EFFECT OF PIROXICAM ON THE CELLULAR RESPONSE IN BUFFALO CALF SKIN

Neelu Gupta¹, A.K. Katiyar² and Madhu Swamy²

ABSTRACT

The present work was conducted in apparently healthy buffalo calves 3-6 months in age. Calves were pretreated with piroxicam intramuscularly 30 minutes prior to intradermal injection of *Steph. epidermidis* suspension and turpentine. Lesions of different time intervals were obtained for the sequential study of cellular responses. Maximal suppression of leukocytes occurred at 3 h in both types of inflammation. In all the cases, neutrophils were more extensively suppressed as compared to other cells.

Keywords: *Staphylococcus epidermidis*, turpentine, piroxicam, neutrophils, monocytes, lymphocytes, basophils

INTRODUCTION

Specific antagonistic drugs have been used earlier in buffalo calves (Gupta et al., 2007, 2008a, 2008b) to study the chemical mediation of acute inflammation in this species. Piroxicam is a cyclooxygenase inhibitor and specifically blocks the synthesis of prostaglandins. However, to our knowledge, no such studies have been conducted in buffaloes. Thus, in the present study, the possible suppression of the cellular response in the inflammation induced by turpentine and *Staph. epidermidis* was studied in the buffalo calves pretreated with piroxicam.

MATERIALS AND METHODS

Twelve healthy male buffalo calves, aged 3 to 6 months, were divided into two groups: control and experimental, for the study of cellular response in the buffalo calf skin. The calves were maintained under hygienic conditions and fed standard feed.

**Substances—Normal saline:** (Wockhardt Ltd. Aurangabad) 0.9% w/v, sterile pyrogen free, isotonic solution was used for the preparation of the bacterial suspension.

**Staphylococcus epidermidis:** (MTCC-35) the culture was obtained from the Institute of Microbial Technology, Chandigarh. A bacterial suspension was prepared, and 0.1 ml was injected intradermally at each site. The concentration of the bacteria per ml of the suspension was determined as $2.3 \times 10^3$ million.

**Turpentine—** (SAM KAM, INDORE) the commercially available turpentine was used intradermally for the induction of inflammation. At each site 0.05 ml turpentine was injected.

**Piroxicam—**(Pfizer Limited Batch No.020-04065 Mumbai) of 20 mg/ml strength was used as a prostaglandin antagonist.

**Preparation of skin—** The site of the cutaneous reaction was prepared according to the method described in horses (Zarrilli and Calhoun, 1970) with suitable modifications. Briefly, one day before induction of the inflammation, hair from the lateral thoraco-abdominal region of the buffalo calves was removed by close shaving. The skin was cleaned with a soft cloth moistened with the sterile distilled water. On the following day, cleaning of the skin was repeated.

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**Induction of inflammation**- Inflammation was induced in the buffalo calf skin as described in the following groups.

**Control Group I**- Six calves of the control group were again divided into two subgroups, i.e., Subgroup IA and Subgroup IB. Each subgroup had three calves.

**Subgroup IA**- Each calf received two intradermal injections of the *Steph. epidermidis* (MTCC-35) suspension (0.1 ml) in the normal saline (Wockhardt Ltd. Aurangabad) for each time interval (0-2 minutes, 30 minutes, 1 h, 3 h, 6 h, 12 h, 24 h and 48 h) at both sides of the thoraco-abdominal region. At the appropriate time (immediately after the 0-2 time interval), the calves were euthanized with a saturated solution of magnesium sulphate given intravenously. The skin specimens were collected and fixed in the Cornoy’s fluid for the histo-pathological studies as described for the chicken by Shrivastava et al., 1997. Sections were cut to 4-5 μm thickness.

Skin sections were stained with the haematoxylin and eosin and with 0.05 percent solution of toluidine blue in acetate buffer (pH 3.8) for basophils as described for the chickens by Dhodapkar et al. (1987).

**Subgroup IB**- Each calf received two i/d injection of the turpentine (SAM KAM, INDORE) @ dose rate of 0.05 ml for each time interval (0-2 minutes, 30 minutes, 1 h, 3 h, 6 h, 12 h, 24 h and 48 h). The rest of the procedure was the same as that for Subgroup IA.

**Experimental Group II**- Six calves of the experimental group were again divided into two subgroups i.e., Subgroup IA and Subgroup IB. Each subgroup had three calves.

**Subgroup IA**- Each calf was pretreated with piroxicam (Pfizer Limited Batch No. 020-04065, Mumbai) i/m, at the dose rate of 0.3 mg/kg body weight 30 minutes prior to the i/d *Steph. epidermidis* and this treatment was repeated every 12 h. The rest of the procedure was the same as that for control group IA.

**Subgroup IB**- Each calf was pretreated with piroxicam i/m, at the dose rate of 0.3 mg/kg body weight 30 minutes prior to i/d injection of the turpentine (0.05 ml), and this treatment was repeated every 12 h. The rest of the procedure was the same as that for control group IA.

