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Some biochemical and immunomodulatory effects of marbofloxacin in sheep vaccinated with live sheep pox vaccine

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SUMMARY

The study was performed to investigate the effect of marbofloxacin (2 mg / kg body weight / day) on some immunological parameters in 9 sheep (35-40 kg. body weight) immunized with live attenuated sheep pox vaccine. Sheep were allocated into 3 equal groups. The first group was left as control; the second group was injected intramuscular (I/M) by marbofloxacin (2mg/kg body weight/day for 5 successive days) then vaccinated by intradermal (I/D) injection of 0.5 ml sheep pox vaccine/sheep. The third group was vaccinated with sheep pox vaccine only. The data revealed stimulation in the level of superoxide anion produced by neutrophil represented by detection of cytochrom C reduction level in the 2nd group ($p<0.05$) on the 1st day post vaccination, while there was significant depression in the level of lysozyme in the 1st two days post vaccination. The level of serum nitric oxide was not affected in the three groups. The lymphocytes transformation depressed significantly ($p<0.05$) in the 1st week post vaccination in the 2nd group as compared with the other two groups. It was concluded that marbofloxacin in its therapeutic level induced initial immunostimulant effect on the neutrophil followed by a transient immunosuppressive effect on other cells as macrophage when used before vaccination with live vaccine.

Key words: marbofloxacin, sheep, lymphocyte, neutrophil, lysozyme, nitric oxide, total antioxidant, super oxide dismutase.

INTRODUCTION

The fluoroquinolones are a class of compounds that comprise a large and expanding group of synthetic antimicrobial agents. Fluoroquinolone derivatives are frequently used therapeutically to treat

bacterial infections owing to, their high potency and wide - spectrum antimicrobial activity combined with good tissue penetration. These agents are widely acknowledged to display intracellular activity against bacteria that reside and/or multiply within phagocytes (Zang and Ward 2008).

Marbofloxacin is a potent synthetic antibiotic of fluoroquinolone, developed for Veterinary use only. It has a broad spectrum of activity, and the bactericidal concentration-dependent killing is observed against many Gram-negative bacteria. Marbofloxacin acts by inhibiting bacterial DNA replication (Aneliya *et al.* 2006).

In this study, the effect of therapeutic dose of marbofloxacin on neutrophil function and lymphocyte transformation was examined with reference to serum levels of total superoxide dismutase and levels of total antioxidant.

MATERIALS AND METHODS

Materials:

Animals: 9 apparently healthy sheep of (35-40 kg/b. wt) were used. They were obtained from the farm of ARRI and fed on the basal ration. They were divided into three equal groups; the first was kept as control, the second group was intramusculary injected by 2mg/kg b. wt marbofloxacin (marbocyl® 10%) for 5 days according to Sidhu *et al.* (2010) followed by vaccination with 0.5ml of sheep pox vaccine intradermally. Third group was only vaccinated with the same dose of the sheep pox vaccine.

Blood samples: serum was collected before vaccination and at 1st, 2nd, and 5th day then weekly till 4th week for determination of nitric oxide level, lysozyme activity, total antioxidant level and activity of superoxide dismutase. Heparinized blood samples were collected at 1st day post vaccination for separation of neutrophil and weekly for lymphocyte separation.

The phagocytic activity of polymorphnuclear leukocyte (PMNL) was assessed by measuring the superoxide anion production by stimulated PMNL using cytochrom reduction assay according to Lander Chacin *et al* (1990).

Lysozyme activity was measured according to Schltz, (1987)

Nitric oxide level in serum is measured using Griess reagent according to Rajaraman *et al.* (1998) Lymphocyte transformation was measured using MTT reduction assay according to Rai - Elbalhaa *et al.* (1985).

Superoxide dismutase was measured in serum according to Nishikimi *et al.* (1972)

Total antioxidant was measured according to Koracevic and Koracevic (2001).

Statistical analysis:

Data were subjected to statistical analysis according to Snedecor and Cochran (1982) by one way Anova employing a completely randomized design.

