

SAFETY EVALUATION OF LONG ACTING OXYTETRACYCLINE ON HAEMAGGLUTINATION TITRE IMMUNOLOGICAL PARAMETERS IN WISTAR ALBINO RATS

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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 mg/kg body weight through intramuscular route in wistar albino rats. Sheep RBC used as antigen. The immunological parameters like Haemagglutination titre (HA Titre) was studied in this study. In the present study, there was no significant ($P > 0.05$) difference in the antibody titre in the antigen plus long acting oxytetracycline low dose and high dose group when compared to antigen and antigen plus pyrrolidone control group. This is suggestive that long acting oxytetracycline at low and high dose does not affect the humoral and cell mediated immune response in rats.

Keywords: Long acting oxytetracycline, HA Titre, 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. Immunomodulation can further be divided as specific and nonspecific. Specific immunomodulation implies a change in the response of the system to a particular antigenic stimulus as brought about by process of vaccination (specific immunostimulation) and desensitization (specific immunosuppression). Nonspecific immunomodulation implies a more fundamental change, where by the “State of Alertness” of the immune system is altered, this infusion affects the nature of its responses to the multiplicity of antigenic stimuli (Goodman and Gillman, 2001).

Long acting oxytetracycline belongs to tetracycline group of antibiotics. It was

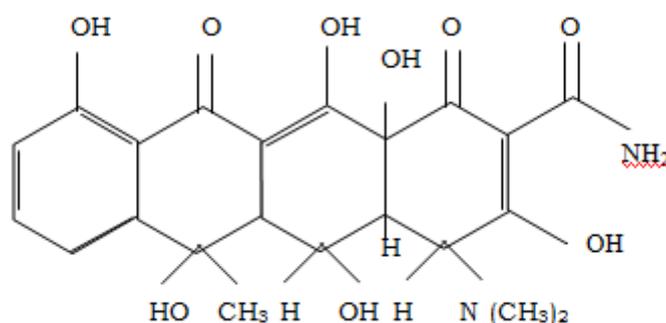
isolated from *Actinomyces streptomyces rimosus*. Oxytetracycline is a broad spectrum antibiotic with bacteriostatic activity widely used in veterinary medicine for the treatment of respiratory and gastrointestinal infectious diseases. It is active against aerobic gram positive and gram negative bacteria, rickettsia, mycoplasma, chlamydial infections anaplasmosis, babesiosis, theilariosis, pasteurellosis, bovine kerato conjunctivitis, ovine foot rot etc (Swift and Thomas,1983; Musset *et al.* 1996).

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 dihydrate



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 0.4ml 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 in heparinized vials for estimation of Haemagglutination titre. 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.

Immunological Parameters:

Haemagglutination test

The titres of the agglutinating antibody in the serum were measured by haemagglutination test as per the procedure adopted by Hudson and Hay (1989). The procedure adopted was as follows.

Antigen

Sheep blood collected in Alsever's solution were washed twice and resuspended in normal saline solution at a concentration of 0.5 per cent.

Serum samples

Serum samples were collected from all antigen stimulated groups (Group V, VI, VII and VIII) at regular weekly intervals without disodium EDTA and subjected to HA test.

Test procedure

To a clean dry microhaemagglutination plate 50 µl of phosphate buffer saline (PBS) was added to all the wells. 50 µl of serum was added to the first well, after mixing serially transferred 50 µl from the first well to the succeeding wells. 50 µl was discarded from the last well. A serial two fold dilution ranging from 1:2 to 1:4096 were made. 50 µl of 0.5 per cent sRBC suspension added to all the wells. The contents were mixed well and incubated at 37°C for one hour. A control was kept consisting of 50 µl serum and 50 µl 0.5 per cent sRBC (positive control), 50 µl PBS and 50 µl 0.5 per cent sRBC suspension (negative control).

The reciprocal of the highest dilution showing complete agglutination of erythrocytes was taken as the antibody titre and the haemagglutination antibody titres were expressed in log₂ (Moharana *et al.*, 2000).