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**RESULT AND DISCUSSION**

**Control Group I**

**Subgroup IA**- The inflammatory reaction in the calf was induced by giving intradermal injections of *Steph. epidermidis*, and lesions of 0-2 minutes, 30 minutes, 1 h, 3 h, 6 h, 12 h, 24 h and 48 h were collected for the histopathology. The vascular and cellular changes were not noticeable at the initial intervals (0-2 minutes and 30 minutes). However, a few neutrophils were found at 0-2 minutes and 30 minutes. At 6 h, hyperaemia of the blood vessels and oedema of the dermis were well marked. From 30 minutes onwards, leukocytes infiltration had significantly increased, and the maximal number of leukocytes was observed at 12 h. The maximum numbers of neutrophils, monocytes, lymphocytes and basophils were recorded at 6, 12, 24 and 1 h, respectively. Infiltration of eosionophils was not noticed at any time interval (Table 1).

**Subgroup IB**- The inflammatory reaction in the calf was induced by giving intradermal inoculations of turpentine (0.5 ml), and lesions of different time intervals were collected for histopathology. The vascular and cellular changes were noticed from 30 minutes onward to 48 h. A few leukocytes were seen adhering to endothelium. From 30 minutes onwards cellular infiltration had significantly increased, and the maximal number of leukocytes was observed at 12 h. The number of neutrophils increased gradually up to 6 h, after which the number gradually decreased, but there were more than in the initial stages. The maximal number of neutrophils was observed at 6 h. The number of monocytes increased gradually up to 12 h, after which it decreased. Marked infiltration of monocytes were observed at 12 h. From 1 h onwards infiltration of lymphocytes increased, and the maximal number of lymphocytes was recorded at 48 h. Toluidine blue sections showed infiltration of basophils. Infiltration of basophils were observed from 30 minutes and gradually increased up to 3 h. Infiltration of eosionophils was not noticed at any time interval (Table 2).

**Experimental Group II**

**Subgroup IA**- Calves were pretreated with Piroxicam intramuscularly 30 minutes prior to intradermal injection of the *Steph. epidermidis* suspension, and lesions of different ages were
Figure 1. Section of buffalo calf skin 30 minutes after intradermal injection of *Staph. epidermidis* suspension. Note - emigration of leukocytes. H&E x 400.

Figure 2. Section of buffalo calf skin 30 minutes after intradermal injection of turpentine. Note - emigration of leukocytes. H&E x 400.

Figure 3. Section of buffalo calf skin 12 h after intradermal injection of *Staph. epidermidis* suspension. Note - intense infiltration of leukocytes. H&E x 400.

Figure 4. Section of buffalo calf skin 12 h after intradermal injection of turpentine. Note - maximal number of leukocytes. H&E x 400.

Figure 5. Section of piroxicam preatreated buffalo calf skin 3 h after intradermal injection of *Satph. epidermidis* suspension. Note - maximal suppression of leukocytes. H&E x 400.

Figure 6. Section of piroxicam preatreated buffalo calf skin 3 h after intradermal injection of turpentine. Note - maximal suppression of leukocytes. H&E x 400.

Figure 7. Section of buffalo calf skin 48 h after intradermal injection turpentine. Note - completely disintegrated neutrophils. H&E x 400.

Figure 8. Section of buffalo calf skin 12 h after intradermal injection turpentine. Note - the presence of giant cells in the interstitium. H&E x 400.

Figure 9. Section of piroxicam preatreated buffalo calf skin 12 h after intradermal injection of Turpentine. Note - the presence of giant cells in the interstitium. H&E x 400.
Table 1. Tissue leukocytosis in response to *Staphylococcus epidermidis* in control and piroxicam pretreated buffalo calves.

<table>
<thead>
<tr>
<th>Time Interval</th>
<th>Neutrophils</th>
<th>Monocytes</th>
<th>Lymphocytes</th>
<th>Basophils</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Staph. epidermidis</td>
<td>Piroxicam</td>
<td>Percent Suppression</td>
<td>Staph. epidermidis</td>
<td>Piroxicam</td>
</tr>
<tr>
<td>0-2 min</td>
<td>0.0 ± 0.221</td>
<td>0.0 ± 0.125</td>
<td>0.0 ± 0.277</td>
<td>0.0 ± 0.125</td>
<td>0.0 ± 0.149</td>
</tr>
<tr>
<td>30 min</td>
<td>0.433 ± 0.992</td>
<td>0.900 ± 0.649</td>
<td>1.533 ± 0.56</td>
<td>1.466 ± 0.223</td>
<td>1.000 ± 0.174</td>
</tr>
<tr>
<td>12 hr</td>
<td>50.266 ± 1.206</td>
<td>45.166 ± 3.164</td>
<td>10.146 ± 1.883</td>
<td>5.846 ± 1.784</td>
<td>14.333 ± 1.763</td>
</tr>
<tr>
<td>24 hr</td>
<td>10.066 ± 3.023</td>
<td>9.500 ± 0.630</td>
<td>7.690 ± 1.530</td>
<td>5.066 ± 0.523</td>
<td>4.933 ± 1.747</td>
</tr>
<tr>
<td>48 hr</td>
<td>0.000 ± 0.000</td>
<td>0.000 ± 0.000</td>
<td>0.000 ± 0.000</td>
<td>0.000 ± 0.000</td>
<td>0.000 ± 0.000</td>
</tr>
</tbody>
</table>
Table 2. Tissue leukocytosis in response to turpentine in control and piroxicam pretreated buffalo calves.

