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SAFETY EVALUATION OF LONG ACTING OXYTETRACYCLINE ON PHAGOCYTIC INDEX IMMUNOLOGICAL PARAMETERS IN WISTAR ALBINO RATS

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Abstract: 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 Phagocytic Index (TIG) were studied in this study. In the present study, there was no significant (P>0.05) difference in the phagocytic Index in the treated group compared to that of control groups. This is suggestive that long acting acting oxytetracycline does not affect the cell mediated immnune response in rats.

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 *Actinoomycete 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

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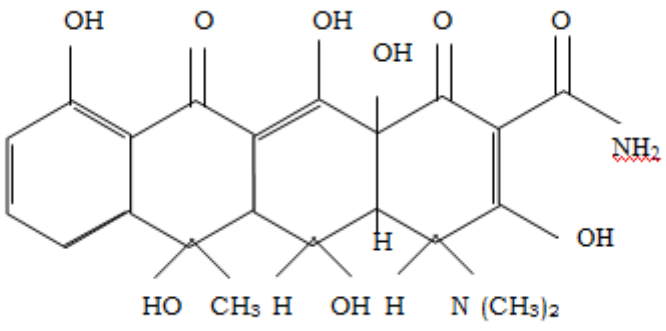
and gram negative bacteria, rickettsia, mycoplasma, chlamydial infections anaplasmosis, babesiosis, theilariosis, pasteurellosis, bovine kerato conjunctivitis, ovine foot rot etc (Swift and Thomas,1983; Musser *et al.* 1996).

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 by providing commercial pellet feed and water *ad libitum* (Alastrain and Warden, 1989).

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 intraperitonaelly.

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 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.

Immunological Parameters:

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.

Phagocytic index (PI)

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

Procedure

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 37^0C 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

Phagocytic Index (PI)

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.

Jayakumar *et al*. (2002) reported that administration of ciprofloxacin (10 mg/kg body weight, iv, twice daily for 4 days) failed to alter phagocytic Index 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 Phagocytic Index 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. This is suggestive that long acting acting oxytetracycline does not affect the cell mediated immnune response in rats.

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

Time interval in days	Saline control (Group I)	Pyrrolidone control (Group II)	Low dose (20 mg/kg) (Group III)	High dose (40 mg/kg) (Group IV)
0	2.05 ± 0.02	2.08 ± 0.08	1.99 ± 0.06	2.10 ± 0.03
1	2.12 ± 0.05	2.18 ± 0.02	2.02 ± 0.12	2.00 ± 0.10
7	2.08 ± 0.02	2.12 ± 0.04	1.96 ± 0.18	1.76 ± 0.12
14	2.10 ± 0.14	2.14 ± 0.18	1.98 ± 0.20	1.85 ± 0.18
21	2.06 ± 0.18	2.10 ± 0.02	2.02 ± 0.10	1.88 ± 0.12
28	1.98 ± 0.04	1.92 ± 0.01	1.80 ± 0.12	1.76 ± 0.10
35	1.90 ± 0.14	1.84 ± 0.12	1.76 ± 0.11	1.65 ± 0.20
42	1.86 ± 0.20	1.78 ± 0.25	1.73 ± 0.15	1.68 ± 0.14

Values: Mean ± SE, n =10, P>0.05

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

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	2.01 ± 0.08	2.06 ± 0.10	2.10 ± 0.04	2.06 ± 0.02
1	2.04 ± 0.10	2.02 ± 0.12	1.99 ± 0.12	1.88 ± 0.13
7	2.06 ± 0.12	2.10 ± 0.18	1.85 ± 0.06	1.74 ± 0.18
14	2.09 ± 0.04	2.08 ± 0.08	1.90 ± 0.14	1.82 ± 0.16

21	2.08 ± 0.04	2.10 ± 0.24	1.94 ± 0.15	1.89 ± 0.18
28	1.98 ± 0.06	1.87 ± 0.18	1.80 ± 0.16	1.74 ± 0.20
35	1.90 ± 0.12	1.80 ± 0.14	1.78 ± 0.10	1.70 ± 0.18
42	1.86 ± 0.20	1.80 ± 0.25	1.72 ± 0.12	1.69 ± 0.19

Values: Mean ± SE, n =10, P>0.05

REFERENCES

[1] Alastrain, N. and Warden, 1989, *Handbook of Laboratory Animals*. Anmol Pub., New Delhi.

[2] Ankari, A.S. and Homeida, A.M. (1996). Effect of antibacterial growth promoters on the immune system of broiler chicks. Vet Immunol Immunopathol. 53(3-4): 277-83.

[3] Chernigov, H.D., (1972), Effect of tetracycline on immunogenesis in piglets vaccinated against Salmonellosis. Veterinariya Mascow, **12**: 45-47.

[4] Goodman and Gillman, 2001, Tha Pharmacological Basis of Therapeutics, 10th Edn, McGraw- Hill Publication.

[5] Jayakumar, K., Honnegowda., Krishnappa, G., Sastry, K,N. and Narayana, K. (2002). Effect of ciprofloxacin on specific immune response in rabbits. Indian J Ex.p Biol. 40(1):111-114.

[6] Lunden, T., Meittinen, S., Lonnstrom, L.G., Lilius, B.M. and Bylund, G., (1998), Influence of oxytetracycline and oxolinic acid on the immune response of rainbow trout. Oncorhynchus mykiss, **8**: 217-230.

[7] Musser, J. , Mechor, G.D. , Gröhn, Y.T , Dubovi, E.J., Shin. S. 1996. Comparision of tilmicosin with long acting oxytetracycline for treatment of respiratory tract disease in calves. J. American. Vet, Med.Association.208(1) 102- 106.

[8] Sharma, S.P. and Bansal, G.C., 1985, Use of long acting oxytetracycline in the treatment of acute anaplasmosis. *Indian Vet. J.*, **61**: 888-889.

[9] Swift,B,L. and Thomas, G.M. 1983, Bovine anaplasmosis: elimination of the carrier state with injectable long acting oxytetracycline J. American. Vet, Med. Assoc., 183(1): 63-65.

[10] Snedecor, G.W. and Cochran, W.G., (1976), Statistical Methods. VI Edn., Oxford and IBH Pub. Co., Calcutta.

[11] Vanfurth, R., Theda, L., Vanzwef and Leijh, P.C.I., 1979, In vitro determination of phagocytosis and intracellular killing by polymorphonuclear and mononuclear phagocytes. In: Handbook of Experimental Immunology. Vol. 2, II Edn., Eds. D.M., Weir, Blackwell Scientific Pub., Oxford.