



Immunological effect of sparfloxacin in goats

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ABSTRACT

In the present study, the immunological effects of a fluoroquinolone, sparfloxacin, were studied in goats after multiple i.m. administration. Three groups of goats (4 goats in each) were selected. Group I consisted of saline control, Group II contained animals with antigen (Ag) control [2 ml of 7% sheep red blood cell (SRBC), Ag given on 1st day as sensitizing dose and on 10th day as challenging dose] and Group III consisted of animals exposed to sparfloxacin and SRBC Ag (Sparfloxacin @ 5 mg/kg i.m. daily for 7 days during sensitizing and challenging period and SRBC as given in Group II). The blood samples were collected on 1, 7, 10, 14, 21, 28, 35 and 42 days of the experiment. The immunological parameters evaluated were haemagglutination (HA) test for humoral immune response (HIR) whereas delayed type hypersensitivity (DTH) for evaluating cell mediated immune response (CMIR). DTH was assessed by injecting 1-chloro-2,4-dinitrobenzene (DNCB) and purified protein derivative (PPD) in the skin of the neck region as well as by absolute lymphocyte count. There was no significant effect of sparfloxacin on humoral immune response as assessed by HA test. DTH assessed by DNCB mitogen was significantly higher in sparfloxacin treated group as compared to antigen treated whereas in case of PPD mitogen there was no significant effect of the drug. Absolute lymphocyte count differed significantly in sparfloxacin treated group (6499.75±19.87) with antigen treated group (5485.00±19.87). Thus, it can be concluded that sparfloxacin has some immunomodulatory effect with respect to cell mediated immune response in goats.

Keywords: Delayed type hypersensitivity, Goats, Immunomodulation, Serum, Sparfloxacin

Several studies indicate that antimicrobial agents have role which affect the host immunity and thereby alter the course of the diseases. It is well known that certain fluoroquinolones especially enrofloxacin have immunomodulatory effects in animals which help in recovery of diseased animals. Sparfloxacin is a potent, long acting third generation bactericidal fluoroquinolone derivative. The drug have shown potent antimicrobial activity against wide range of Gram negative and Gram positive bacteria including glucose non-fermentors and anaerobes, *Legionella*, *Mycoplasma*, *Chlamydia* and *Mycobacterium* spp. Methicillin resistant *Staphylococcus aureus* is also susceptible to Sparfloxacin (Shimada *et. al.* 1993). The major indication of this drug is in respiratory tract infections which include pneumonia, exacerbation, chronic bronchitis and sinusitis (Tripathi 2006). Several studies indicate that antimicrobial agents have role which affect the host immunity and thereby alter the course of the diseases. It is well-known that certain fluoroquinolones

especially enrofloxacin have immunomodulatory effects in animals which help in recovery of diseased animals. Fluoroquinolones are known to exert immunomodulatory effects on immune response when given to treat bacterial diseases (Badari *et al.* 2015).

Goat is a versatile animal and mainly reared in tropical countries like India. It is mainly reared by poor sections of society and serves as an easy alternative for uplifting the economic conditions of poor farmers because of its versatile traits like greater adaptability, maintenance on low grade ration, least rearing expenses and early economic return. In order to use a drug effectively, it is important to investigate that the drug has shown immunological effects on particular species in which the drug is to be clinically used. Keeping in view the aforesaid facts, the immunological effects of a fluoroquinolone, Sparfloxacin were studied in the present investigation, undertaken after the approval of IAEC as per guideline of CPCSEA and further approved by Rajendra Agricultural University, Bihar, Pusa, Bihar.

MATERIALS AND METHODS

Experimental animals and administration of drugs: For conducting immunological study, clinically healthy goats were divided into three groups consisting of six animals in each group (aged 1.5-2 years) weighing 25-30 kg, and were maintained under standard conditions and feeding

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schedule with availability of water *ad lib*. The animals were adapted to laboratory conditions for 2 weeks prior to the commencement of experiment. Sparfloxacin powder, (Wockhardt Pharmaceutical Pvt. Ltd., Mumbai) was dissolved in sterile distilled water to make strength of 10 mg of Sparfloxacin/ml. The drug was injected @ 5 mg/kg body weight by intramuscular (i.m.) route in each goat.