RESULTS

(Table 1) shows the level of superoxid anion produced by neutrophil represented by cytochrom-C reduction assay. A significant increase occurred in the marbofloxacin treated vaccinated group at the 1st day post vaccination as compared with other two groups.

(Table 2) indicates that lysozyme activity was significantly inhibited in the marbofloxacin treated vaccinated group at the 1st and 2nd days post vaccination as compared with the other two groups.

(Table 3) indicate that nitric oxide level was nonsignificantly affected allover the experiment period in all sheep groups

(Table 4) indicate that lymphocyte transformation due to phytohemagglutinin (PHA) mitogen using MTT reduction assay showed inhibition in the 2nd group in the 1st week only as compared with the other two groups.

Effect of marbofloxacin on the levels of serum total antioxidant and super oxide dismutase was not significantly affected allover the experiment period in all sheep groups. (Table 5 and 6)

Table (1): Effect of marbofloxacin on the level of superoxide anion produced by neutrophil using cytochrom –c reduction assay among sheep groups

Group	1st day
Control group	0.175±0.02
Marbofloxacin treated vaccinated group	0.445±0.09*
Vaccinated non treated group	0.137±0.044

Data as optical density (O.D) at 550nm *Significante at ($p<0.05$)
Data represent means ± SE.

Table (2): Effect of marbofloxacin on serum lysozyme level in sheep (mmol/ml)

Group	Bef drug and vaccine.	1day	2nd day	5th day	7day	14day	21day	28 day
Control Group	89.7±0.005	75.±14.7	119.1±7.3	97.1±26.5	111.8±33.7	111.8±7.3	111.78±13	97.07±7.3
Marbo.. Treated vaccinated. Group	89.7±7.4	38.23±7.4*	82.3±7.3*	75.01±7.2	97.08±14.7	111.76±0.02	75.01±7.4	82.36±7.3
Vaccinated. Non treated group	82.4±7.35	185.3±36	133.8±7.3	97.07±7.3	126.5±29.5	133.8±2	104±7.3	97.07±7.3

*Significant at P < 0.05 Data represent Means ±SE
Marbo.: means marbofloxacin.

Table (3) Effect of marbofloxacin on serum nitric oxide level in sheep (umol/ml):

Group	Bef drug and vacc.	1day	2nd day	5th day	7day	14day	21day	28 day
Control group	14.52±1.94	14.77±1.85	18.53±1.75	12.41±1.87	12.4±1.9	15.5±1.3	15.1±2	16.3±1.8
Marbo.. Treated vaccinated. group	14.53±2	19.5±5.19	16.57±1.9	15.2±4.23	14.3±2.4	15.5±3.6	13.4±0.4	15.1±1
Vaccinated. Non treated group	13.58±1.94	18.90±2.10	14.65±3.03	11.38±1.26	11.2±1.3	17±3.9	14.94±3.8	15.36±3.2

Data represent Means ±SE Marbo.: means marbofloxacin.

Table (4) Effect of marbofloxacin on lymphocyte proliferation in response to (PHA) mitogen using MTT reduction assay

Group	7day	14day	21day	28 day
Control Group	2.07±0.339	1.607±0.080	1.66±0.082	1.66±0.072
Marbo.. Treated vaccinated. Group	1.716±0.062*	1.445±0.168	1.66±0.124	1.54±0.152
Vaccinated. Non treated group	2.59±0.127	1.609±0.025	1.75±0.088	1.756±0.089

*Significante at (p<0.05) data represent as Mean ± SE
Marbo.: means marbofloxacin (Data represented as O.D at 570nm)

Table (5) Effect of marbofloxacin on serum level of total antioxidant(mmol/L) in all sheep groups

Gp	Bef drug	1day	2nd day	5th day	7thday	14day	21day	28 day
Control Group	1.38±0.1	1.64±0.1	1.63±0.05	1.53±0.01	1.51±0.09	1.52±0.008	1.46±0.08	1.48±0.10
Marbo. Treated vaccinat group	1.79±0.09	1.57±0.04	1.72±0.10	1.61±0.08	1.36±0.052	1.48±0.04	1.37±0.05	1.66±0.02
Vaccinat non treated group	1.65±0.06	1.63±0.08	1.46±0.19	1.54±0.07	1.36±0.13	1.61±0.03	1.24±0.04	1.28±0.02

