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IMMUNOSUPPRESSIVE ROLE OF FLORFENICOL IN BUFFALO CALVES

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SUMMARY

A study was designed to investigate the effect of florfenicol therapeutic dose, 20mg/kg body weight intramuscularly ; 2 injections 48 hours apart , on the immune competence of Egyptian female buffalo calves (3- 6 months) vaccinated with brucella abortus strain 19 . Florfenicol administration elucidated a significant decline in lymphocyte blastogenesis , chemotaxis and phagocytic activity.

Throughout the estimation of antibody titre , the results disclosed remarkable decrease of antibody levels by standard tube agglutination (SAT) and rivanol tests . It was concluded that the drug significantly suppress both cellular and humoral immune performance in Egyptian buffalo calves.

INTRODUCTION

Florfenicol roots trace back to chloramphenicol, whose P-nitro group caused human cancers. The florfenicol molecule differs structurally from chloramphenicol in two therapeutically important ways . First , The P- nitro group on chloramphenicol has been replaced by methyl sulphonyl group because p-nitro group is implicated in chloramphenicol induced idiosyncratic aplastic anemia . Second , the hydroxyl group on the carbon 3 of chloramphenicol has been replaced with fluorine atom .The replacement of this group with a non acetylable fluorine atom protect the molecule from inactivation by acetyl transferase , the common mechanism of bacterial resistance to chloramphenicol (Sams ,1994) .

During studding the immunophramacological effect of florfenicol in non lactating dairy cows ,

fect of florfenicol in non lactating dairy cows , Bretzalff et al. (1987) reported that florfenicol significantly inhibited phagocytosis at all concentrations.

On the same line , Khodary and Rizk (2000) indicated that florfenicol at the recommended dose reduced the leucocytic count in calves. Recently, Khalil (2002) concluded that florfenicol treatment induced immunosuppressive effects in calves , fish and poultry .

Florfenicol seem to have a promising forthcoming immunotherapeutic application in bovine .The present work was designed to investigate its possible effects on both cellular and humoral immune response of buffalo calves.

MATERIAL AND METHODS

1-Drug: Florfenicol (Nuflor® Produced by Schering -Plough Animal Health , USA.

2-Animals : Ten , clinically healthy, Egyptian female buffalo calves (3- 6 months) were used in this work. They were divided into two equal groups. The first received the vaccine (Brucella abortus strain 19, Coopers Animal Health Inc., Kansas , USA) alone and served as a control. The second group was injected with florfenicol therapeutic dose, 20mg/kg body weight intramuscularly ; 2 injections 48 hours apart simultaneous with vaccination process .

3-Blood sampling :From each animal ,two samples (serum and heparinized blood) were collected 2 hours prior to drug injection and weekly during 8 weeks post treatment.

A -Cellular immune response : -

1. Lymphocyte transformation:

Blast transformation of peripheral blood lymphocytes was measured after De Cock et al. (1980) ; Ishikawa and Shirahata (1985) and Shimakuira et al. (1985). Lymphocytes were separated according to Brulles and Wells (1977). The cells were washed three times then suspended in RPMI - 1640 medium with 10% fetal calf serum . The lymphocytes were cultured with mitogen, phytohaemaglutinin (PHA), in flat bottom microtitre plate. The plates were covered and incubated at 37°C in a humidified Co_2 atmosphere (5-10%) for 48 - 72 hours. The concentration of residual glucose in RPMI - 1640 medium used in this technique was estimated in control and stimulated cells, according to by Charles et al.(1978).

2. Phagocytic activity:

Isolation and cultivation of phagocytic cells was done according to Antley and Hazen (1988). The mononuclear cells were prepared for phagocyte activity with freshly prepared culture of Candida albicans (Richardson and Ssmith, 1981).The total number of phagocytic cells (the number of phagocytes with ingested yeast cells) and the total number of ingested yeast cells in individual phagocytes were determined to calculate the percent-

age of phagocytosis.

3. Chemotaxis:

Chemotaxis under agarose was performed according to Nelson et al. (1975).

B-Humoral immune response : -

1- Brucella antibody titer :

The titer of antibodies was measured in serum using serum tube agglutination test according to the method adopted by Alton et al.,1988 . Simultaneously, rivanol test was carried out according to the procedures of the National Veterinary Services Laboratories, Ames Iowa, USA (1984).

Statistical analysis :

The results are represented as the mean ± S.E. .Statistical significance was determined by student's (t) test for paired observations according to Snedecor (1971).

RESULTS

A- Effect of florfenicol on the cellular immune response:

Florfenicol administration displayed a significant reduction of chemotaxis. This reduction continued through the 4th week post treatment. After the 5th week, it increased to reach the control level (Table, 1).

Table (1) : Effect of florfenicol on the chemotaxis index in calves. (Mean ± S.E.) (n=5)

Week (post vaccination)	Vaccinated Group	Treated vaccinated group
Prevaccination	2.45 ± 0.31	2.56± 0.20
1	2.51 ± 0.15	1.72 ± 0.07*
2	3.26 ± 0.24	1.90 ± 0.16*
3	3.67 ± 0.11	1.57 ± 0.25*
4	3.80 ± 0.17	1.83 ± 0.17*
5	2.05 ± 0.28	2.11 ± 0.22
6	2.25 ± 0.35	2.31 ± 0.29
7	2.58 ± 0.36	2.08 ± 0.40
8	1.72 ± 0.22	1.93 ± 0.24

*: Significant at P< 0.05

The group treated with florfenicol depicted a significant reduction in lymphocyte stimulation indices after 1st. week till the end of experiment (in the presence of PHA). Phagocytic cell percentage showed a significant decrease for 6 weeks (Table, 2).