The treatment given to different groups was: Group I, Saline control; Group II, Antigen control – 2 ml of 7% SRBC [Sheep red blood cell (SRBC) suspension (i.v.) was given to each goat on the first day of treatment, i.e. sensitizing dose, and on the 10th day of experiment, i.e. challenging dose]; Group III, Sparfloxacin + Antigen – Apart from antigen (SRBC) given as in Group II, sparfloxacin was administered @ 5 mg/kg, i.m. daily for 7 days during sensitizing and challenging period (Pre and post challenge).

Collection of biological samples: Blood samples were collected on day 1, 7, 10, 14, 21, 28, 35 and 42 days of the experiment.

Analysis of immunological parameters: Preparation of buffer solution was done, i.e. Phosphate buffer saline and Alsever's solution. Mitogen were used for delayed type hypersensitivity, namely, DNCB (1-chloro-2, 4 – dinitrobenzene), 1% DNCB solution was prepared in acetone (10 mg/ml) and PPD (Tuberculin), a commercial preparation of tuberculin (purified protein derivative) available in the diluted form of 10 i.v./0.1 ml (Beacon Diagnostic Pvt. Ltd.) was procured.

Preparation of sheep red blood cell (SRBC) suspension: Blood (10 ml) was collected from jugular vein of sheep in a clean sterilized test tube containing equal volume of Alsever's solution as anticoagulant and left for 24 h. The blood was pooled and mixed with equal amount of phosphate buffer saline (PBS) and centrifuged at 500-800 rpm for 10 min. The supernatant was removed and packed cell volume was washed 3 times with PBS. Finally, 7% SRBC suspension and 0.8% SRBC suspension was made in fresh PBS and stored at 4°C. This SRBC suspension was used only for four days from the day of preparation. For further work, fresh SRBC suspension was prepared as and when required.

Collection of serum sample from goats: Blood (5-10 ml) was taken from the jugular vein of each goat with the help of 18 G needle by venepuncture. The blood was taken into a sterilized test tube and kept in slanting position for 4 to 5 h at room temperature. The separated serum was collected in a clean sterilized vial of 2 ml capacity and was preserved by adding sodium azide (1:10,000) and stored at 0°C until used.

Assessment of immune response after drug administration

Humoral immune response (HIR): Humoral Immune response (HIR) was assessed by HA test. Antigen used for haemagglutination reaction was sheep red blood cell. HA test was performed and goat sera control of the anti-SRBC

antibody titres were measured by using micro-titration technique as described by Beard (1980).

HA test was performed in 'U' shaped micro perspex plate. By taking 0.5 ml of serum, two fold serial dilution was made in PBS except in control well in which only PBS (0.5 ml) was added. In next step, 0.5 ml of 0.8% SRBC suspension was added to all the wells. A known positive and negative control was also included. The plate was stirred gently for mixing and uniform distribution of erythrocyte and left at room temperature for 40 min. The goat's serum produced a diffuse sheet of agglutination RBC covering the bottom of well where as negative control well showed circumscribed compact button at the bottom.

Cell mediated immune response (CMIR): Cell mediated immune response was assessed by Delayed type hypersensitivity (DTH) reaction, mitogen used for cutaneous basophilic hypersensitivity reaction are given below.

DNCB skin sensitivity test: The test was done as per the method described by Chauhan and Verma (1983) with minor modification. Three goats from each group were taken. Area of about 10 – 15 cm² was shaved with the help of razor on left and right lateral neck for DNCB application. These areas were cleaned with acetone and dried. DNCB (0.25 ml; 10 mg/ml) in acetone vertical was applied on right side. On left side, 0.25 ml of acetone was applied which served as control. DNCB was applied on 5th day and challenged on 15th day of experiment by applying 0.25 ml of DNCB (10 mg/ml) in acetone on right side and 0.25 ml acetone on the left side at the same site of first application. The skin thickness was measured with the help of slide calliper at 4, 8, 12, 24, 48, 72 and 96 h during pre and post challenge. The CMIR was calculated by subtracting the thickness of right side from left side.

