رقم البحث (6)

EFFECT OF MARBOFLOXACIN ON THE IMMUNE RESPONSE OF RABBITS UNDER VACCINATION BY

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ABSTRACT

The effect of intramuscular administration of marbofloxacin at a dose of 2 mg/kg b.wt on humoral immune response was investigated in twenty Newzealand white rabbits of 3-4 months old and weighing about 2-2.5 kg b.wt. Rabbits were vaccinated with the inactivated rabbit viral hemorrhagic disease (RVHD) vaccine. The haemagglutination inhibition (HI) antibody titers, total serum protein; albumin; and globulin levels, and the percentage of each protein fraction were evaluated. The results showed that the administration of marbofloxacin in vaccinated group induced a significant decrease in the HI antibody titers, total serum protein level, globulin level, and gamma globulin percentage concurrently with a significant increase in alpha and beta globulins percentages only at the 1st week post vaccination and treatment. On the other hand, no significant changes could be detected in the total serum protein; albumin; and globulin levels, alpha, beta and gamma globulin percentages of non-vaccinated treated group. Therefore, it was concluded that administration of marbofloxacin is not recommended to be used in vaccinated rabbits as it possess an immunosuppressive effect but can be used safely in non-vaccinated rabbits.

INTRODUCTION

Rabbits are important farm animals raised for a variety of purposes including meat, fur and wool production from some breeds. They are also used as laboratory animals and kept as pets **(Okerman, 1988)**. From the most serious viral diseases attacking rabbit populations is the viral hemorrhagic disease (RVHD) which causes high mortalities reaching 100%

especially in adults **(Hanaa, et al., 2009)**. Vaccination of rabbits in industrial rabbitries against RVHD is the only way to control the disease where it is endemic **(Taha, et al, 2009)**.

Antibiotics are widely used in rabbit industry either for prophylaxis or treatment of bacterial diseases affecting rabbits **(Hanaa, et al., 2009)**. Many antibiotics are capable of depressing the immune system even at therapeutic levels. Immunosuppressive properties of some antibiotics are effective in inhibition of both cellular and humoral immune responses to a variety of vaccines **(Shalaby, 1989)**.

Marbofloxacin is a new third generation of fluoroquinolones intended for veterinary use **(Ismail and El-Kattan, 2007)**. Many studies have shown an immunotropic action of fluoroquinolones; they can either stimulate or inhibit the functions of the immune system **(Szczypka and Obminska-Mrukowicz, 2003)**.

There have been no published reports on the immuno-pharmacological effect of marbofloxacin in rabbits. Therefore, the present work was aimed to explore the possible effect; if any; of marbofloxacin on humoral immune response of non-vaccinated and RHDV-vaccinated rabbits.

MATERIALS AND METHODS

Drug: Marbofloxacin (Marbocyl® 10%):

It is present as injectable vial of 50 ml. It was produced by Vétoquinol SA, France.

Vaccine:

Inactivated rabbit haemorrhagic disease virus vaccine was used for active immunization of experimental rabbits. It was purchased from Veterinary Serum and Vaccine Research Institute (VSVRI), Abbasia, Cairo, Egypt.

Rabbit haemorrhagic disease virus (RHDV):

RHDV (local isolate Giza 97) with a titre of 10^3 LD_{50}/ml identified against reference immune serum by **Salman, (1999).** It was kindly supplied from Newcastle disease vaccines department, Veterinary Serum and Vaccine Research Institute, Abbasia, Cairo.

Animals:

A total of twenty (20) Newzealand white rabbits of 3-4 months, weighing about 2-2.5 kg were used in this work. They were purchased from a private rabbitry without previous history of RHDV outbreaks or vaccination against RHDV.

Experimental design:

Rabbits were housed in disinfected metal cages in a well ventilated, well lightened and disinfected room. They received commercial pellet ration and clean water (ad-libitum), and kept under observation for 1 week before being used. They were classified into 4 groups (each of 5 rabbits) as the following:

- Group (1) was left as control, non-vaccinated non-treated group.
- Group (2) was intramuscularly injected with marbofloxacin 10% at a dose of 2 mg/kg b.wt. according to **Abo-El-Sooud and Goudah, (2009)** daily for 5 successive days.
- Group (3) was subcutaneously injected with the inactivated rabbit haemorrhagic disease virus vaccine at a dose of 0.5 ml per rabbit **(Hanaa, et al., 2009)**.
- Group (4) was given marbofloxacin 10% (2 mg/kg b.wt.) then vaccinated at the 5th day of treatment.