Table (2) : Effect of florfenicol on lymphocyte transformation (LYT) indicies (in the presence of PHA), phagocytic cell % (Phag %) in calves. (Mean ± S.E.) (n=5).

Week (post vaccination)	Vaccinated group		Treated vaccinated group	
	LYT	Phag%	LYT	Phag%
Prevaccination	1.80 ± 0.13	75 ± 4.7	1.83 ± 0.11	77.8 ± 4.7
1	2.23 ± 0.27	77.3 ± 6.7	0.93 ± 0.17*	61.7 ± 3.6*
2	2.97 ± 0.05	81.5 ± 5.3	1.07 ± 0.17*	52.3 ± 4.1*
3	2.43 ± 0.21	82.7 ± 4.5	0.95 ± 0.11*	50.7 ± 4.2*
4	2.51 ± 0.34	77.5 ± 4.7	1.06 ± 0.05*	56.2 ± 5.7*
6	2.80 ± 0.31	85 ± 4.7	0.98 ± 0.06*	55 ± 3.7*
7	2.71 ± 0.10	80.3 ± 6.7	0.85 ± 0.18*	70.3 ± 6.7
8	2.60 ± 0.11	77.6 ± 4.8	1.36 ± 0.13*	73.6 ± 3.7

*: Significant at P< 0.05

B- Effect of florfenicol on humoral immune response.

Table (3) displayed a significant lowering in brucella antibody titres using either rivanol or standurd tube agglutination titre (SAT) due to administration of florfenicol with strain 19 brucella vaccine . This reduction observed 1-4 weeks post vaccination.

Table (3): Effect of florfenicol on the antibody titre using Rivanol and Standard agglutination test (SAT) in vaccinated buffalo calves. (Mean ± S.E.)

Week (post vaccination)	Vaccinated group		Treated vaccinated group	
	SAT	Rivanol	SAT	Rivanol
Prevaccination	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
1	66 ± 10.3	25 ± 0.0	0.0 ± 0.0*	0.0 ± 0.0*
2	299.3±15.2	90 ± 10	90.82±10.5*	25.0±0.0*
3	897 ± 91.6	160 ± 15	305.0±20.64*	65.81 ±5..64*
4	417 ±42.2	400 ± 37	211.6±3.55*	190.87±13.28*
5	382.6± 35.1	200 ± 20	351.48± 55.8	188.57±21.81
6	245 ± 25.7	150 ± 15	219.98 ± 5.42	159.37 ± 10.28
7	90.81± 9.52	30.0 ± 5	82.80± 10.4	55.64± 10.87
	90.0 ± 5.82	30.0 ± 5	86.28 ± 8.40	35.8 ± 5.5

*: Significant at P< 0.05

DISCUSSION

Florfenicol is a new molecule belonging to the family of agents that includes thiamphenicol and chloramphenicol compounds. Because of their structural similarities, all compounds in this family have the same mechanism of antibacterial activity (Smas, 1994).

The present investigation was planned to gain more information on the effect of florfenicol on the immune competence of Egyptian buffalo calves.

Results of this study indicated that intramuscular administration of florfenicol restrain the immune system of calves . The inhibitory effect was pronounced at the 20mg/kg body weight intramuscularly ; 2 injections ,48 hours apart ,which have been recommended as a therapeutic dosage in many animal species . The immune suppressive effect was associated with decline in lymphocyte transformation , phagocytic ,chemotaxis activity , and antibody titre.

The previous data substantially reinforced the findings reported by Bretzlaff et al. (1987) and Paape et al. (1990) in cows . They documented that florfenicol induced immune suppression via inhibition of phgocytosis and alteration of neutrophil morphology .

Immunosuppressive effect of florfenicol may be attributed to the ability of the drug to penetrate lymphocyte and thereby inhibits its protein and DNA synthesis and eventually suppress the cellular function (Zhakov et al.,1980). Florfenicol was found to be highly effective against number of gram positive and negative bacteria (Graham et al., 1988) .Given this background , it could be predicted that vaccination with Br. abortus strain 19 would be subjected to static effect of florfenicol consequently reducing the reducing the body immune responsiveness to vaccine . Furthermore, the decrease of antibody titre may be refered to the suppressive effect of florfenicole on the haematopoietic activity resulting in acute depletation of circulating B and T lymphocytes , consequently declining the antibody titre (Zhakov et al. ,1980).

Unfortunately, our data do not provide a satisfactory clue for the hitherto documented immunossuppressive activity of florfenicol. Nevertheless, comparable effects to ours have been observed using other antibiotics such as chloraphenicol, that inhibited phytohaemagglutinin-induced lymphocyte transformation (Becker et al. , 1974). The immunosuppressive effect of chloramphenicol was tested in detailed study by Voiculescu et al (1983). They demonstrated that chloramphenicol strongly inhibited the antigen - dependent B cell blstogenesis in vitro . In addition chloramphenicol suppressed phagocytosis and completely blocked neutrophils activity

(Paape et al. , 1990).

Pieced together , our findings did clearly demonstrated a substantial immunosuppressive activity of florfenicol. In this context, the present study bias the possible future contraindicative use of this drug at least as antibacterial therapy in vaccinated animals.

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