**Experimental groupings:**

Four groups of animals (1 experimental and 3 control groups) with seven animals for each group (N=7) were used in this study. Three control groups were divided as follows: (1) HEALTHY (saline - saline) (2) HEALTHY TREATED WITH MBV (MBV - saline) and (3) INFLAMMATION (saline - CR). Animals in the fourth experimental group (INFLAMMATION TREATED WITH MBV) were treated with MBV prior injected with CR.

**MBV and saline administration:**

Thirty minutes prior to CR injection, MBV (dissolved in 0.9% saline at a dose of 0.1 mg/kg) or saline was injected subcutaneously into Zusanli acupoint on the right hind limb as previously described by Lee et al., (2001).

**CR administration:**

0.1 mL of 1% CR (1 mg/100 µL of saline) was administered subcutaneously at the subplantar area of the right hind paw to induced inflammation.

**Measurement of paw volume:**

The paw was marked with marker pen at the level of tibio-tarsal joint. Paw volume volumes were measured using plethysmometer (Ugo Basile, Italy) before (V0) CR administration. The measurements were consistently taken at 30 minute intervals for seven hours and at 24 hours (Vt) after CR administration.

**% of Inflammation calculation:**

V0 was used as the basal volume and Vt represented the volume for each time point respectively. The degree of inflammation was indicated by the difference between the basal volume and subsequent reading of paw measurement. Data were then converted to a percentage of inflammation using the following equation:

\[
\text{% of inflammation} = 100 \times \left( \frac{V_0 - V_t}{V_0} \right)
\]

The results are expressed as mean± SEM.

**Statistical analysis:**

Results from the experimental group which is INFLAMMATION TREATED WITH MBV was compared with INFLAMMATION group to determine the effect of MBV on CR-induced inflammation in rat paw. Analysis of the variance (ANOVA) was applied for the evaluation of the result. Probability of p<0.05 were considered as statistically significant. The SPPS 12.0 programme was used for the statistical analysis.
Results

We have tested and evaluated the effect of MBV on carrageenan-induced inflammation in rat paw. Paw volume was increased in the INFLAMMATION group after the administration of the phylogistic agent (carrageenan), as compared with the HEALTHY group, demonstrating that inflammation was evoked by the injection of carrageenan. Maximal increase in paw size was noted after 4.5 hr.

Inflammation group evoked by carrageenan and treated with MBV (INFLAMMATION TREATED WITH MBV) manage to reduce the inflammation as compared to those untreated with MBV (INFLAMMATION) (Table 1). MBV treatment has reduced inflammation started at T2.5 until the end T7. The results obtained indicate that MBV had a significant anti-inflammatory effect (P<0.05).

Discussion

Our findings suggests that MBV has anti-inflammatory effect on carrageenan induced inflammation on rat paw and confirm observations by Lee et al., (2001).

The animal model of carrageenan induced inflammation which was developed by Winter and his team (Winter et al., 1962) has been used extensively since 1962. This model has been shown to be one of the best methods to screen anti-inflammatory drugs (Chan et al., 1995) since it has shown an acceptable level of reproducibility (Winter et al., 1962). In this experimental model, oedema which is one of the signs of inflammation, was used as a parameter to measure the anti-inflammatory effect of MBV. Oedema formation is characterised by the presence of several mediators including histamine, serotonin, bradykinin, prostaglandins and nitric oxide at the area of inflammation (Di Rosa et al., 1971; Seibert et al., 1994; Salvemini et al., 1996; Nantel et al., 1999, Damas et al., 1986; Heller et al., 1998). In the carrageenan-induced inflammation experimental model, there were two phases involved known as early and late phases.

Table 1. Percentage of inflammation at different times of MBV anti-inflammatory effect on carrageenan induced inflammation on rat's paw.

