

Immunomodulatory and Therapeutic Potential of Enrofloxacin in Bovine Sub Clinical Mastitis

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ABSTRACT : Immunomodulatory and therapeutic potential of Enrofloxacin was studied in bovine sub clinical mastitis (SCM). The therapeutic efficacy was adjudged by Somatic cell count and Total bacterial count of the milk, whereas, the immuno modulatory potential of the drug was assessed by measuring myeloperoxidase (MPO) and acid phosphatase (ACP) enzyme level in the milk leukocytes. Forty-five cows were divided into three equal groups. Gr I consisting 15 cows served as healthy control, whereas, 30 cows (SCM), Gr II and Gr III, selected on the basis of California Mastitis Test (CMT) positive reaction. Gr II cows received 150 mg of Enrofloxacin, once a day for three days and Gr.III received sterile 5 ml PBS (pH 7.4) for 7days, both the treatment were given by intramammary route. The observation was made up to 30 days post-treatment (PT). The CMT of the healthy milk was negative (0), whereas, it ranged between 1 point score and 2 point score in SCM. The Somatic cell count (SCC) and Total bacterial count (TBC) decreased significantly ($p < 0.05$) on day 3 PT in GrII cows in Enrofloxacin treated group, however, such changes were insignificant in PBS treated group. Traces of MPO and ACP enzyme were found in the healthy milk. The mean ACP level enhanced by 70% on day 3 PT in GrII and only 18.7% in Gr. III cows. The mean MPO level enhanced to 32% in Gr. II and 18 % in Gr. III cows on day 3 PT. Concomitant use of Enrofloxacin in SCM at sub optimal dose was found to reduce the bacterial load by increasing the bactericidal enzyme level in the milk polymorphonuclear cells (PMNs) in bovine SCM, which indicates its immunomodulatory potential in mastitis. (*Asian-Aust. J. Anim. Sci. 2003. Vol 16, No. 6 : 889-893*)

Key Words : Acid Phosphatase, Enrofloxacin, Myeloperoxidase, Polymorphonuclear Cells, Somatic Cell Count , Total Bacterial Count

INTRODUCTION

Mastitis is the disease of economic importance since it causes serious economic losses to the dairy industry (Shem et al., 2002). The incidence of the disease increases between parturition and peak lactation. The large variation in severity of the disease depends on the susceptibility of individual cows (Vandeputte-Van Messon et al., 1988). The SCC increases in mastitis, which is directly correlated to high bacterial load (Kurzhal et al., 1985). The outcome of the bacterial infection is greatly dependent on the function of polymorphonuclear cells, which plays vital role in udder immunity (Kehrli and Shuster, 1994, Paape et al., 1979). Impaired functioning of the PMN cells attributes to the pathogenesis of mastitis, which is observed in post parturient period (Dosogne et al., 1999). PMN leukocyte contains Myeloperoxidase, is the lysosomal enzyme of leucocyte granules and forms a system of defence against bacterial infection (Cooray and Bjorek, 1995). Similarly Acid phosphatase is also a lysosomal enzyme releases in soluble form in response to mastitis and during phagocytosis. ACP is positively correlated to high leukocyte count in the milk (Andrews, 1976).

Efficient removal of the invading bacteria requires both activity of the antibiotic against microorganism and an optimal activity of the immune system of the animal

(Hoeben et al., 1997). Mastitis is mostly treated by intramammary antibiotics, these antibiotics may affects body defences (Hoeben et al., 1998). It may be important that antibiotics used should not have adverse effect on PMN cell functioning, but might improve the competence of the immune cells. The purpose of this study was to see the therapeutic efficacy and immunomodulatory potential of enrofloxacin by measuring the lysosomal enzymes of the PMN cells isolated from the milk of the cows affected with sub clinical mastitis.