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

Haemagglutination test

The haemagglutinating antibody titres in the serum of antigen stimulated rats are presented in Table. 1. The antibody titres in the serum of the antigen treated rats were expressed in \log_2 .

The antibody titres in the antigen stimulated rats were measured by HA test. Any alteration in the antibody titre will affect the humoral immune response. The first dose of antigen was given on Day 0 and second dose of antigen was given on Day 7 of experiment. The HA titre in the group given antigen alone (Group V) ranged from 0.172 ± 0.056 to 1.875 ± 0.032 . The antibody titre in the group given antigen and pyrrolidone ranged from 0.205 ± 0.053 to 1.825 ± 0.046 . The group given antigen and long acting oxytetracycline (Group VI) antibody titre varied from 0.152 ± 0.026 to 1.792 ± 0.030 . The group given antigen and long acting oxytetracycline (Group VIII) antibody titre varied from 0.132 ± 0.032 to 1.635 ± 0.022 .

There was no significant ($P > 0.05$) difference in the HA titre in the low and high dose long acting oxytetracycline treated groups (Group VII and VIII) compared to antigen (Group V) and antigen and pyrrolidone (Group VI) groups.

In contrary, Vanzini *et al.* (1988) reported that administration of long acting oxytetracycline at 20 mg/kg body weight through intramuscular route after inoculation with *Babesia bovis* organism showed a decrease in antibody level in the serum of calves.

Exon *et al.* (1989) reported that administration of long acting oxytetracycline (liquamycin-200) at 20 mg/kg body weight for 12 days by intramuscular route suppressed the γ -interferon production and at high doses suppressed both specific and nonspecific cell mediated immune response in rats.

Smith *et al.*, (1983). reported that concurrent administration of oxytetracycline and subcutaneous vaccination with *Brucella abortus* strain-19 organism caused a reduction in humoral antibodies to *Brucella abortus* compared to untreated vaccinated calves.

Jayakumar *et al.* (2002) reported that administration of Ciprofloxacin (10 mg/kg body weight, iv, twice daily for 4 days) failed to alter specific antibody titres against Brucella plain killed antigen and indicates did not adversely affect specific immune response in normal New Zealand White rabbits.

Conclusion

The present study was conducted evaluate the effect of long acting oxyteracycline on humoral and specific immune response by using Haemagglutination in 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 Haemagglutination titre, there was no significant ($P>0.05$) difference in the antibody titre in the antigen plus long acting oxytetracycline low dose and high dose group when compared to antigen and antigen plus pyrrolidone control group. This is suggestive that long acting acting oxytetracycline does not affect the humoral and cell mediated immune response in rats.

Table 1. The effect of long acting oxytetracycline on Haemagglutinating antibody titre in antigen stimulated rats expressed in \log_2

Time interval in days	Antigen control (Group V)	Antigen + Pyrrolidone control (Group VI)	Antigen + Low dose (20 mg/kg) (Group VII)	Antigen + High dose (40 mg/kg) (Group VIII)
0	0	0	0	0
1	0.172 ± 0.056	0.205 ± 0.053	0.152 ± 0.026	0.132 ± 0.032
7	1.875 ± 0.032	1.825 ± 0.046	1.792 ± 0.030	1.635 ± 0.022
14	1.860 ± 0.016	1.815 ± 0.030	1.720 ± 0.028	1.620 ± 0.042
21	1.784 ± 0.022	1.724 ± 0.064	1.684 ± 0.032	1.624 ± 0.032
28	1.720 ± 0.008	1.692 ± 0.021	1.594 ± 0.018	1.520 ± 0.014
35	1.680 ± 0.012	1.642 ± 0.040	1.536 ± 0.018	1.486 ± 0.030
42	1.620 ± 0.010	1.598 ± 0.032	1.510 ± 0.022	1.475 ± 0.028

Values: Mean ± SE, n =10, $P>0.05$

VII. REFERENCES

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