EFFECT OF LONG ACTING OXYTETRACYCLINE ON IMMUNE RESPONSE BASED ON PHAGOCYTIC INDEX IN RATS

Ravikumar C.*, Jagadeesh S. Sanganal1, Shridhar N. B.1, Narayanaswamy H. D.2, Sunilchandra U.3 and Shivashankar B. P.4

*Department of Veterinary Pharmacology and Toxicology, Veterinary College, Hassan, Karnataka.
1Department of Veterinary Pharmacology and Toxicology, Veterinary College, Hebbal, Bengaluru.
2Department of Pathology, Veterinary College, Hebbal, Bengaluru.
3Department of Pharmacology and Toxicology, Veterinary College, Bidar.
4Scientist, Institute of Animal Health and Veterinary Biologicals (IAH&VB), Bengaluru.

ABSTRACT

The immune system expresses an adaptive response in all the vertebrates against invading microorganisms. The role of immune system is to sustain host defense mechanisms and maintain homeostasis. The Long acting oxytetracycline administered at 20 and 40 mg/kg body weight through intramuscular route in wistar albino rats. Sheep RBC used as antigen. The immunologica parameter of Phagocytic Index (PI) was studied in this study. There was no significant (P>0.05) difference in the phagocytic Index in the treated group compared to that of control groups. This study was suggestive of long acting oxytetracycline formulation having no effect on the cell mediated immune response in rats.

KEY WORDS: Long acting Oxytetracycline, Phagocytic Index, Immune response.

INTRODUCTION

Immunomodulation may involve either an increase in the magnitude of immune response i.e. immunostimulation or a decrease in the magnitude of the immune response i.e. immunosuppression. Long acting oxytetracycline belongs to tetracycline group of
antibiotics. It was isolated from *Actinomycete streptomyces rimosus*. Oxytetracycline is a broad spectrum antibiotic with bacteriostatic activity widely used in veterinary medicine for the treatment of respiratory and gastrointestinal infectious diseases. It is active against aerobic gram positive and gram negative bacteria, rickettsia, mycoplasma, chlamydial infections anaplasmosis, babesiosis, theilariosis, pasteurellosis, bovine keratoconjunctivitis, ovine foot rot etc. To prevent repeated administration, to reduce the cost of treatment and to avoid stress condition, a long acting formulation of oxytetracycline was developed. The prolonged effect of this new preparation was claimed to be due to use of 2-pyrrolidone based formulation which should lead to provide prolonged circulating antibacterial concentration of the active agent for three to five days and controlled precipitation of oxytetracycline at the site of injection without significant tissue damage.

Wister Albino rats aged between two to three month old within body weight ranging from 150 to 200 g were procured from Small Animal House, Veterinary college, UAS, Bangalore. The animals were divided into eight experimental groups consisting of ten animals each group with equal number of male and female rats. Animals were housed in standard polypropylene rat cages and allowed for acclimatization for one week before the start of actual study and maintained hygienically under standard laboratory conditions (Alastrain and Warden, 1989), by providing commercial pellet feed and water *ad libitum*.

Long acting oxytetracycline available as Oxytetracycline dihydrate injectable solution / L.A. (Oxytetracycline dihydrate 200 mg/ml in 2-pyrrolidone) manufactured by Pfizer Limited, Mumbai was used in the experiment. This preparation was further diluted with 2-pyrrolidone and a single administration to experimental animal by intramuscular route was carried out.

**Structure of Oxytetracycline dehydrate**

![Structure of Oxytetracycline dehydrate](image_url)

**Experimental protocol:** The animals were divided into eight experimental groups. The details of the treatments given were as follows.
Group I    Saline control (no treatment)  
Group II  Vehicle control i.e. 2-pyrrolidone (0.5 ml) administered through intramuscular route.  
Group III    Single dose administration of long acting oxytetracycline at 20 mg/kg body weight through intramuscular route  
Group IV    Single dose administration of long acting oxytetracycline at 40 mg/kg body weight through intramuscular route.  
Group V    Administered 0.4 ml antigen on Day 0 and Day 7 intraperitoneally.  
Group VI    Administered 0.4 ml antigen on Day 0 and Day 7 intraperitoneally and 0.5 ml 2-pyrrolidone through intramuscular route.  
Group VII    Administered 0.4 ml antigen on Day 0 and Day 7 intraperitoneally and long acting oxytetracycline at 20 mg/kg body weight through intramuscular route.  
Group VIII    Administered 0.4 ml antigen on Day 0 and Day 7 intraperitoneally and long acting oxytetracycline at 40 mg/kg body weight through intramuscular route.  