PPD skin sensitivity test: The test was done as per the method described by Singh (1987). About 10 – 15 cm² area of both side of the neck were shaved, cleaned with acetone and dried. PPD (0.3 ml) (tuberculin) emulsified in 0.7 ml of Freund's complete adjuvant was injected intradermally on right side of neck and similar volume of PBS was inoculated on the left side. On 10th day post-sensitization, 0.1 ml of PPD (tuberculin) was injected on right side of neck skin and left neck skin received 0.1 ml PBS measurement of skin thickness was made by the aid of slide caliper on 4, 8, 12, 24, 48, 72 and 96 h post injection. The results were expressed as the difference of swelling on PPD injected site and PBS injected site at 4, 8, 12, 24, 48, 72 and 96 h post injection during pre and post challenge period.

Cell-mediated immune response was also assessed by the method described in Schalm's Veterinary Haematology. Absolute lymphocyte count for CMIR was done using a thin blood smear made from whole blood. After proper drying of the smear, it was stained with Leishman's stain and air dried. The edge of the smear was examined under oil immersion of the microscope. A total no. of 100 leukocytes were counted using the blood cell counter.

RESULTS AND DISCUSSION

Sparfloxacin a member of fluoroquinolones, possesses many advantages such as bactericidal and broad spectrum activity, no cross resistance with other groups of chemotherapeutic agents and better distribution in different tissues and body fluids in various species of animals. Effect of sparfloxacin on humoral immune response in goats was recorded using sheep red blood cell (SRBC) as an indicator of humoral immunity. It is well known that many drugs including antimicrobials are known to either stimulate or depress the immune system and thereby alter the course of the diseases. Therefore, it is highly desirable to carry out immunological studies of antimicrobials to find out the immunological responses of these drugs when given during routine therapy for various systemic infections.

Table 1 depicts HIR of sparfloxacin against SRBC antigen (LS mean±SE) to HA antibody titre (log₂ value) in goats. The study reveals no immunomodulatory effect on humoral immunity by sparfloxacin. The HA antibody titre recorded in sparfloxacin treated group was 1.70126 ± 0.0770 which had higher titre value as compared to antigen treated group, 1.57102±0.07569 but did not differ significantly. The antibody forming response against SRBC was recorded to be increased gradually from 7th day onwards and highest HA titre was observed on 21st days and then declining trend was observed. Pefloxacin and ciprofloxacin were also reported to alter the humoral immune response of mice against sheep red blood cells (Shoeb *et al.* 1990).

Effect of sparfloxacin on CMIR in goats after multiple intramuscular administrations was observed by using two different mitogen (DNCB and PPD/Tuberculin) and absolute lymphocyte count as indicator of cell mediated immunity.

Table 2 depicts CMIR of sparfloxacin to DNCB mitogen (LS mean±SE) in increase in skin thickness (mm) in goats after multiple intramuscular administrations. The study indicates the immunomodulatory action on cell mediated

Table 1. Humoural immune response (HIR) (LS mean±SE) of sparfloxacin against SRBC to antibody titre (log₂ value) in goats

Overall mean (μ)	1.3917±0.123
<i>Factor A (Groups)</i>	
Saline control ^A	0.90308 ^A ±0.07569
Antigen control ^A	1.57102 ^B ±0.07569
Sparfloxacin + Antigen	1.70126 ^B ±0.07700
<i>Factor B (Days)</i>	
1 ^A	0.90308 ^a ±0.123
7 ^A	0.90308 ^a ±0.123
10 ^A	1.48014 ^b ±0.123
14 ^A	1.80617 ^c ±0.123
21 ^A	2.25771 ^d ±0.123
28 ^A	1.73091 ^c ±0.123
35 ^A	1.20411 ^{ca} ±0.123
42 ^A	0.84911 ^a ±0.129

p<0.01.

