International Journal of Science, Environment and Technology, Vol. 5, No 2, 2016, 424 – 430

ISSN 2278-3687 (O)
2277-663X (P)

EFFECT OF LONG ACTING OXYTETRACYCLINE FORMULATION ON IMMUNITY BASED ON TOTAL SERUM IMMUNOGLOBULIN CONCENTRATION IN RATS

Ravikumar C[*], Jagadeesh S Sanganal[1], Shridhar NB[1], Narayanaswamy H D[2], Sunilchandra U[3] and Shivashankar B P[4]

*Department of Veterinary Pharmacology and Toxicology, Veterinary College, Hassan. Karnataka.[1]Department of Veterinary Pharmacology and Toxicology, Veterinary College, Hebbal, Bengaluru.[2]Department of Pathology, Veterinary College, Hebbal, Bengaluru.[3]Department of Pharmacology and Toxicology, Veterinary College, Bidar. [4]Scientist, Institute of Animal Health and Veterinary Biologicals (IAH&VB), Bengaluru.
E-mail: ravivet4u@rediffmail.com (**Corresponding Author*)

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 antimicrobial agents aid in killing or inhibiting the growth of microorganisms. There has been considerable recent interest on the nature of interaction that occurs among antibiotics, micro-organisms and the host defense mechanisms. The present study was done to assess the effect of long acting oxyteracycline formulation on humoral and specific immune response by using total serum immunoglobulin (TIG) parameter in both non antigen and antigen stimulated rats. Sheep RBC used as antigen in this study. In antigen stimulated rats, antigen and long acting oxytetracycline at high dose treatment group show significant ($P<0.05$) decrease in the TIG concentration on Day 7 and 14 when compared to antigen and antigen plus pyrrolidone control groups. This indicated the depression of the specific immune response in the high dose treated antigen stimulated rats.

Keywords: Oxytetracycline, Long acting, total serum immunoglobulin.

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. Pharmacological manipulation of the immune system is important in the management of autoimmune diseases, prevention or treatment of malignancies, control of infection in immunocompromised patients and organ transplanted individuals. Any alteration in the components of the immune function may have deleterious consequences on the health of infected animals (Goodman and Gillman, 2001).

*Received Mar 7, 2016 * Published April 2, 2016 * www.ijset.net*

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 and chlamydial infections. The long acting formulation of oxytetracycline is the drug of choice for the treatment of acute diseases as well as supportive therapy and prophylaxis like anaplasmosis, babesiosis, theilariosis, pasteurellosis, bovine kerato conjunctivitis, ovine foot rot etc. The prolonged effect of long acting formulation 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 Oxytetracyclinedihydrate injectable solution / L.A. (Oxytetracyclinedihydrate 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.

MATERIALS AND METHODS

The animals were divided into eight experimental groups. The details of the treatments given were as follows.

Groups	Treatment
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/kgbody 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.

The blood samples were collected disodium ethylene diamine tetraacetic acid (EDTA) vials for estimation of hematological parameters and in 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. Total serum immunoglobulin concentration (TIG) was estimated by following the procedure as described by Mullen (1975). 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 TIG concentration (mg/ml; Table 1 and 2) in the saline control (Group I) and pyrrolidone control (Group II) varied from 25.36±0.51 to 29.55±0.98 mg/ml and 26.33±0.47 to 28.65±0.32 mg/ml respectively. The group given long acting oxytetracycline at 20 mg/kg body weight showed a TIG concentration of 22.73±0.81 to 27.60±0.62 mg/ml and the group given to 40 mg/kg body weight (Group IV) showed TIG concentration ranging from 22.53±0.34 to 27.24±0.77 mg/ml.

There was no significant ($P>0.05$) difference in the TIG values of rats in low dose (Group III) and high dose (Group IV) long acting oxytetracycline treated group when compared to saline group (Group I) and pyrrolidone group (Group II). In the antigen stimulated group, the antigen control group (Group V) showed TIG concentration ranging from 25.87±0.98 to 30.25±0.58 mg/ml. The group given antigen plus pyrrolidone showed TIG values ranging from 26.08±0.73 to 29.85±0.58 mg/ml. The TIG values ranged between 23.95±0.87 to 28.98±0.68 mg/ml and 22.78±0.43 to 26.92±0.62 in the low dose group (Group VII) and high dose group (Group VIII), respectively.

There was a significant ($P<0.05$) decrease in the total serum immunoglobulin concentration in the group given antigen and high dose (Group VIII) on Day 7 and 14 when compared to antigen and antigen plus pyrrolidone control groups (Group V and VI).Serum globulin has α, β and γ-globulin fractions. The immunoglobulins are present in γ-globulin fraction. So, if there is any variation in the γ-globulin, it indicates the variation in immune response. In nonantigen stimulated rats, long acting oxytetracycline in both low and high dose did not produce any significant ($P>0.05$) difference in the TIG concentration when compared to saline and pyrrolidone control group. In antigen stimulated rats, antigen and long acting oxytetracycline at high dose treatment group show significant ($P<0.05$) decrease in the TIG concentration on Day 7 and 14 when compared to antigen and antigen plus pyrrolidone control groups. This indicates that there will be depression of the specific immune response in the high dose treated antigen stimulated rats.