Group I, II, III and IV were normal non antigen stimulated groups. In these Group I was Saline control, Group II was given vehicle i.e. 2-pyrrolidone control, Group III, and Group IV were given long acting oxytetracycline at 20 and 40 mg/kg body weight through intramuscular route, respectively.

The vehicle or long acting oxytetracycline given on Day '0'. These groups were used to assess the effect of long acting oxytetracycline on non-specific natural host defense mechanisms in rats.

Group V, VI, VII and VIII were antigen stimulated groups. In these, Group V was given antigen, Group VI was given antigen and pyrrolidone, Group VII was given antigen and long acting oxytetracycline at 20 mg/kg body weight through intramuscular route, Group VIII was given antigen and long acting oxytetracycline at 40 mg/kg body weight through intramuscular route. Antigen was given on Day '0', Vehicle and drug at two different doses were administered Day 1 after the administration of antigen. A second dose of antigen was given on Day 7 as a booster dose. These groups were used to assess the effect of long acting oxytetracycline on specific immune response.

**Collection of blood samples:** The rats were anaesthetized with diethyl ether and blood was collected from retro-orbital plexus. The blood samples were collected heparinized vials for estimation of phagocytic index. The blood was also collected in separate test
tubes for serum separation which was used for estimation of serological parameters. In all the groups blood was collected on Day '0' i.e. immediately before administering the drug/antigen and then on Day 1, 7, 14, 21, 28, 35 and 42 of the experiment.

**Phagocytic index (PI):** The phagocytic index was assessed followed the procedure outlined by Vanfurth *et al.* (1979) using *Staphylococcus* organisms.

The blood samples were collected individually in sterile heparinized vials. To each tube containing one ml heparinized blood sample, 0.1 ml of killed whole suspension of *Staphylococcus* antigen was added. The vials were incubated at 37°C for one hour. Then smears were prepared and stained with Giemsa’s stain. The means number of bacteria ingested per 100 phagocytes were calculated.

The phagocytic index was calculated by using the formula:

\[
PI = \frac{\text{The number of bacteria ingested by phagocytes}}{\text{The number of phagocytes involved}}
\]

**Statistical analysis:** The data generated from the experimental study was subjected to one-way ANOVA by statistical analysis (Snedecor and Cochran, 1976) using computerized Graph Pad Prism software.

**RESULTS AND DISCUSSION**

The PI values of experimental groups of rats are presented in Table 1 and 2. The PI values in the saline control group (Group I) ranged from 1.86±0.20 to 2.12±0.05. The PI values in the pyrrolidone group (Group II) ranged from 1.78±0.25 to 2.18±0.02. In the group given long acting oxytetracycline low dose (Group III) PI values ranged from 1.73±0.15 to 2.02±0.12. In the group given long acting oxytetracycline high dose (Group IV) the PI values ranged from 1.65±0.20 to 2.10±0.03.

There was no significant (P>0.05) variation in PI values of the groups treated with long acting oxytetracycline (Group III and IV), when compared to saline and pyrrolidone groups (Group I and II). Among the antigen stimulated groups, the antigen control group (Group V) has PI values ranged from 1.86±0.20 to 2.09±0.04. The group given antigen and pyrrolidone (Group VI) the PI values ranged from 1.80±0.14 to 2.10±0.24. The group received antigen and long oxytetracycline low dose (Group III) PI values ranged from
1.72±0.12 to 2.10±0.04. The group received antigen and long acting oxytetracycline high
dose (Group VIII) PI values ranged from 1.69±0.19 to 2.06±0.02.

There was no significant (P>0.05) difference in the PI in the groups treated antigen plus
long acting oxytetracycline groups (Group VII and VIII) when compared to control
groups (Group V and VI). But, there was decrease in PI values in antigen and high dose
group (Group VIII) when compared to antigen (Group V) and antigen plus pyrrolidone
group (Group VI) from Day 21 onwards.