Table 2. Cell Mediated Immune Response (CMIR) (LS mean±SE) of sparfloxacin to DNCB mitogen in goats

Overall mean (μ)	1.0698±0.051
<i>Factor A (Challenge)</i>	
Pre challenge ^a	0.8732 ^a ±0.027
Post challenge ^a	1.2663 ^b ±0.027
<i>Factor B (Group)</i>	
Saline control ^A	0.9073 ^A ±0.034
Antigen control ^A	1.1064 ^B ±0.034
Sparfloxacin + Antigen ^A	1.1955 ^C ±0.034
<i>Factor C (Hour)</i>	
4 ^a	0.5898 ^a ±0.051
8 ^a	0.7494 ^b ±0.051
12 ^a	1.9577 ^c ±0.051
24 ^a	1.4507 ^d ±0.051
48 ^a	1.1069 ^e ±0.051
72 ^a	0.9182 ^f ±0.051
96 ^a	0.7157 ^{ab} ±0.051

p<0.01.

immunity or host immunity by sparfloxacin. Significant increase in skin thickness was observed in sparfloxacin treated group (1.1955±0.0335 mm) as compared to both the control group (0.9073±0.0335 mm – saline control and 1.1064±0.0335 mm - antigen control). Increase in cutaneous basophilic hypersensitivity (CBH) response to DNCB was gradually increased significantly from 4 h (0.5898±0.0512 mm) and thereafter the highest CBH reaction was found at 12 h (1.9577±0.0512 mm) and significantly declined trend up to 96 h (0.7157±0.0512 mm) during pre challenge period i.e. 5th day and post challenge period i.e. 15th day of experiment.

Table 3 presents CMIR of sparfloxacin (LS mean±SE) to PPD/tuberculin. An increase in skin thickness (mm) in goats after multiple intramuscular administration of drug as well as increase in CBH response was observed

Table 3. Cell mediated immune response (CMIR) (LS mean±SE) of sparfloxacin to PPD mitogen in goats

Overall mean (μ)	0.7280±0.023
<i>Factor A (Challenge)</i>	
Pre challenge ^a	0.6379 ^a ±0.001
Post challenge ^a	0.818 ^b ±0.001
<i>Factor B (Group)</i>	
Saline control ^a	0.7592 ^a ±0.015
Antigen control ^a	0.5792 ^b ±0.015
Sparfloxacin + Antigen ^a	0.8456 ^b ±0.015
<i>Factor C (Hour)</i>	
4 ^a	0.3427 ^a ±0.023
8 ^a	0.6310 ^b ±0.023
12 ^a	1.2435 ^c ±0.023
24 ^a	0.9552 ^d ±0.023
48 ^a	0.7923 ^c ±0.023
72 ^a	0.6319 ^b ±0.023
96 ^a	0.4994 ^f ±0.023

p<0.01.

Table 4. Cell mediated immune response (CMIR) (LS mean±SE) of Sparfloxacin to absolute lymphocyte count/cubic mm in goats

Overall mean (μ)	5737.125±1.4702
<i>Factor A (Group)</i>	
Saline control ^A	5226.62 ^A ±19.867
Antigen control ^A	5485.00 ^B ±19.867
Sparfloxacin + Antigen ^A	6499.75 ^C ±19.867
<i>Factor B (Days)</i>	
1 ^A	5642.33 ^a ±32.442
7 ^A	5677.66 ^a ±32.442
10	5762.66 ^b ±32.442
14 ^A	5808.33 ^c ±32.442
21 ^A	5805.00 ^c ±32.442
28 ^A	5758.66 ^b ±32.442
35 ^A	5729.33 ^{ab} ±32.442
42 ^A	5713.00 ^{abc} ±32.442

p<0.01

in sparfloxacin treated group (0.8456±0.0153 mm). The increase was not significantly higher as compared to antigen control (0.5792±0.0153 mm). The study did not show immunomodulatory action on cell mediated immunity. Gradual increase in CBH response to PPD/ Tuberculin was noted at initial hours and mean peak skin thickness was noted at 12 h during pre challenge and post challenge period of experiment, which later on significantly declined.

The result of absolute lymphocyte count (per cubic millimeter) reveals that simultaneous administration of sparfloxacin caused significantly increased lymphocyte count in sparfloxacin treated group (6499±19.8670 cubic/mm) as compared to both control (5226.6250±19.8670 cu/mm – saline control and 5485±19.8670 cu/mm – antigen control). Lymphocyte count gradually increased from 1st day and the highest count was found at 14th day and thereafter, a declining trend was observed (Table 4).

