International Journal of Science, Environment and Technology, Vol. 5, No 2, 2016, 672 – 677

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

ASSESSMENT OF EFFECT OF LONG ACTING OXYTETRACYCLINE FORMULATION ON IMMUNE STATUS BASED ON DINITROCHLOROBENZENE SKIN SENSITIVITY TEST IN 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 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 based on dinitrochlorobenzene (DNCB) skin sensitivity test in both non antigen and antigen stimulated rats. In sheep RBC antigen stimulated rats, antigen and long acting oxytetracycline at high dose treatment group did not show significant ($P>0.05$) difference in skin thickness, indicating that non specific as well as specific cell mediated immune responses were not altered with long acting oxytetracycline formulation in rats.

INTRODUCTION

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).

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 prolonged effect of long acting formulation

*Received Mar 3, 2016 * Published April 2, 2016 * www.ijset.net*

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 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.

MATERIALS AND METHODS

Experimental animals

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.

Dinitrochlorobenzene (DNCB) skin sensitivity test was performed on the lines of Brummerstedt and Basse (1973). An area of three cm in diameter was marked on the left flank region. The site was clipped off the hair close to the skin. A metallic ring of three cm diameter was used to mark the area. For primary sensitization, two per cent solution of 2,4-Dinitrochlorobenzene (DNCB) in acetone was used and 0.4 ml of this solution was applied slowly drop by drop to the marked area keeping the metallic ring at the site. The solution was allowed to evaporate quickly by blowing gently during application. After 14 days of primary sensitization, a challenge dose of 0.25 ml was applied similarly. Skin thickness was measured using slide calipers before challenge dosing i.e., at zero hour and at 21 and 48 hours intervals after challenge dosing.

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 skin thickness (mm) of the rats measured after primary and challenge sensitization are depicted in the Table 3 and 4 and Fig. 3 and 4.

There was no significant ($P>0.05$) difference in the skin thickness of non antigen stimulated (Group III and IV) and antigen stimulated (Group VII and VIII) long acting oxytetracycline treated groups, when compared with their respective control groups.

In non antigen and antigen stimulated groups did not produce any significant ($P>0.05$) difference in skin thickness of long acting oxytetracycline treated groups when

compared with their respective control groups. This indicates that both non specific as well as specific cell mediated immune responses were not altered. On the contrary, Sharma and Bansal (1985) reported that long acting oxytetracycline at a dose rate of 30 mg/kg body weight by intramuscular route during incubation period of anaplasma infection increased the cell mediated immune response in calves. Nakagawa *et al.* (1988) reported Cyclosporine applied topically to the challenge site also resulted in a reduction of retest and flare-up reactions of contact sensitivity to DNCB, but did not affect the production of generalized rash in animals. These results indicate that local topical application of cyclosporine may make treatment of human cutaneous immune-mediated disorders a possibility without serious side effects. Exon *et al.* (1989) reported that administration of long acting oxytetracycline intramuscularly or subcutaneously at 20 mg/kg body weight for 12 days in rats suppressed both specific and nonspecific cell mediated immune response. Zakharov *et al*. (1992) reported that doxycycline at 25 mg/kg body weight decreased delayed type of hypersensitivity in mice against tularemia antigen. Jayakumar *et al*.(2002) reported that administration of ciprofloxacin (10 mg/kg body weight, iv, twice daily for 4 days) not alter skin thickness in DNCB skin sensitivity test against Brucella plain killed antigen in normal New Zealand White rabbits.

CONCLUSION

The present study was conducted evaluate the effect of long acting oxyteracycline formulation on humoral and specific immune response by assessment of DNCB skin sensitivity in both non antigen and antigen stimulated rats. In non antigen and antigen stimulated groups long acting oxyteracycline formulation did not produce any significant ($P>0.05$) difference in skin thickness in long acting oxytetracycline treated groups when compared with control groups. This indicated that both non specific as well as specific immune responses were not altered in rats administered with long acting oxytetracycline formulation.

Table 1. The effect of long acting oxytetracycline on dinitrochlorbenzene skin sensitivity test (mm) non antigen stimulated in rats

Time interval in hours	Saline control (Group I)	Pyrrolidone control (Group II)	Low dose (20 mg/kg) (Group III)	High dose (40 mg/kg) (Group IV)
0	1.392 ± 0.048	1.412 ± 0.091	1.342 ± 0.068	1.322 ± 0.100
24	1.894 ± 0.082	2.023 ± 0.154	1.998 ± 0.092	1.943 ± 0.054
48	1.686 ± 0.121	1.742 ± 0.063	1.468 ± 0.083	1.394 ± 0.067

Values: Mean ± SE, n =10, P>0.05

Table 2. The effect of long acting oxytetracycline on dinitrochlorbenzene skin sensitivity test (mm) in antigen stimulated rats

Time interval in hours	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	1.285 ± 0.081	1.302 ± 0.098	1.189 ± 0.112	1.212 ± 0.084
24	1.948 ± 0.169	2.014 ± 0.055	1.914 ± 0.055	1.875 ± 0.122
48	1.480 ± 0.063	1.504 ± 0.105	1.465 ± 0.069	1.450 ± 0.064

Values: Mean ± SE, n =10, P>0.05

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