The Study of anti-inflammatory activity of Buspirone in rats

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Abstracts:
Inflammation is fundamentally a protective response of the body. It is a complex biological response of vascular tissues to harmful stimuli. It is the body’s natural reaction to protect itself from infection and foreign substances. Inflammation continues to be an area of great interest for research probably due to non-availability of a safer and more effective anti-inflammatory agent. The study was aimed at answering a few important questions and issues if buspirone is having anti-inflammatory effect. In the present study we describe the effects of buspirone in animal models of acute as well as subacute inflammation. Buspirone, is a 5-HT$_{1A}$ receptor partial agonist which is widely used for treating anxiety.$^{1, 2}$ Study was carried out in 4 groups of rats (6 rats/group) of either sex for anti-inflammatory tests. In acute and subacute model of inflammation of present study, buspirone (16 mg/kg), a 5HT$_{1A}$ receptor partial agonist, exerted anti-inflammatory effect. From the present study we can conclude that buspirone in high dose has anti-inflammatory effect.

Key words: Buspirone, Inflammation

Introduction:
Inflammation is fundamentally a protective response of the body. It is a complex biological response of vascular tissues to harmful stimuli. It is the body’s natural reaction to protect itself from infection and foreign substances. In the practice of medicine, the importance of inflammation is that it can sometimes be inappropriately triggered or poorly controlled, and is thus the cause of tissue injury in many disorders. Inflammation continues to be an area of great interest for research probably due to non-availability of a safer and more effective anti-inflammatory agent. The conventional nonselective non steroidal anti-inflammatory drugs (NSAIDs) like aspirin, diclofenac etc possess side effects like gastrointestinal disturbances, ulcerations, impairment of renal functions and hypersensitivity reactions. The newer COX-2 selective agents such as celecoxib & etoricoxib etc retain the anti-inflammatory effect characteristic of NSAIDs with a marked increase in gastrointestinal tolerability as compared to classic non selective ones. But even these agents show cardiotoxic, renotoxic and hepatotoxic side effects. Hence the search for alternatives or adjuvants to these drugs is still going on in an attempt to produce an ideal anti-inflammatory drug.

In the present study we describe the effects of buspirone in animal models of acute as well as subacute inflammation. Buspirone, is a 5-HT$_{1A}$ receptor partial agonist which is widely used for treating anxiety.$^{1, 2}$ 5-HT$_{1A}$ receptors are located presynaptically on cell bodies in the raphe nuclei (somatodendritic receptors) and postsynaptically in 5-HT forebrain projecting areas. By activating somatodendritic receptors, 5-HT and 5-HT$_{1A}$ receptor agonists decrease the firing of 5-HT neurons in the...
raphe, and, consequently decrease 5-HT terminal release.\(^3\)

Data suggest that a tonic release of serotonin in the spinal cord may occur during ongoing peripheral inflammation, modulating the neurogenic component of edema either by an inhibitory action on 5-HT\(_1\) receptors or by a stimulatory action on 5-HT\(_2\) receptors.\(^4\)

Aims and objectives:
1. To find out the anti-inflammatory activity of Buspirone.
2. To compare the anti-inflammatory activity of Buspirone with NSAIDs.

Material and Methods:
Study was carried out in 4 groups of rats (6 rats/group) of either sex for anti-inflammatory tests at Dept of pharmacology and central animal house, Bharati Vidyapeeth Deemed University, Medical College and Hospital Sangli. The protocol and synopsis was discussed in IAEC (Institutional Animal Ethical Committee), including number of animals, reuse of animals and end point in each test. IAEC approved the project, IAEC approval registration no. – BVDUMC&H/Sangli/CAH/IAEC/2012-13/04.

Animals-
Male and female (non pregnant) Wistar rats weighing (200-250 g) of body weight were used. They were housed under standardized conditions (temperature 25\(^\circ\) Celsius, relative humidity 60% & 12 hour light/dark cycle). They had access to standard pellet diet and water ad. libitum. Experiments were conducted between 9:00 to 16:00 hours. All animal procedures were in accordance with the recommendations for the proper care and use of laboratory animals. The doses of drugs employed in the study were based upon the human dose after conversion to that of rat.\(^5\)

Drugs: The clinical doses of various drugs were converted to rat equivalent doses. The volume of drug administered intraperitoneally was < 1ml/100gm of rat. The drug requirement for each animal was calculated by weight and the required amount of drug was dissolved in appropriate volume of the vehicle. All the drugs were freshly prepared on the day of the experiment and used the same day.

Acute inflammation was produced by injecting carrageenan in the hind paw of Wistar rats and subacute inflammation by implanting sterile cotton pellets subcutaneously as described below.