<table>
<thead>
<tr>
<th>Time Interval</th>
<th>Neutrophils</th>
<th>Monocytes</th>
<th>Lymphocytes</th>
<th>Basophils</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Turpentine</td>
<td>Proxicam</td>
<td>Percent Suppression</td>
<td>Turpentine</td>
<td>Proxicam</td>
</tr>
<tr>
<td>0-2 min</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>30 min</td>
<td>10.200</td>
<td>9.20</td>
<td>±1.206</td>
<td>9.088</td>
<td>0.433</td>
</tr>
<tr>
<td>1 hr</td>
<td>21.433</td>
<td>1.066</td>
<td>±6.762</td>
<td>12.518</td>
<td>2.300</td>
</tr>
<tr>
<td>3 hr</td>
<td>98.200</td>
<td>1.410</td>
<td>±5.002</td>
<td>28.823</td>
<td>18.300</td>
</tr>
<tr>
<td>12 hr</td>
<td>159.433</td>
<td>±1.701</td>
<td>±3.32</td>
<td>19.014</td>
<td>95.833</td>
</tr>
<tr>
<td>24 hr</td>
<td>118.560</td>
<td>±2.824</td>
<td>±2.995</td>
<td>11.916</td>
<td>72.560</td>
</tr>
<tr>
<td>48 hr</td>
<td>85.433</td>
<td>±3.541</td>
<td>±4.086</td>
<td>10.007</td>
<td>48.633</td>
</tr>
</tbody>
</table>
obtained and processed for histopathological examination. The vascular and cellular changes were noticed from 30 minutes onward to 48 h. The maximal number of leukocytes was observed at 12 h, but there were fewer than in the control group. However, maximal suppression of leukocytes was observed at 3 h. Infiltration of neutrophils was more marked at 6 h. While maximal suppression of neutrophils was recorded at 3 h. The maximal number of the monocytes was recorded at 12 h, while maximal suppression of monocytes at 6 h. Marked infiltration of lymphocytes was observed at 24 h. However, maximal suppression of lymphocytes was seen at 3 h. Maximal basophil suppression was observed at 1 h. Infiltration of eosionophils was not noticed at any time interval (Table 1).

Subgropup II B- Thirty minutes before intradermal injection of turpentine, the calves were pretreated with the piroxicam intramuscularly, and the lesions as per the non-pretreated group were obtained and processed for histopathological examination. The vascular and cellular changes were noticed from 30 minutes onward to 48 h. The maximal number of leukocytes were observed at 24 h. However, maximal suppression of leukocytes was observed at 3 h. Neutrophils were more marked at 6 h. While maximal suppression of neutrophils was recorded at 3 h. Monocytes were noted from 30 minutes onwards up to 24 h. The maximal number of monocytes was recorded at 48 h while maximal suppression of monocytes was at 6 h. Multinucleated giant cells were observed at 12 and 24 h. Lymphocytes were noted at 1 h to 48 h. Marked infiltration of lymphocytes was observed at 48 h. However, maximal suppression of lymphocytes was seen at 3 h. Few basophils were noted at 30 minutes onward to 24 h. The maximal number of the basophils was observed at 3 h, and suppression of the basophils was noted at 3 h. Infiltration of eosionophils was not noticed at any time interval (Table 2).

Pretreatment with the prostaglandin antagonist piroxicam caused suppression of the total leukocyte infiltration in both turpentine and Staph. Epidermidis-induced buffalo inflammation. At 3 h, maximal suppression of leukocytes occurred in both types of injury. However, in the turpentine induced reaction, piroxicam caused maximal suppression of the neutrophils, lymphocytes and basophils at 3 h, and of monocytes at 6 h. Whereas, in the Staph. Epidermidis-injury it resulted in maximal suppression of neutrophils and lymphocytes at 3 h, monocytes at 6 h, and basophils at 1 h. Issekutz and Movat (1982) studied the effect of prostaglandin on polymorphonuclear leukocyte infiltration. They concluded that prostaglandins enhance chemotactic-factor-mediated poly-morphonuclear infiltration. Since, piroxicam is a cyclooxygenase pathway inhibitor through which the prostaglandins are formed, pretreatment with the drug may also cause suppression of neutrophil infiltration. Gupta et al. (2008) reported that intradermal injection of S. epidermidis suspension and turpentine resulted in an inflammatory reaction in buffalo calf skin. The inflammation-induced vascular permeability was significantly suppressed by injection of the prostaglandin antagonist drug piroxicam, indicating that the prostaglandins might be responsible for increased vascular permeability in inflammation. In the present study, the greater suppression of neutrophils indirectly indicates the suppression of prostaglandin synthesis due to piroxicam pretreatment. Taken together our results indicate that the prostaglandins may play a role in the mediation in the cellular response in buffalo inflammation.

REFERENCES


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REFERENCES