All vertebrate animals have two arms of immune response i.e. humoral immune response (HIR) and cell mediated immune response (CMIR). The efficacy of both the arms can be judged. The humoral immune response can be judged by the demonstration of antibody titre against particular antigen (Kolmer *et al.* 1952) whereas, CMIR can be judged by delayed type hypersensitivity (DTH) and lymphocyte Count (LC). Sheep red blood cell (SRBC) is more often used as an antigen for the evaluation of HIR in different experimental animals by assessing the antibody titre in the sensitized host (Kabat *et al.* 1961). In the present study, the serum antibody titre to SRBC was evaluated using haemagglutination test for the assessment of HIR as per Beard *et al.* (1980). Likewise, CMIR was determined by DTH (Chauhan *et al.* 1983) reaction *in vivo* and lymphocyte test *in vitro* (Baounous *et al.* 1993).

In the present investigation, the haemagglutination antibody titre in sparfloxacin treated group was recorded to be higher (1.70126±0.0756) as compared to antigen treated group (1.57102±0.0756) though it was found to be non-significant. Therefore, it is suggested that no significant immune-modulatory effect on humoral immune response

happened due to this drug (Table 1). The above finding is more or less in agreement with the result of Jose *et al.* (1999) who observed that the simultaneous administration of pefloxacin produced a marginal suppression of humoral immune response, which was not statistically significant in rabbits. Valera *et al.* (1995) reported that humoral immune response by fluoroquinolones is a complex phenomenon which is affected by many factors like dose of the drug, duration of therapy and time of administration of antigen. In the present study, the antibody formation was observed on 7th day of antigen exposure and highest HA titre was observed on 21st day followed by declining trend thereafter up to 42nd days. The above findings are also in agreement with the findings of Jose *et al.* (1999) in rabbit for pefloxacin, Nitesh *et al.* (2005) in goat for pefloxacin and Dhiraj *et al.* (2007) for enrofloxacin respectively, who observed more or less similar observations up to 42nd day.

DTH reaction is considered as a valid measure for the *in vivo* assessment of CMIR (Thompson *et al.* 1975). T-cell provoke development of DTH which is measured by increase in the thickness of skin at the site of antigen inoculation in sensitized goat. Thickening of skin is produced by infiltration of various cells including sensitized T-lymphocyte at the site of mitogen inoculation (Reuben *et al.* 1979). In the present study, the sensitized skin in contact with mitogens like DNCB, PPD were used as one of the parameters of CMIR. The inoculated area was reddened, oedematous, swollen and subsequently indurated. The reaction were more prominent in DNCB and PPD inoculated group, respectively (Tables 2, 3). The DNCB skin sensitivity showed significant variation in the mean skin thickness between sparfloxacin treated group as compared to antigen treated group. In contrast, PPD skin sensitivity test did not show significant variation in the mean skin thickness between antigen treated group as compared to sparfloxacin treated group. It is well known that PPD possesses weak antigenic properties in comparison to DNCB. Probably, PPD did not show any marked mononuclear cell proliferation in goats by this drug.

In the present study, DNCB showed immuno-modulatory action on cell-mediated immunity or T-cell mediated immunity as significant increases in mean skin thickness in sparfloxacin treated group. The above findings are also in agreement with the finding for enrofloxacin (Dhiraj 2007) and for pefloxacin (Nitesh 2005) in goats.

The lymphocyte count is used as a method for detection of CMIR. The technique is preferred due to its low cost and accuracy as compared to other sophisticated techniques used for the evaluation of CMIR. In the present experiment, the lymphocyte count was employed as one of the techniques for the evaluation of CMIR in different experimental group such as SRBC inoculated group (Gr. II), sparfloxacin + SRBC treated group (Gr. III), normal saline inoculated group (Gr. I), by using the lymphocyte from peripheral blood of the experimental goats. The stimulation index (SI) of the lymphocytes stimulated with concanavalin-A, was

significantly higher in sparfloxacin + SRBC treated at 14 days post SRBC inoculated group as compared to SRBC alone treated group (Table 4). The result demonstrates that sparfloxacin potentiates the cell-mediated immunity. The present finding is in agreement with the result of Dhiraj *et al.* (2007) who observed that enrofloxacin potentiate cell-mediated immunity. Based on the immunological studies of Sparfloxacin in the present investigation, it is concluded that it can be safely and effectively used in mild to severe systemic infections.

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