Each group was housed separately under well hygienic measures with daily observation until the end of the experiment.

Sampling:

Blood samples were collected from the ear vein of each rabbit without an anticoagulant to be used for serum separation at zero time (prior vaccination and drug administration), and subsequently at the 3rd, 7th, 14th, 21st and 28th days post vaccination and/or drug administration for studying the humoral immune response.

Assessment of humoral immune response:

1- The haemagglutination (HA) and the haemagglutination inhibition (HI) tests were carried out according to **Pu, et al., (1985)** in a round-bottom micro-titer plate (U-shape). The HA test was carried out to determine the HA titer of the virus before carrying out the HI test.

2- Estimation of serum total protein was carried out according to the Biuret method described by **Gornal, et al., (1949)**, serum albumin was determined according to **Dumas, et al., (1971)** modified by use of citrate instead of succinate buffer, a lower buffer concentration and reading of the final color at 550 instead of 630 nm. Globulins were determined by difference, where: Serum globulins= Total protein - Albumin

3- Qualitative fractionation of serum proteins for determination of serum alpha, beta, and gamma globulins was carried out using polyacrylamide gel columns according to the technique described by **Davis, (1964)** and **Ornstein, (1964)**.

Statistical analysis:

The obtained data in the present study were statistically analyzed for analysis of variance (ANOVA) and least significant difference (LSD) as described by **Snedecor and Cochran, (1981)** by using computerized SPSS (1996) version 10.0. The results of haemagglutination inhibition test were analyzed using a student's "T" test employed for analysis of variance according to **Snedecor, (1967)**.

RESULTS AND DISCUSSION

In veterinary practice, antibiotics are involved in the treatment of bacterial infection. Many of these antibiotics are capable of modifying the immune system even at therapeutic levels. The present work was aimed to explore the possible effect of using marbofloxacin on humoral immune response of both non-vaccinated and vaccinated rabbits (rabbit viral hemorrhagic disease). The haemagglutination inhibition (HI) antibody titers, total serum protein; albumin; and globulin levels, and the percentage of each protein fraction were evaluated.

1- Effect of marbofloxacin on the HI antibody titers:

The HA titer of the virus was determined to be (2^6= 1/64) and calculated as one HA unit. The HI test was performed using the dilution of virus containing 8 HA units of the virus (2^3=1/8). The obtained results, illustrated in Table (1), displayed a significant decrease in HI antibody titers only at the 1st week in vaccinated group as a result of marbofloxacin treatment compared with vaccinated non-treated group. On the other hand, a non-significant decrease was observed at 3 days, 2, 3 and 4 weeks post treatment and vaccination. These results are in agreement with that recorded by **Khalifeh et al., (2009)** who observed that, enrofloxacin reduced the production of Newcastle disease (ND) antibody in the first 3 weeks after the last ND vaccination measured by HI test. Likewise, these results were reinforced with that obtained by **Zahra and Abd El-Azem, (2003)** who found that, concurrent administration of marbofloxacin with live attenuated Rift Valley fever virus vaccine and 7 days post-vaccination induced a significant depression of antibody titers. Furthermore, **Ayad, (1987)** found that flumequine decreased the antibody titers of poults vaccinated with Newcastle virus vaccine. Similarly, **Bianciardi et al., (2004)** recorded a transient decrease in antibody titers after oral administration of enrofloxacin in *leishmania infantum* infected dogs.

2- Effect of marbofloxacin on serum total protein, albumin and globulin levels:

Tables (2) and (4) clearly demonstrated that the administration of marbofloxacin evoked non-significant changes in total serum protein and globulin levels of both vaccinated and non-vaccinated rabbits along the course of the study when compared with their respective control groups. Whereas, only at 1 week, a significant decrease was recorded in vaccinated treated group in comparison with vaccinated non-treated group. The decrease in total serum proteins at the 1st week of the experiment could be referred to the decrease in serum globulin level as a result of the inhibition of antibody (immunoglobulin) production by B-cells as previously shown in this study. These results are in complete agreement with that of **Zahra and Abd El-Azem, (2003)** who demonstrated a significant decrease in the level of serum total proteins and globulins in calves treated with marbofloxacin either simultaneously with Rift Valley fever virus vaccine or 7 days post-vaccination. Similarly, **Yosef, (2007)** found that intramuscular injection of marbofloxacin (10 mg/kg b.wt.) to healthy chicks produced a significant decrease in serum total protein levels on the 1st day post treatment, then it was returned to normal level on the 7th and 14th days post treatment. Moreover, **Takizawa et al., (1999)** mentioned that total globulin was significantly lower in grepafloxacin-treated rats than for controls.