MATERIALS AND METHODS

Selection of dairy cows

The experiment was conducted at the organized dairy farm, it was started in the month of November upto mid January. Forty-five, crossbred lactating cows were selected from the organized dairy farm (Cattle and Buffalo), IVRI, Izatnagar. These cows were maintained in the animal shed of the institute under identical environmental conditions, they were supplied with green fodder, concentrate ration, wheat straw and clean drinking water. These cows were milked twice daily, both machine and hand milking were in vogue in the dairy farm.

Experimental design

The cows were divided in 3 equal groups. Gr. I, served as normal healthy control (average milk yield 9-15 lit/day). Gr.II and Gr.III (30 cows) screened for SCM on the basis of

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Table 1. CMT (point score), SCC ($\times 10^5$ cells/ml) and TBC ($\times 10^3$ cells/ml) in response to Enrofloxacin treatment

Parameters	Days post treatment				
	0	3	7	15	30
CMT					
GrI	0	0	0	0	0
GrII	1.87 \pm 0.091 ^a	0.53 \pm 0.133 ^b	0.07 \pm 0.067 ^c	0	0
GrIII	1.40 \pm 0.131 ^a	1.73 \pm 0.118 ^b	1.93 \pm 0.182 ^b	1.60 \pm 0.131 ^a	1.13 \pm 0.091 ^c
SCC					
GrI	3.400 \pm 0.162	3.48 \pm 0.145	3.48 \pm 0.145	3.400 \pm 0.162	3.100 \pm 0.106
GrII	8.73 \pm 0.182 ^a	2.43 \pm 0.142 ^b	1.66 \pm 0.119 ^c	1.64 \pm 0.072 ^c	1.56 \pm 0.107 ^c
GrIII	7.38 \pm 0.338 ^a	10.03 \pm 0.514 ^b	12.15 \pm 0.516 ^b	9.68 \pm 0.346 ^b	6.42 \pm 0.193 ^a
TBC					
GrI	0.40 \pm 0.131	0.40 \pm 0.131	0.47 \pm 0.165	0.33 \pm 0.159	0.40 \pm 0.131
GrII	5.93 \pm 0.483 ^a	0.20 \pm 0.107 ^b	0.067 \pm 0.067 ^c	0.067 \pm 0.067 ^c	0.067 \pm 0.067 ^c
GrIII	2.87 \pm 0.165 ^a	3.067 \pm 0.118 ^a	3.27 \pm 0.118 ^a	2.33 \pm 0.126 ^b	1.72 \pm 0.118 ^c

*Values with different superscripts in each rows (a, b, c, d) differ significantly ($p < 0.05$).

CMT positive reaction (Schalm et al., 1971), with average milk yield 8-14 lits. and 9-12 lits. per day in Gr II and in Gr III respectively. Enrofloxacin (Indoflox, M/S INDO Biocare, Indo Biocare Pvt. Ltd., Vadodara, 10% injection) was infused in Gr.II cows at the rate of 150 mg per teat by intramammary route, after diluting the drug in 5 ml sterile PBS (pH 7.4), once a day for three days, 5 ml sterile PBS (pH 7.4) was infused in Gr.III cows once a day for 7 days. The observations were made up to 30 days PT.

Collection of milk samples

The quarter milk samples from each cow were collected in sterile vials after cleaning the teat orifice with 70% ethyl alcohol and after discarding few streams of milk. 60 ml of milk sample was collected aseptically in sterile polyethylene screw capped vials and carried to the laboratory on ice. The milk was collected on day 0, 3, 7, 15 and 30 PT.

Somatic cell count and total bacterial count

The SCC in the milk samples was done as per the method described by Schalm et al. (1971). TBC carried out by the method of Griffin et al. (1977), on 5% bovine blood agar, after incubation at 37°C for 24 h, number of colonies counted by colony counter, later colony characteristics were noted and organism identified by gram's staining.

Isolation of polymorphonuclear cells (PMNs)

The isolation of PMNs was carried out as per the method described by Daley et al. (1991). The viability of the PMN cell was checked by Trypan blue exclusion technique and the cell suspension was adjusted to 1×10^7 cells/ml, in sterile PBS (pH 7.4).