Carrageenan-induced rat paw oedema\(^6\)
Test drugs were administered intraperitoneally according to the body weight 30 minutes before injecting 0.05 ml of 1% of sterile carrageenan in normal saline into subplantar region of the left hind paw. A mark was made at the left ankle joint. Contralateral limb received equal volume of saline. Paw volume up to the ankle joint was measured in drug treated & untreated group. The edema volume was measured by mercury displacement with the help of a plethysmograph before carrageenan injection and 0, 30 min and 1, 3, 5 hours, 5\(^{th}\) day and 14\(^{th}\) day after injecting carrageenan. The difference between 0 hour & subsequent readings was considered as edema volume.
Percentage reduction in edema = 
\[
\frac{\text{Mean edema in control group} - \text{Mean edema in drug treated group}}{\text{Mean edema in control group}} \times 100
\]

At the end of this study, no animal showed grave injury or permanent disability after 8 weeks. After 6-8 weeks all animals gained a healthy status on careful examination. During the recovery period the animals were followed up by standard procedures as outlined by CPCSEA guidelines. After washout period of 8 weeks the healthy animals were mixed with common pool to minimise animal use. From the common pool healthy animals were screened for cotton pellet – induced granuloma test.

**Cotton pellet –induced Granuloma**

This method is widely used to study the exudative & proliferative phases of inflammation. Autoclaved cotton pellets (5mg) were used. Under ether anaesthesia, pellets were inserted subcutaneously through skin incision in the axilla of the animals.

Drug treatment was started 2 hours after cotton pellet implantation & continued for 5 consecutive days. Vehicle treated animals received normal saline for the same duration as the drug. On the 6th day, under anaesthesia granulomas were removed surgically and skin was sutured. The granulomas were dried for 24 hours at 60° celcius and the dry weights were determined. The weight of granulomatous tissue formed was calculated by subtracting initial weight from the final dry weight of cotton pellets and percentage protection by the drug was calculated.

Mean granuloma dry weight for the various groups were calculated and expressed as mg/100gm body weight using following formula. The percentage inhibition of granuloma dry weight was calculated using formula.

\[
\text{Percentage inhibition of granuloma dry weight} = \frac{(Tc - Tt)}{Tc} \times 100
\]

Where
- Tc = dry weight of granuloma in control groups.
- Tt = dry weight of granuloma in treated groups.

At the end of this study, no animal showed grave injury or permanent disability after 8 weeks. After 8-10 weeks all animals gained a healthy status on careful examination. During the recovery period the animals were followed up by standard procedures as outlined by CPCSEA guidelines. At the end of anti-inflammatory studies, no animal showed permanent disability after 8 weeks.

**Statistical Analysis**: Statistical analysis was carried out using one way ANOVA (Analysis of variance) for significance between groups. Data was expressed as mean ±S.E. The level of significance between individual groups was detected using unpaired “t” test. For all tests, effects with a probability of P < 0.05 were considered to be significant.
**Results:**

**Table 1:- Effect of various treatments on carrageenan induced paw oedema**

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>Drug</th>
<th>Mean paw volume at the end of each observation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>30 min</td>
</tr>
<tr>
<td>I</td>
<td>6</td>
<td>Control (0.2ml NS)</td>
<td>0.417+/−</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.083</td>
</tr>
<tr>
<td>II</td>
<td>6</td>
<td>Ibuprofen (30mg/kg)</td>
<td>0.500+/−</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.000</td>
</tr>
<tr>
<td>III</td>
<td>6</td>
<td>Buspirone (8 mg/kg)</td>
<td>0.500+/−</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.000</td>
</tr>
<tr>
<td>IV</td>
<td>6</td>
<td>Buspirone (16mg/kg)</td>
<td>0.500+/−</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.000</td>
</tr>
</tbody>
</table>

p < 0.05 is significant  * p < 0.05 compared with control  

p < 0.001 is highly significant  # p < 0.05 compared with Ibuprofen  
@ p < 0.05 compared with Buspirone 16 mg/kg

**Graph 1:- Percentage inhibition of paw edema at 3 hr**

Graph showing percentage inhibition of paw edema at 3 hr for groups II, III, and IV.

Gr II Ibuprofen 30 mg/kg;
Gr III  Buspirone 8 mg/kg ;  Gr IV Buspirone 16 mg/kg

**Graph 2: Percentage inhibition of paw edema at 5 hr**

<table>
<thead>
<tr>
<th></th>
<th>% inhibition of paw edema at 5 hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gr II</td>
<td>88</td>
</tr>
<tr>
<td>Gr III</td>
<td>65</td>
</tr>
<tr>
<td>Gr IV</td>
<td>88</td>
</tr>
</tbody>
</table>

Gr II Ibuprofen 30 mg/kg ;
Gr III Buspirone 8 mg/kg ;  Gr IV Buspirone 16 mg/kg

**Graph 3: Percentage inhibition of paw edema on 5th day**

<table>
<thead>
<tr>
<th></th>
<th>% inhibition of paw edema on 5th day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gr II</td>
<td>100</td>
</tr>
<tr>
<td>Gr III</td>
<td>87</td>
</tr>
<tr>
<td>Gr IV</td>
<td>100</td>
</tr>
</tbody>
</table>