Similar results was observed by Cooper and Allen (1959) who reported that chlortetracycline incorporated diet of mice before immunization with avirulent live culture of *Erysipeleas bacilli* and the antibiotic fed continuously for a period of two weeks after immunization and observed decrease immunoglobulin level in the serum. Oxytetracycline administered intraperitoneally decreased serum immunoglobulin level in

carp fish (Rijkers*et al*., 1981). Exon *et al*. (1989) reported that administration of long acting oxytetracycline (liquamycin-200) at 20 mg/kg body weight for 12 days through intramuscular route suppressed the γ-interferon production and at high doses suppressed both specific and nonspecific cell mediate immune response in rats. Joks *et al*. (2010) reported that suppression of murine IgE responses in vivo was dose dependent and lasted for 5-7 day after injected intraperitoneally with benzylpenicilloyl(14)-Keyhole limpet hemocyanin (BPO(14)-KLH) in alum on days 0, 21 and 42, fed with minocycline or doxycycline (10-100 mg/kg) in mice. Jayakumar *et al*. (2002) reported that administration of Ciprofloxacin (10 mg/kg body weight, iv, twice daily for 4 days) did not alter serum immunoglobulin concentration against Brucella plain killed antigen indicates did not adversely affect specific immune response in normal New Zealand White rabbits.

Table 1. The effect of long acting oxytetracycline formulation on total serum immunoglobulin concentration (mg/ml) 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	26.12 ± 0.59	28.46 ± 0.75	25.13 ± 0.73	24.13 ± 0.48
1	25.36 ± 0.51	26.93 ± 0.75	22.73 ± 0.81	23.90 ± 0.48
7	26.00 ± 0.17	28.65 ± 0.32	25.23 ± 0.55	22.53 ± 0.34
14	27.40 ± 0.39	28.13 ± 0.35	24.66 ± 0.59	23.78 ± 0.42
21	29.12 ± 0.56	26.33 ± 0.47	26.01 ± 0.86	25.70 ± 0.54
28	29.55 ± 0.98	28.65 ± 0.92	27.54 ± 0.70	26.27 ± 0.70
35	28.35 ± 0.39	28.12 ± 0.79	27.60 ± 0.62	27.24 ± 0.77
42	27.70 ± 0.39	26.80 ± 0.74	26.20 ± 0.72	25.98 ± 0.64

Values: Mean ± SE, n =10, P>0.05

Table 2. The effect of long acting oxytetracycline on total serum immunoglobulin concentration (mg/ml) 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	28.30 ± 0.95	26.73 ± 0.74	24.44 ± 0.64	25.14 ± 0.60
1	25.87 ± 0.98	27.73 ± 1.02	23.95 ± 0.87	24.96 ± 0.89
7	28.37 ± 0.86	26.08 ± 0.73	24.08 ± 0.52	22.78 ± 0.43*
14	30.03 ± 0.98	28.41 ± 0.89	24.92 ± 0.66	23.56 ± 0.58*
21	26.33 ± 0.62	29.20 ± 0.78	25.47 ± 0.62	24.70 ± 0.32
28	30.25 ± 0.58	29.25 ± 0.62	26.84 ± 0.60	25.60 ± 0.56
35	28.22 ± 0.80	29.85 ± 0.58	28.98 ± 0.68	26.92 ± 0.62
42	28.85 ± 0.70	27.94 ± 0.69	27.10 ± 0.74	26.14 ± 0.82

Values : Mean ± SE, n=10, * P<0.05

CONCLUSION

The present study was conducted evaluate the effect of long acting oxyteracycline formulation on humoral and specific immune response by analyzing total serum immunoglobulin concentration in both non antigen and antigen stimulated rats. Sheep RBC used as antigen in this study. For Total serum immunoglobulin concentration (TIG), In non antigen stimulated rats, long acting oxytetracycline at low and high dose treatment group show did not significant ($P<0.05$) decrease in the TIG concentration when compared control groups. In antigen stimulated rats, antigen and long acting oxytetracycline at high dose treatment group show significant ($P<0.05$) decrease in the TIG concentration on Day 7 and 14 when compared to antigen and antigen plus pyrrolidone control groups. This indicated the depression of the specific immune response in the high dose long acting oxytetracycline formulation treated antigen stimulated rats.

REFERENCES

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

[2] Brummerstedt, E. and Basse, A., 1973, Cutaneous hypersensitivity to 2-4-dinitrochlorobenzene in calves. Nord. Vet. Med., 25: 392-398.

[3] Cooper, M.S. and Allen, G.A., 1959, The effect of CTC on the immunosuppression. I. Influence on development of protective antibodies after vaccination with a live, avirulent strain of Erysipelothrixrhusipathae. J. Immunol., 83: 232-236.

[4] Exon, J.H., Stevens, M.G., Koller, I.D. and Mathes, G.G., 1989, Immunotoxocity assessment of gentamicin and liquamycin. Vet. Hum. Toxicol, 31: 127-130.

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

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

[7] Joks, R., Norowitz, S., Nowakowski, M., Bluth, M.H and Durkin, H.G. 2010. Tetracycline-mediated IgEisotype-specific suppression of ongoing human and murine IgE responses in vivo and murine memory IgE responses induced in vitro. IntImmunol., 22(4): 281-288

[8] Mullen, P.A., 1975, Zinc sulphate turbidity test as an aid to diagnosis. Vet. Annual, 15: 451-455.

[9] Nakagawa, S., Oka, D., Jinno, Y,, Takei, Y., Bang, D. andUeki, H.1988. Topical application of cyclosporine on guinea pig allergic contact dermatitis. Arch Dermatol., 124(6): 907-910.

[10] Rijckers, G.T., Oosterom, R.V. and Muiswinkel, W.V., 1981, The immune system of cyprinid fish. Oxytetracycline and the regulation of humoral immunity in the carp (Cyprinuscarpio). Aquaculture., 2: 281-290.

[11] 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.

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

[13] Zakharov, V.F., Besednova, N.N., Krylova, N.V. and Nikitin, A.V., 1992, Combined action of doxycycline and mytilan on the immune response to tularemia antigen. Antibiot. Khimioter, 37: 43-45.