Enzyme assay

The milk PMN cell pellet (1×10^7 cells per ml.) was lysed in 1 ml. of 0.1% Triton x-100 in sterile PBS by gentle vortexing, centrifuged at 200 g. for 10 minutes at 5°C, the

supernatant thus obtained was used for enzyme assay. Enzyme level was assayed before initiation of treatment and on day 3 PT.

Myeloperoxidase assay (EC 1.11.1.7)

MPO was assayed by using o-dianisidine as electron donor (Bretz and Baggiolini, 1974) and enzyme concentration was calculated by using molar extinction coefficient for oxidized o-dianisidine.

Acid phosphatase assay (EC 3.1.3.2)

ACP was assayed by the standard kit (Qualigen, Glaxo, Mumbai, India) as per the method of King and Jagtheesan (1959), later, the KA (King Armstrong) unit was converted to international unit.

Statistical analyses

The data were analyzed using one-way analysis of variance. The mean \pm SE of the same group of treatment were analyzed by using Duncan's Multiple Range Test and the mean \pm SE of different groups were analyzed by using Paired 't' test as per the standard methods.

RESULTS

Results pertaining to the therapeutic activity of the enrofloxacin in SCM are presented in table 1.

CMT, SCC and TBC

The CMT point score of the healthy milk was negative ('0'), whereas, it ranged between 1 point score and 2 point score in SCM. The CMT point score showed a decreasing trend just after the initiation of the treatment with enrofloxacin in Gr II cows, the CMT point score decreased to an extent of 71.6% on day 3 PT, to 96.4% on day 7 PT, as compared to '0' day value. The CMT point score was negative from day 15 PT, onwards till day 30 PT. The

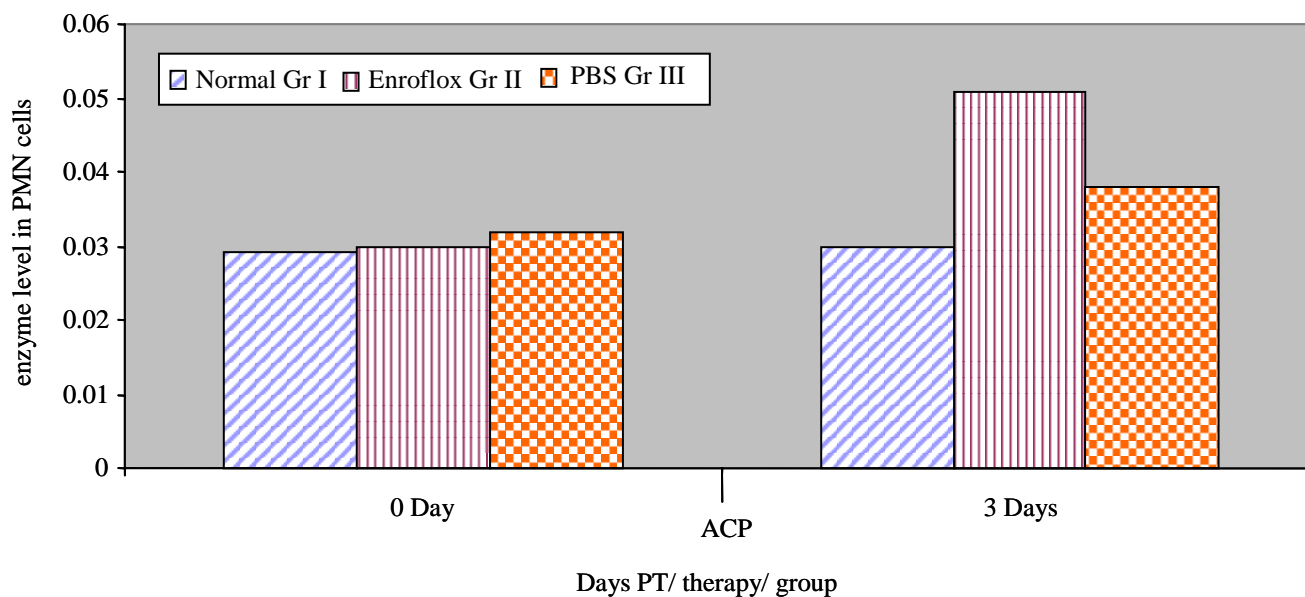


Figure 1. Effect of Enrofloxacin on ACP level in milk PMN cells.