Gr II Ibuprofen 30 mg/kg ;
Gr III Buspirone 8 mg/kg ;  Gr IV Buspirone 16 mg/kg
Table 2:- Effect of various treatments on granuloma dry weight

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>Drug</th>
<th>Mean granuloma dry weight-- mg/100 gm body weight(Mean +/- SEM)</th>
<th>Percentage inhibition of dry weight of granuloma</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>6</td>
<td>Control (0.2ml NS)</td>
<td>23.00 +/- 0.365</td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>6</td>
<td>Ibuprofen (30mg/kg)</td>
<td>12.50 +/- 0.224                                             *</td>
<td>45.65</td>
</tr>
<tr>
<td>III</td>
<td>6</td>
<td>Buspirone (8 mg/kg)</td>
<td>16.50 +/- 0.224                                             * # @</td>
<td>28.26</td>
</tr>
<tr>
<td>IV</td>
<td>6</td>
<td>Buspirone (16mg/kg)</td>
<td>13.17 +/- 0.307                                             *</td>
<td>42.73</td>
</tr>
</tbody>
</table>

p < 0.05 is significant
p < 0.001 is highly significant
*p < 0.05 compared with control
# p < 0.05 compared with Ibuprofen
@ p < 0.05 compared with Buspirone 16mg/kg

Graph 4:- Mean granuloma dry weight

Gr I Control 0.2 ml NS;  Gr II Ibuprofen 30 mg/kg ;
Gr III Buspirone 8 mg/kg ; Gr IV Buspirone 16 mg/kg
**Graph 5:- Percentage inhibition of dry weight of granuloma**

<table>
<thead>
<tr>
<th>Group</th>
<th>Percentage Inhibition of Dry Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gr I</td>
<td>Control 0.2 ml NS</td>
</tr>
<tr>
<td>Gr II</td>
<td>Ibuprofen 30 mg/kg</td>
</tr>
<tr>
<td>Gr III</td>
<td>Buspirone 8 mg/kg</td>
</tr>
<tr>
<td>Gr IV</td>
<td>Buspirone 16 mg/kg</td>
</tr>
</tbody>
</table>

**Discussion:**

Buspirone is a 5HT<sub>1A</sub> receptor partial agonist and mainly used in the treatment of anxiety and depression.<sup>8</sup> Data suggest that a tonic release of serotonin in the spinal cord may occurs during ongoing peripheral inflammation, modulating the neurogenic component of edema either by an inhibitory action on 5-HT<sub>1</sub> receptors or by a stimulatory action on 5-HT<sub>2</sub> receptors.<sup>9</sup> So we were interested in evaluating anti-inflammatory of buspirone.

In acute and subacute model of inflammation of present study, buspirone (16 mg/kg), a 5HT<sub>1A</sub> receptor partial agonist, exerted anti-inflammatory effect. Carrageenan-induced rat paw oedema is a widely used test to determine anti-inflammatory activity and constitutes a simple and routine animal model for evaluation of pain at the site of inflammation without any injury or damage to the inflamed paw.<sup>10</sup>

Carrageenan is the phlogistic agent of choice for testing anti-inflammatory drugs as it is known to be non-antigenic and is devoid of apparent systemic effects. Local and systemic inflammation is associated with enhanced levels of the pro-inflammatory cytokines TNF-α, IL-1, and IL-6.<sup>11</sup>

In the present study degree of Paw edema inhibition by buspirone (16 mg/kg) was less than that of ibuprofen (30 mg/kg) at 3hr i.e. 69% for buspirone and 77% for ibuprofen. The cotton pellet induced granuloma method has been widely used to assess the transudative, exudative and a proliferative phase of subacute inflammation. The fluid adsorbed by the pellet correlates the wet weight of the granuloma, whereas the dry weight correlates well with the amount of granulomatous tissue formed.<sup>12</sup>

Present study showed that percentage inhibition of mean granuloma weight by buspirone (16 mg/kg) was less than that of ibuprofen (30 mg/kg) i.e. 42.73% for buspirone and 45.65% for ibuprofen.
Dahu et al. reported that buspirone injected intrathecally in rats decreased the inflammatory paw oedema caused by subcutaneous carrageenan.

**Conclusion:**
The present study indicates a comparable anti-inflammatory effect of buspirone (16mg/kg) to ibuprofen (30mg/kg). In comparison, previous studies have shown comparable promise of buspirone (8 and 16 mg/kg) as anti-inflammatory. No other studies fail to show such results. Our study endorses previous findings in the studies showing comparable use of buspirone (8 and 16mg/kg) as anti-inflammatory. From the present study we can conclude that buspirone in high dose has anti-inflammatory effect. Hence we hold promise in study involving buspirone (16mg/kg) in animals or humans in future for anti-inflammatory effect.

**References:**