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EFFECT OF LONG ACTING OXYTETRACYCLINE FORMULATION ON IMMUNE STATUS BASED ON ORGAN TO BODY WEIGHT RATIO IN RATS

[1]Ravikumar C[*], [2]Jagadeesh S Sanganal, [3]Sunilchandra U, [2]Shridhar NB, Narayanaswamy HD and Shivashankar BP

*Department of Veterinary Pharmacology and Toxicology, Veterinary College, Hassan, Karnataka

[1]Department of Veterinary Pharmacology and Toxicology, Veterinary College, Hebbal, Bengaluru

[2]Department of Pharmacology and Toxicology, Veterinary College, Bidar

[3]Department of Pathology, Veterinary College, Hebbal, Bengaluru

[4]Scientist, Institute of Animal Health and Veterinary Biologicals (IAH&VB), Bengaluru

E-mail: ravivet4u@rediffmail.com (*Corresponding Author*)

Abstract: The role of immune system is to sustain host defense mechanisms and maintain homeostasis. The immune system maintains a constant surveillance against invading pathogenic microorganisms, 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 broad spectrum antibiotic with bacteriostatic activity widely used in veterinary medicine for the treatment of respiratory and gastrointestinal infectious diseases. This study depicts the effect of long acting oxytetracycline on immune response with reference to the organ to body weight ratios in respect of spleen and lymph node. There was no significant ($P>0.05$) difference in the organ body weight ratio of spleen and lymphnode in long acting oxytetracycline treated groups and antigen plus long acting oxytetracycline groups in nonantigen and antigen stimulated groups when compared with their respective control groups respectively. The present study indicated that single dose of long acting oxytetracycline formulation did not affect the organ body weight ratio of spleen and lymphnode in rats, concluding that the formulation had no effect on the immune status in rats.

Keywords: Oxytetracycline, Long acting, Immunomodulation, Organ to body weight ratio

INTRODUCTION

The role of immune system is to sustain host defense mechanisms and maintain homeostasis. The immune system maintains a constant surveillance against invading pathogenic microorganisms, which is essential for survival of animals. The antimicrobial agents aid in killing or inhibiting the growth of microorganisms. There has been

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considerable recent interest on the nature of interaction that occurs among antibiotics, micro-organisms and the host defense mechanisms.

The immunomodulation by drugs and chemicals appears to have its rudimentary beginning in the use of herbs and teas (decoctions), which were used to cure several maladies in oriental cultures. In Ancient Chinese medicine, use of ginseng tea was found to have immunomodulating activity by virtue of its content of trace mineral germanium (Goodman, 1988). 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. Immunomodulatory regimens offer an attractive approach as they often have fewer side effects than existing drugs, including less potential for creating resistance in microbial diseases and Immunotherapy is the "treatment of disease by inducing, enhancing, or suppressing an immune response (Masihi, 2001). Immunological screening tests could act as an adjunct to traditional pharmacokinetic or toxicity studies which are required for safety evaluation of newer drugs.

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 use of organ-to-body weight ratios is often helpful for clarifying treatment-related organ weight changes, particularly in non-rodents in which there can be notable variations in organ and body weights (Wooley, 2003). Splenic and thymic weights should always be interpreted in conjunction with histopathologic findings because of the inherent variability in lymphoid organ weights. Lymphoid organ weight changes that are measured in the absence of a corresponding histopathological alteration should be interpreted with caution (Haley *et al*., 2005).

In treatment-associated alterations in splenic or thymic weights are not identified in general toxicology studies of short duration in non-rodents, these weights may be omitted from studies of longer duration, where data interpretation may be confounded by aging changes (Bucci *et al*., 2002).

The pharmacokinetic parameters and the pharmacological effects of long acting oxytetracycline on various system in the body have been studied, however adequate information is not available regarding the effect of long acting oxytetracycline on the natural host defense mechanisms and specific immune response in rats. The present study was conducted to study the effect of long acting oxytetracyclineorgan to body weight ratio in rats.

MATERIALS AND METHODS

The present study was designed to evaluate the effect of long acting oxytetracycline on the innate immunity i.e., natural host defense mechanisms in normal non antigen stimulated rats and immune response in antigen stimulated rats.

Experimental animals

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*.

Drug

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.