Table (3) demonstrated that serum albumin level was non-significantly changed as a result of marbofloxacin administration in vaccinated and non-vaccinated rabbits along the 4 weeks of the study when compared with their respective control groups.

3- Effect of marbofloxacin on serum protein fractions (%) using electrophoresis :

Table (5), (6) and (7) demonstrated that a significant decrease in gamma-globulin percentage concomitant with a significant increase in alpha- and beta-globulin percentages was recorded only at the 1st week of the study in vaccinated treated group compared with vaccinated non-treated group. Meanwhile, there were no significant changes in alpha, beta and gamma globulins between non-vaccinated treated group and non-vaccinated non-treated group along the 4 weeks of the study. The significant increase in the alpha-globulin and beta-globulin percentages at the 1st week of the experiment might be linked to the decrease in gamma-globulin (immunoglobulin) percentage a result of the inhibition of antibody production by B-cells as previously shown in this study. In keeping with these lines, **El-Sayed and Manal, (2007)** found that there was a significant increase in alpha and beta globulins associated with a significant decrease in gamma globulins in florfenicol-treated

buffalos. These results coincide with that of **Jimenez-Valera et al., (1995)** who found that, mice injected intraperitoneally with ciprofloxacin (10 mg/kg b.wt) for 3 consecutive days then immunized with sheep erythrocytes after 24 hour from the last injection showed significant suppression of IgG-forming cells. On a similar ground, **Forsgren et al., (1987)** reported that the secretion of immunoglobulins by pokeweed mitogen-stimulated B-lymphocytes were inhibited in the presence of a clinically achievable concentration of ciprofloxacin. Moreover, **Tokarzewski, (2002)** reported that enrofloxacin has an immune suppressive effect on the level of IgY antibody present in serum and egg yolk after immune stimulation with live *Salmonella enterica* in laying hens. In the same direction, **Oda et al., (1996)** showed that oral administration of prulifloxacin (750 mg/kg) for 4 successive weeks increased the level of alpha-globulins in dogs.

It was concluded that marbofloxacin has a suppressive effect on humoral immune response of vaccinated rabbits, so it is not recommended to be used during vaccination programs but can be used safely in non-vaccinated rabbits.

Table (1): Effect of marbofloxacin (2 mg/kg b.wt.) given intramuscularly for 5 successive days on rabbit hemorrhagic disease virus (RHDV) haemagglutination inhibition (HI) antibody titers (Log2) in vaccinated and non-vaccinated rabbits. (Mean ± S.E.) n=5

Group	Time post vaccination and/or treatment					
	Zero time (before vacc. And/or treat.)	3rd day	1st week	2nd week	3rd week	4th week
G1 (Non-vacc. Non-treat.)	0	0	0	0	0	0
G2 (marbofloxacin treat.)	0	0	0	0	0	0
G3 (RHDV vacc.)	0	2.60±0.24 a	5.60±0.24 a	6.00±0.44 a	7.20±0.37 a	7.60±0.50 a
G4 (vaccinated treated)	0	2.40±0.24 a	4.80±0.20 b	5.80±0.37 a	6.60±0.40 a	6.80±0.37 a

Means within the same row bearing different superscripts are significant at($p<0.05$).