Data represent as Mean ± SE

Table (6) Effect of marbofloxacin on level of serum superoxide dismutase in all sheep groups (mmol/L)

Gp	Bef drug	1day	2nd day	5th day	7thday	14day	21day	28 day
Control Group	64.96±6.3	63.96±0.03	62.66±6.06	65.6±7.3	61.4±13.7	64.06±7.3	63.33±5.8	65.03±5.3
Marbo. Treated vaccinat group	64.26±4.2	75.33±3.81	75.73±0.35	76.4±10	57±6.12	59±5.4	52.8±2.4	52.6±2.1
Vaccinat non treated group	71.6±11	64.4±2.39	86.53±5.8	75.6±5.0	66.8±8.82	60.2±0.8	59.76±0.6	60.1±1.9

Data represent as Mean ± SE

DISCUSSION

Fluoroquinolone derivatives are frequently used therapeutically to treat bacterial infections, these antibiotics demonstrate activity against several bacteria and are frequently used in the treatment of a wide range of Gram-positive and Gram negative bacterial infections (Gooding et al., 1992; Prescott et al.2000; Meunier et al.., 2004). These antimicrobial agents act on DNA Gyrase and/or topoisomerases. They have been demonstrated to be potential chemotherapeutic agents for antitumour and antiparasitic pathogens (Burri *et al.*, 1996).

Marbofloxacin (marbocyl1) is a third-generation fluoroquinolone developed for Veterinary use. It is used in sheep during treatment of *Actinobacillus Seminis* which was isolated from testis of ram (Puente-Redondo *et al.* 2000). Its plasma protein binding is low (Schneider *et al.*, 1996). In addition, the molecule shows a very high volume of distribution, which is widely diffused throughout the organism. Its elimination half-life (14 h) facilitates a single daily administration (Schneider *et al.*, 1996).

In the present work a significant increase in the production of the superoxid anion represented by significant increase in the level of cytochrom-c reduction at the 1st day post vaccination that was coincided with the results of Gladue *et al.* (1989) who mentioned that grepafloxacin as a fluoroquinolone is transported by the immune phagocytic cells increasing its production of superoxid anion then diffused to the infected tissues and El Bekay *et al.* (2002) who found that, generation of superoxide anion was significantly greater in phagocytic cells when incubated with norfloxacin. Also, Masayuki *et al.* (2002) suggested that there is priming effect of grepafloxacin on superoxide generation from human. In contrast, the pharmacological concentrations of fluoroquinolone did not influence the superoxide anion production by neutrophil. (Massimo *et al.* 1988)

Lysozyme is mainly produced from the macrophage (Tizard 1994)

.The obtained results showed that its activity in serum was significantly depressed in the marbofloxacin treated group than the other two groups at 1st and 2nd day post vaccination. This may be attributed to the depressant effect of the drug on the macrophage function as mentioned by Szczypka, *et al.* (2003) who used the same concentration in mice and found that marbofloxacin impairs the killing activity of peritoneal macrophages in non-infected mice.

The level of nitric oxide did not significantly affected that might be due to the transient effect of the therapeutic dose of the drug.on the immune cells, this was disagree with that of Vouldoukis *et al.* (2006) who found an increase in the nitric oxide level during incubation of macrophage with

500ug/ml of marbofloxacin in presence of canine leishmaniasis. Moreover, the results were not in agree with that of Hall *et al.*(2003) who found that alatrofloxacin possesses immunosuppressive activity but in the first four hours after bacteria the drug activates a lytic mechanism involving the release of nitric oxide, with elevations in lysosomal hydrolytic enzyme activities. The differences in the results may be attributed to the difference in dose and type of stimulating antigen.