intramammary infusion of enrofloxacin in Gr II cows significantly ($p < 0.05$) decreased the mean SCC to an extent of 68.9% on day 3 PT, which gradually decreased to 81.6% on 7th day PT, as compared to '0' day values, thereafter the values steadily decreased from day 7 PT, onwards till day 30th PT. Whereas, in Gr.III cows, there was steady rise of CMT point score till day 15 PT, however, the CMT point score remained more than 1 point score till 30th day PT, similarly, the mean SCC also enhanced up to day 15 PT, thereafter, nonsignificant reduction in SCC was observed on day 30 PT. The TBC in Gr.II dropped significantly ($p < 0.05$) on day 3 PT, the TBC decreased to an extent of 96.6% on day 3 PT, compared to pretreatment bacterial load, thereafter, it continued to decrease till day 15 PT, the TBC remained the same on day 30 PT as it was observed on day 15 PT. Whereas, the TBC remained $> 1,000$ cfu/ml of milk till day 30 PT, in Gr III cows. The initiation of clinical recovery was appreciated after 3 days of treatment and over all recovery rate was 93.3% in Gr. II cows. The colony characteristic on blood agar revealed 26.4% hemolytic and 73.3% nonhemolytic colonies, mainly gram positive cocci.

MPO and ACP enzyme level

There was release of MPO from the milk PMNs with the treatment of enrofloxacin. Compared to initial average MPO level of 6.94 ± 0.112 μ moles per 10^7 Cells, there was 32% rise on day 3 PT. The variation observed on the day 3 ranged from 8.60 to 11.67 μ moles per 10^7 cells (average 9.74 ± 0.263 μ moles per 10^7 cells). However the average MPO level rise was only 18% in Gr III cows, which did not differ significantly from the pretreatment values.

The infusion of enrofloxacin significantly ($p < 0.05$)

increased the ACP level to an extent of 70% on day 3 PT, with average value of 0.051 ± 0.003 μ moles $\times 10^7$ cells, ranging from 0.036 to 0.069 μ moles $\times 10^7$ cells. However quarters treated with sterile PBS enhanced the ACP level to 18.7% (0.038 ± 0.001 μ moles per 10^7 cells) as compared to pretreatment (0.030 ± 0.001 μ moles per 10^7 cells) level, which did not differ significantly.

DISCUSSION

It is evident from the result of the present trial that there is marked reduction of total bacterial count as early as 72 h PT, similarly, the SCC also reduced significantly. Enrofloxacin, is a synthetic potent antimicrobial agent with long lasting bactericidal effect with extended spectrum of activity, it belongs to fluoroquinolones group (Neer, 1998). The intramammary infusion of suboptimal dose of enrofloxacin shows significant fall of average CMT point score, and TBC as early as day 3 PT, and significant progressive fall was appreciated from day 7th to 15th. Fluoroquinolone group of antibiotics are potentially useful drugs for the treatment of mastitis caused by pathogenic organism (Kaartinen et al., 1995). Bovine sub clinical mastitis is commonly caused by *Staphylococcus aureus*, *Streptococcus* sp. and coliform bacilli (Shem et al., 2001). Choudhuri (2000) in a clinical trial reported 88.23% recovery by 3 days intramammary infusion of enrofloxacin in clinical mastitis.