Neutrophil is a phagocytic cell. The function of phagocytic cell is phagocytosis. If there
is change in phagocytic index after treatment with a drug it indicates that the drug had
altered the phagocytic function of phagocytic cell. This test was conducted to assess the
cell mediated immune response. In the present study long acting oxytetracycline in both
low and high dose did not show any significant (P>0.05) difference on phagocytic index
in non antigen and antigen stimulated rats when compared to respective control groups.
On the contrary, Sharma and Bansal (1985) reported that long acting oxytetracycline at
dose rate of 30 mg/kg body weight by intramuscular route during incubation period of
anaplasma infection increased cell mediated immune response in cattle.

Ankari and Homeida (1996) observed after administration of 0.05 g/kg oxytetracycline in
feed to broiler chicks for 50 days caused an increased serum concentration of the drug and
significantly reduced the macrophage phagocytic activity compared to control group. It is
suggested that the prolonged administration of oxytetracycline to chickens may induce an
immunosuppressant effect. Lunden et al. (1998) reported that rainbow trouts were
incubated orally with oxytetracycline at the dose of 75 mg/kg with Aeromonas
salmonicida and Listonella anguillarum suppressed phagocytic activity of whole blood
leucocytes. Chernigav (1972) reported that piglets given two daily intramuscular doses of
10,000 units/kg tetracycline for 14 days increased phagocytic activity of neutrophils.

<table>
<thead>
<tr>
<th>Time interval in days</th>
<th>Saline control (Group I)</th>
<th>Pyrrolidone control (Group II)</th>
<th>Low dose (20 mg/kg) (Group III)</th>
<th>High dose (40 mg/kg) (Group IV)</th>
</tr>
</thead>
</table>

Table 1. The effect of long acting oxytetracycline on phagocytic index in non antigen
stimulated rats
Table 2. The effect of long acting oxytetracycline on phagocytic index in antigen stimulated rats

<table>
<thead>
<tr>
<th>Time interval in days</th>
<th>Antigen control (Group V)</th>
<th>Antigen + Pyrrolidone control (Group VI)</th>
<th>Antigen + Low dose (20 mg/kg) (Group VII)</th>
<th>Antigen + High dose (40 mg/kg) (Group VIII)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>2.01 ± 0.08</td>
<td>2.06 ± 0.10</td>
<td>2.10 ± 0.04</td>
<td>2.06 ± 0.02</td>
</tr>
<tr>
<td>1</td>
<td>2.04 ± 0.10</td>
<td>2.02 ± 0.12</td>
<td>1.99 ± 0.12</td>
<td>1.88 ± 0.13</td>
</tr>
<tr>
<td>7</td>
<td>2.06 ± 0.12</td>
<td>2.10 ± 0.18</td>
<td>1.85 ± 0.06</td>
<td>1.74 ± 0.18</td>
</tr>
<tr>
<td>14</td>
<td>2.09 ± 0.04</td>
<td>2.08 ± 0.08</td>
<td>1.90 ± 0.14</td>
<td>1.82 ± 0.16</td>
</tr>
<tr>
<td>21</td>
<td>2.08 ± 0.04</td>
<td>2.10 ± 0.24</td>
<td>1.94 ± 0.15</td>
<td>1.89 ± 0.18</td>
</tr>
<tr>
<td>28</td>
<td>1.98 ± 0.06</td>
<td>1.87 ± 0.18</td>
<td>1.80 ± 0.16</td>
<td>1.74 ± 0.20</td>
</tr>
<tr>
<td>35</td>
<td>1.90 ± 0.12</td>
<td>1.80 ± 0.14</td>
<td>1.78 ± 0.10</td>
<td>1.70 ± 0.18</td>
</tr>
<tr>
<td>42</td>
<td>1.86 ± 0.20</td>
<td>1.80 ± 0.25</td>
<td>1.72 ± 0.12</td>
<td>1.69 ± 0.19</td>
</tr>
</tbody>
</table>

Values: Mean ± SE, n=10, P>0.05

CONCLUSION

The present study was conducted evaluate the effect of long acting oxytetracycline on specific immune response by using Phagocytic Index parameter in both non antigen and antigen stimulated rats. Sheep RBC used as antigen in this study. The Long acting oxytetracycline administered at 20 and 40 mg/kg body weight mg/kg body weight through intramuscular route in wistar albino rats. Sheep RBC used as antigen. For Phagocytic Index, there was no significant (P>0.05) difference in the phagocytic Index in the treated group compared to that of control groups. Thus the study concluded that long acting acting oxytetracycline formulation did not affect the cell mediated immune response in rats.

REFERENCES