Lymphocyte transformation in response to phytohaemagglutinin was significantly depressed in the 1st week post vaccination that may attributed to the direct depressant effect of the drug on the DNA synthesis of the lymphocyte that was coincided with that of Szczypka *et al.* (1986) and Gollapudi *et al.*(1986) who mentioned that difloxacin diminish proliferation by preventing the activity of enzymes involved in DNA replication. Also agreed with Gollapudi *et al.* (1992) who investigated the effects of rufloxacin, a new, long acting fluoroquinolone, on the growth and differentiation of human peripheral blood mononuclear cells stimulated with phytohemagglutinin and Pokweed mitogen, they found marked inhibition of B-cell differentiation., and Gorla *et al.* (1999) who mentioned that chromosomal aberrations occur when peripheral lymphocytes are exposed to the antimicrobial enrofloxacin. The obtained result were agreed with that of Kaminski *et al.* (2010) who found that fluoroquinolone antibiotic, exerts immunosuppressive effects on human t cells due to its suppressive effect on mitochondrial DNA of the lymphocyte.

Superoxide dismutase and total antioxidant levels in the serum did not affected by the drug allover the experiment the present result were disaagree with that mentioned by Goswami *et al.* (2006) who found that antioxidants gave protection against the production of reactive oxygen species by fluoroquinolones against *E.coli*.

In conclusion, marbofloxacin when used with its therapeutic level in sheep has a good effect on neutrophil function. Its depressant effect on the other immune function was transient for short period, so its use could be safe, especially before vaccination program.

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بعض التاثيرات البيوكميائية و المناعية للماربوفلوكساسين على الاغنام المحصنة بتحصين الجدرى الحى

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أجريت هذه الدراسة لمعرفة تأثير عقار الماربوفلوكساسين على الإستجابة المناعية ضد التحصين بجدرى الأغنام. وقد استخدم عدد ٩ من الأغنام بوزن يتراوح بين(٣٥-٤٠ كجم) قسمت الى ثلاث مجموعات. الأولى كانت ضابطة اما الثانية فقد حقنت فى العضل بالعقار بجرعة ٢مجم/ كجم من وزن الحيوان لمدة ٥أيام متصلة ثم حصنت بالحقن تحت الجلد بتحصين جدرى الأغنام ٠.٥مل/حيوان. اما المجموعة الثالثة فقد تم تحصينها فى نفس التوقيت وبنفس الجرعة ولكنها لم تأخذ الدواء وأخذت العينات فى الأيام (الأول ،الثالث، وأسبوعياً حتى الأسبوع الرابع بعد التحصين)وحللت النتائج وكانت النتائج كالتالى: كان هناك زيادة معنوية فى مستوى السوبر اكسيد انين المنتج بواسطة الخلايا متعددة الأنوية والممثل باختزال السيتوكروم سى فى المجموعة الثانية عند ($P<0.05$) وذلك فى اول يوم بعد التحصين. وقد انخفض نشاط الليزوزيم معنوياً فى اول يومين بعد التحصين فى نفس المجموعة. اما بالنسبة لمستوى أكسيد النيتريك فلم يتأثر معنوياً طول فترة التجربة بالنسبة للمجموعات الثلاثة.

بينما كان هناك تثبيط معنوى فى قابلية خلايا الليمفوسايت على الإنقسام الناتج من معاملة هذه الخلايا ب فيتوهيم أجليوتينين) فى المجموعة الثانية بالنسبة للمجموعات الأخرى وذلك فى الأسبوع الأول بعد التحصين وبالنسبة لمستوى مضادات الأكسدة ومستوى السوبر أكسيد ديسميوتاز فلم يكن هناك تغير معنوى يذكر خلال فترة التجربة.

مما تقدم تبين لنا ان استخدام الجرعة العلاجية من عقار الماربوفلوكساسين ادى الى تنشيط وقتى فى أول يوم لبعض منتجات الخلايا متعددة الأنوية (النيوتروفيل) وتبع ذلك تثبيط وقتى حتى الأسبوع الأول بعد التحصين بجدرى الأغنام مما يجعل استخدامه لنوع ما آمن قبل التحصين بهذا التحصين.