<table>
<thead>
<tr>
<th>Time (hr)</th>
<th>Healthy</th>
<th>Healthy treated with MBV</th>
<th>Inflammation</th>
<th>Inflammation treated with MBV</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>0.5</td>
<td>12.19 ± 0.86</td>
<td>12.72 ± 0.83</td>
<td>15.13 ± 0.37</td>
<td>11.04 ± 1.04</td>
</tr>
<tr>
<td>1</td>
<td>8.99 ± 0.35</td>
<td>12.00 ± 0.60</td>
<td>17.12 ± 0.40</td>
<td>15.38 ± 1.78</td>
</tr>
<tr>
<td>1.5</td>
<td>11.71 ± 0.50</td>
<td>12.13 ± 0.73</td>
<td>21.24 ± 0.65</td>
<td>13.78 ± 2.04</td>
</tr>
<tr>
<td>2</td>
<td>8.01 ± 0.52</td>
<td>11.48 ± 0.60</td>
<td>31.50 ± 0.99</td>
<td>20.51 ± 2.69</td>
</tr>
<tr>
<td>2.5</td>
<td>9.66 ± 0.83</td>
<td>11.06 ± 0.67</td>
<td>39.18 ± 1.25</td>
<td>31.44 ± 2.66*</td>
</tr>
<tr>
<td>3</td>
<td>9.60 ± 0.95</td>
<td>10.35 ± 0.66</td>
<td>44.69 ± 1.48</td>
<td>40.78 ± 2.74*</td>
</tr>
<tr>
<td>3.5</td>
<td>4.55 ± 0.43</td>
<td>9.71 ± 0.65</td>
<td>55.44 ± 3.03</td>
<td>45.02 ± 3.48*</td>
</tr>
<tr>
<td>4</td>
<td>3.86 ± 0.23</td>
<td>9.21 ± 0.48</td>
<td>61.08 ± 3.11</td>
<td>44.93 ± 3.26*</td>
</tr>
<tr>
<td>4.5</td>
<td>2.99 ± 0.30</td>
<td>9.11 ± 0.46</td>
<td>63.03 ± 2.57</td>
<td>44.16 ± 3.11*</td>
</tr>
<tr>
<td>5</td>
<td>2.81 ± 0.29</td>
<td>8.78 ± 0.55</td>
<td>55.32 ± 2.88</td>
<td>37.43 ± 3.37*</td>
</tr>
<tr>
<td>5.5</td>
<td>2.98 ± 0.31</td>
<td>7.90 ± 0.66</td>
<td>50.46 ± 2.25</td>
<td>35.95 ± 3.24*</td>
</tr>
<tr>
<td>6</td>
<td>2.90 ± 0.37</td>
<td>7.25 ± 0.63</td>
<td>48.64 ± 2.18</td>
<td>32.54 ± 2.89*</td>
</tr>
<tr>
<td>6.5</td>
<td>3.85 ± 0.22</td>
<td>7.01 ± 0.48</td>
<td>42.54 ± 1.44</td>
<td>42.54 ± 1.44</td>
</tr>
<tr>
<td>7</td>
<td>3.60 ± 0.18</td>
<td>5.54 ± 0.40</td>
<td>41.06 ± 1.67</td>
<td>25.89 ± 3.27*</td>
</tr>
<tr>
<td>24</td>
<td>3.18 ± 0.64</td>
<td>3.77 ± 0.44</td>
<td>30.21 ± 1.50</td>
<td>12.38 ± 1.36</td>
</tr>
</tbody>
</table>

* p<0.05 when compared with control (INFLAMMATION). Values are expressed as mean±SEM (n=7).
phase (Gamache et al., 1986). Both phases were termed as biphasic (Vinegar et al., 1969). The early phase which occurred during the early 60 minutes after CR administration showed the liberation of histamine, serotonin and bradykinin while the prostaglandin-like compounds were abundance during the late phase (Crunkhorn & Meacock, 1971; Suleyman et al., 2004) as well as the released of free radicals including hydrogen peroxide, superoxide and hydroxyl radicals (Marzocco et al., 2004; Weissmann, 1993). The period of late phase begins at 1 hour subsequently after the CR injection (Honda et al., 2002).

Furthermore, the late phase of carrageenan-induced inflammation was also influenced by the role of enzymes such as cyclooxygenase and lipoygenase (Marzocco et al., 2004). Carrageenan induced inflammation was reported to increase the production of interleukin-1 (IL-1) and tumour necrosis factor (TNF), as both are proinflammatory cytokines (Pinheiro and Calixto, 2002).

Overall, we suggest that MBV may possess anti-inflammatory effect by inhibiting signalling pathways that are related with inflammatory mediators and suppress the complement cascade induced by CR including inhibited the synthesis of prostaglandins (PG), as well as blocking the production of superoxide and metabolism of arachidonic acid. Further investigation is, however, needed to test this hypothesis.

In addition, MBV may inhibit the production of pro-inflammatory cytokines such as IL-1 and TNF. Interestingly, therapeutic strategies which related with the inhibition of TNF production are widely used for the management of inflammatory associated diseases including rheumatoid arthritis (Van & Rutgeerts, 1995; Feldmann, 2002).

Conclusion

Our results demonstrate that MBV has anti-inflammatory effect on CR induced inflammation in rat paw. This might explain the traditional use of bee venom in the treatment of inflammatory associated diseases. However, further clinical studies are essential for the development of anti-inflammatory agent to treat various inflammatory associated diseases.

Acknowledgements

This study was funded by University of Malaya Postgraduate Research Fund (PS179/2007B). We would like to thank to Mr Johgalingam from Dept of Physiology for his excellent technical advice during this study.

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