Antibiotics are the only proven method for the prevention and control of mastitis, most of the antibiotics used for the therapeutic measures may affect immune mechanism directly by depressing the respiratory burst of

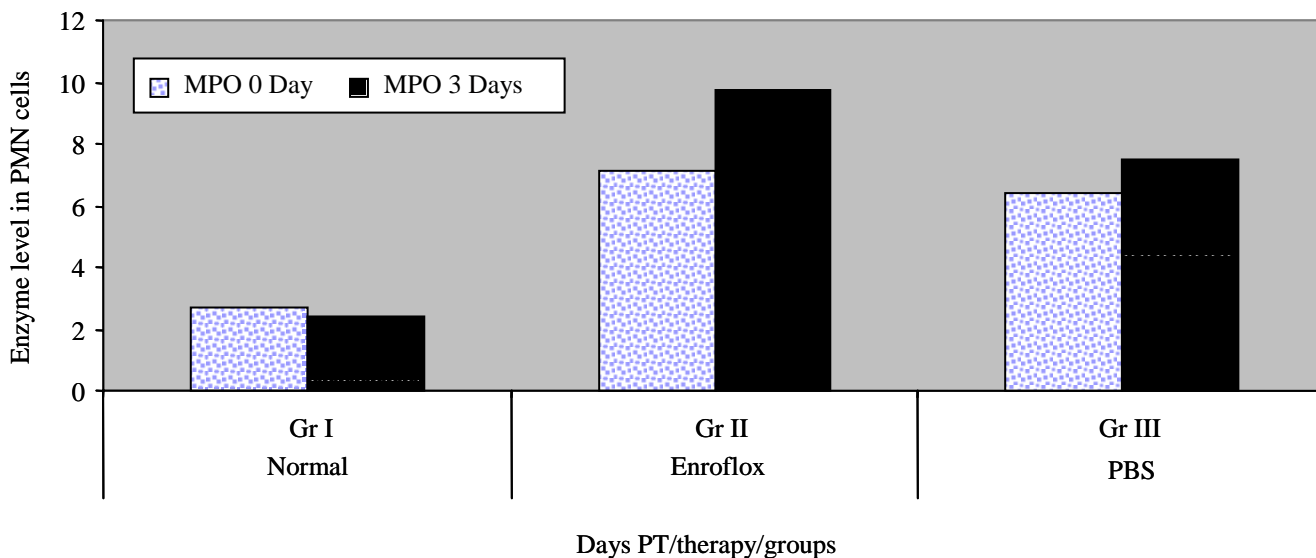


Figure 2. Effect of Enrofloxacin on MPO level in milk PMN cells

the PMN cells, and indirectly, by changes in the microorganism (Vanden, 1989). For removal of the pathogenic bacteria from the mammary gland the leukocytic defence should be potent enough to destroy the invading pathogens by internal cellular killing mechanism. In the periparturient period high producing cross bred cows have impaired PMN cellular functioning (Burvenich et al., 1994), therefore, antibiotic used to treat mastitis in such period must not impede the competence of the immune cells.

The immunomodulatory potential of enrofloxacin was assessed in the milk before and after treatment by measuring ACP and MPO enzyme level in the milk leukocytes. The MPO level raised to 32% and ACP to 70% as compared to pretreatment values. The bactericidal activities of PMN leukocytes are attributed to the presence of various antibacterial peptides and acid hydrolases in the Lysosomal granules, it has the capacity to generate lethal reactive oxygen intermediates (Lehrer and Ganz, 1990). Enrofloxacin might have stimulated the production of MPO and ACP in the PMN cells, both of these lysosomal enzymes are bactericidal in nature. MPO is an important component of oxygen dependent antimicrobial activity of the cell (Klefanoff and Clark, 1978), MPO together with H_2O_2 and halide creates potent antibacterial system against pathogen (Cooray et al., 1993). Hoeben et al. (1997), reported that enrofloxacin is the antibiotic which increases the chemiluminescence of the milk PMN cells and release of respiratory burst enzyme like myeloperoxidase. The immunomodulatory potential of enrofloxacin might be due to improvement of its penetration into the PMN cells and stimulation of H_2O_2 production.

It is concluded that no adverse reaction was observed after enrofloxacin treatment in bovine SCM, except milk discard for four days during the treatment. Significant fall

of TBC and enhancement of bactericidal enzymes in the leukocytes in the treated milk samples indicates the immunomodulatory potential of enrofloxacin in bovine sub clinical mastitis.

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