Table (2): Effect of marbofloxacin (2 mg/kg b.wt.) given intramuscularly for 5 successive days on total serum protein level (gm/dl) in vaccinated and non-vaccinated rabbits. (Mean ± S.E.) n=5

Group	Time post vaccination and/or treatment					
	Zero time (Before vacc. And/or treat.)	3rd day	1st week	2nd week	3rd week	4th week
G1 (Non-vacc. Non-treat.)	6.88 ± 0.39 a	6.53 ± 0.15 b	6.12 ± 0.10 b	6.03 ± 0.29 b	6.44 ± 0.30 b	6.44 ± 0.33 b
G2 (marbofloxacin treated)	7.37 ± 0.48 a	6.23 ± 0.24 b	6.81 ± 0.65 b	6.10 ± 0.55 b	6.90 ± 0.24 b	6.70 ± 0.42 b
G3 (RHDV vaccinated)	6.61 ± 0.29 a	7.39 ± 0.37 a	8.33 ± 1.03 a	7.73 ± 0.26 a	8.97 ± 0.29 a	8.67 ± 0.38 a
G4 (vaccinated treated)	6.91 ± 0.48 a	6.93 ± 0.20 ab	6.21 ± 0.29 b	7.54 ± 0.77 a	8.30 ± 0.16 a	8.20 ± 0.47 a

Means within the same column bearing different superscripts are significant at($p<0.05$).

Table (3): Effect of marbofloxacin (2 mg/kg b.wt.) given intramuscularly for 5 successive days on serum albumin level (gm/dl) in vaccinated and non-vaccinated rabbits. (Mean ± S.E.) n=5

Group	Time post vaccination and/or treatment					
	Zero time (Before vacc. And/or treat.)	3rd day	1st week	2nd week	3rd week	4th week
G1 (Non-vacc. Non-treat.)	4.60 ± 0.60 a	4.00 ± 0.28 a	4.34 ± 0.19 a	3.78 ± 0.35 a	4.10 ± 0.28 a	3.90 ± 0.27 a
G2 (marbofloxacin treated)	4.69 ± 0.27 a	4.13 ± 0.21 a	5.05 ± 0.53 a	3.97 ± 0.30 a	4.10 ± 0.31 a	4.00 ± 0.54 a
G3 (RHDV vaccinated)	4.24 ± 0.32 a	4.03 ± 0.32 a	4.63 ± 0.20 a	3.29 ± 0.29 a	4.34 ± 0.17 a	4.16 ± 0.23 a
G4 (vaccinated treated)	4.37 ± 0.42 a	4.36 ± 0.11 a	4.23 ± 0.06 a	3.92 ± 0.10 a	4.20 ± 0.30 a	4.02 ± 0.35 a

Means within the same column bearing different superscripts are significant at($p<0.05$).

Table (4): Effect of marbofloxacin (2 mg/kg b.wt.) given intramuscularly for 5 successive days on serum globulin level (gm/dl) in vaccinated and non-vaccinated rabbits. (Mean ± S.E.) n=5

Group	Time post vaccination and/or treatment					
	Zero time (Before vacc. And/or treat.)	3rd day	1st week	2nd week	3rd week	4th week
G1 (Non-vacc. Non-treat.)	2.28 ± 0.45 a	2.15 ± 0.28 b	1.78 ± 0.16 b	2.25 ± 0.10 b	2.34 ± 0.31 b	2.53 ± 0.12 b
G2 (marbofloxacin treated)	2.68 ± 0.50 a	2.10 ± 0.33 b	1.76 ± 0.49 b	2.13 ± 0.63 b	2.80 ± 0.21 b	2.70 ± 0.63 b
G3 (RHDV vaccinated)	2.37 ± 0.17 a	3.35 ± 0.63 a	3.69 ± 0.87 a	4.44 ± 0.44 a	4.63 ± 0.26 a	4.51 ± 0.21 a
G4 (vaccinated treated)	2.54 ± 0.24 a	2.57 ± 0.18 ab	1.98 ± 0.33 b	3.62 ± 0.86 a	4.10 ± 0.33 a	4.18 ± 0.73 a

Means within the same column bearing different superscripts are significant at($p<0.05$).

Table (5): Effect of marbofloxacin (2 mg/kg b.wt.) given intramuscularly for 5 successive days on serum alpha-globulin (%) in vaccinated and non-vaccinated rabbits. (Mean ± S.E.) n=3

Group	Time post vaccination and/or treatment			
	1st week	2nd week	3rd week	4th week
G1 (Non-vacc. Non-treat.)	15.95 ± 1.51 a	15.89 ± 1.66 a	15.39 ± 2.08 a	14.19 ± 0.71 a
G2 (marbofloxacin treated)	14.29 ± 1.10 a	14.86 ± 1.62 a	14.30 ± 0.08 a	14.75 ± 1.30 a
G3 (RHDV vaccinated)	11.36 ± 1.25 b	10.12 ± 1.75 b	12.97 ± 1.50 b	11.36 ± 0.08 b
G4 (vaccinated treated)	14.04 ± 0.79 a	12.52 ± 0.82 b	12.82 ± 0.64 b	10.97 ± 0.55 b

Means within the same column bearing different superscripts are significant at($p<0.05$).