Sheep red blood cell (sRBC) was used as the antigen. Sheep blood was collected in Alsever's solution and stored at 4^0C for one week then washed three times in pyrogen free sterile normal saline and two per cent sRBC suspension was prepared at the time of administrations (Shah and Gupta, 1998).

Two dose level i.e. low dose and high dose were selected in the present study. The therapeutic dose 20 mg/kg body weight (Musser *et al.*, 1996) used in animals was considered as low dose. Twice the concentration of therapeutic dose i.e. 40 mg/kg bodyweight was considered as high dose.

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/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 (0.5ml, Intramuscular route), 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 (0.4ml) on Day '0', Vehicle and drug at two different doses were administered Day 1 after the administration of antigen. The 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.

At the end of the experimental period of six weeks, the animals were sacrificed and observed for gross lesions of internal organs. The spleen and lymph node were separated

from adhering tissue using saline and collected by placing on blotting paper and gently pressed to remove excess of saline. The organs were weighed on analytical balance and weights of each organ were recorded. Organ to body weight ratio were calculated and expressed in per centage. 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 percent organ body weight ratio of spleen and lymph node in nonantigen and antigen stimulated groups are depicted in Table 1.

In nonantigen stimulated groups, the organ body weight ratio of spleen of control group (Group I) was 0.43±0.05. The organ body weight ratio of spleen in pyrrolidone group (Group II) was 0.44±0.03. The spleen body weight ratio in groups of rats which received long acting oxytetracycline low dose (Group III) and high dose (Group IV) groups were 0.52±0.03 and 0.55±0.02, respectively.

In antigen stimulated groups, the organ body weight ratio of spleen in antigen control group (Group V) was 0.53±0.04. The organ body weight ratio of spleen in antigen plus pyrrolidone group (Group VI) was 0.47±0.03. The organ body weight ratio of spleen in group given antigen plus long acting oxytetracycline low dose (Group VII) and high dose (Group VIII) groups were 0.52±0.03 and 0.50±0.02 respectively.

There was no significant ($P>0.05$) difference in the organ body weight ratio of spleen in both non antigen stimulated and antigen stimulated treated groups when compared to respective control groups.

Ankari (1996) observed administration 0.05 g/kg oxytetracycline in feed to broiler chicks for 50 days resulted in a significant decrease of the total number of leukocytes, lymphocytes and the size of bursa of Fabricius and thymus but not spleen or body weight.

Table 1: Effect of long acting oxytetracycline on organ – body weight of spleen and lymphnode in rats

Group	Body weight (g)	Spleen weight (g)	Organ body weight ratio of spleen (%)	Lymphnode weight (g)	Organ body weight ratio of lymphnode (%)
I	201.50 ± 2.36	0.91 ± 0.11	0.43 ± 0.05	0.031 ± 0.003	0.017 ± 0.001
II	198.00 ± 3.96	0.93 ± 0.06	0.44 ± 0.03	0.034 ± 0.002	0.018 ± 0.002
III	199.90 ± 3.18	1.09 ± 0.07	0.52 ± 0.03	0.031 ± 0.002	0.015 ± 0.001
IV	197.00 ± 3.35	1.06 ± 0.07	0.55 ± 0.02	0.036 ± 0.001	0.018 ± 0.003
V	209.50 ± 3.45	1.07 ± 0.07	0.53 ± 0.04	0.033 ± 0.002	0.016 ± 0.001
VI	204.50 ± 4.04	0.97 ± 0.07	0.47 ± 0.03	0.028 ± 0.001	0.013 ± 0.002
VII	202.50 ± 2.71	1.05 ± 0.07	0.52 ± 0.03	0.035 ± 0.003	0.017 ± 0.001
VII	199.00 ± 3.39	1.02 ± 0.06	0.50 ± 0.02	0.035 ± 0.002	0.018 ± 0.001

Values: Mean ± SE; n =10; P>0.05

CONCLUSION

Immunomodulation occurs as a result of changes in lymphoid organs. So taking the weights of lymphoid organ is important. There was no significant (P>0.05) difference in the organ body weight ratio of spleen and lymphnode in long acting oxytetracycline treated groups and antigen plus long acting oxytetracycline groups in nonantigen and antigen stimulated groups when compared with their respective control groups respectively. In conclusion, single dose of administration of long acting oxytetracycline formulation did not affect the organ body weight ratio of spleen and lymphnode in rats.

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