Table (6): Effect of marbofloxacin (2 mg/kg b.wt.) given intramuscularly for 5 successive days on serum beta-globulin (%) in vaccinated and non-vaccinated rabbits. (Mean ± S.E.) n=3

Group	Time post vaccination and/or treatment			
	1st week	2nd week	3rd week	4th week
G1 (Non-vacc. Non-treat.)	14.37 ± 1.29 a	12.14 ± 0.34 a	13.25 ± 0.63 a	12.91 ± 1.61 a
G2 (marbofloxacin treated)	13.92 ± 0.94 a	14.16 ± 0.38 a	14.15 ± 1.18 a	10.93 ± 0.66 a
G3 (RHDV vaccinated)	11.05 ± 1.04 b	10.93 ± 0.73 b	9.56 ± 1.56 b	8.91 ± 1.46 b
G4 (vaccinated treated)	14.45 ± 1.07 a	10.11 ± 0.36 b	10.69 ± 0.98 b	8.42 ± 0.58 b

Means within the same column bearing different superscripts are significant at($p<0.05$).

Table (7): Effect of marbofloxacin (2 mg/kg b.wt.) given intramuscularly for 5 successive days on serum gamma-globulin (%) in vaccinated and non-vaccinated rabbits. (Mean ± S.E.) n=3

Group	Time post vaccination and/or treatment			
	1st week	2nd week	3rd week	4th week
G1 (Non-vacc. Non-treat.)	15.56 ± 1.49 b	15.68 ± 0.83 b	15.29 ± 1.60 b	16.02 ± 0.94 b
G2 (marbofloxacin treated)	15.69 ± 0.99 b	15.52 ± 1.25 b	15.13 ± 0.25 b	15.91 ± 1.59 b
G3 (RHDV vaccinated)	18.34 ± 0.73 a	19.66 ± 0.50 a	20.24 ± 1.57 a	22.32 ± 0.65 a
G4 (vaccinated treated)	15.88 ± 0.78 b	18.86 ± 0.75 a	19.94 ± 1.47 a	21.65 ± 0.77 a

Means within the same column bearing different superscripts are significant at($p<0.05$).

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تأثير الماربوفلوكساسين على المناعة السوائلية فى الأرانب المحصنة

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الملخص العربى

تم دراسة تأثير الحقن العضلى للماربوفلوكساسين بجرعة قدرها ٢ مجم/ كجم من وزن الجسم على المناعة السوائلية فى عدد ٢٠ أرنب نيوزيلاندى يتراوح عمرهم بين ٣- ٤ شهور و أوزانهم بين ٢- ٢,٥ كجم، تم تحصين الأرانب بلقاح النزف الدموى الفيروسى. تم قياس مستوى الأجسام المضادة الخاصة بفيروس النزف الدموى الأرنبى و مستوى البروتينات الكلية ، الزلال ، و الجلوبيولين بالإضافة إلى تحديد نسبة كل بروتين (الجلوبيولينات المختلفة) عن طريق الفصل الكهربى. أوضحت نتائج الدراسة أن إعطاء الماربوفلوكساسين فى الأرانب المحصنة أدى إلى نقص معنوى فى مستوى الأجسام المضادة الخاصة بفيروس النزف الدموى الأرنبى و مستوى البروتينات الكلية و الجلوبيولين و نسبة جلوبيولينات الجاما يصاحبه زيادة معنوية فى نسبة كلا من جلوبيولينات البيتا و جلوبيولينات الألفا فى الأسبوع الأول من التجربة. على الجانب الآخر لم يكن هناك تغيرات معنوية فى كلا من مستوى البروتينات الكلية و الجلوبيولين و نسبة جلوبيولينات الجاما و البيتا والألفا فى الأرانب الغير محصنة التى تم إعطائها الماربوفلوكساسين. و نستخلص من هذه الدراسة أنه لا ينصح باستخدام الماربوفلوكساسين فى الأرانب المحصنة حيث أن له تأثير مثبط للمناعة و لكن يمكن استخدامه بصورة آمنة فى الأرانب الغير محصنة.