

Some Immunomodulating Effects of Diclazuril in New Zealand Rabbits

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Abstract

This study was conducted to investigate the immunomodulating effects of the diclazuril anticoccidial drug (1 mg/kg BW) given orally for 5 successive days to rabbits vaccinated with rabbit haemorrhagic viral disease (RHVD). Two blood samples were collected at the 1st, 2nd, 3rd, 7th, 14th and 21st days post drug and vaccine administration to study the effect of diclazuril on the innate immune response (phagocytic activity, serum lysozyme activity and nitric oxide production) and the humoral immune response (serum total proteins, determination of serum globulins and gamma globulins fractions using electrophoretic technique). The results revealed that diclazuril had adverse effect on both the innate and humoral immune response as evidenced by a significant decrease in phagocytic activity, lysozyme activity, nitric oxide production, serum total proteins, globulins and gamma globulins in rabbits. Therefore, diclazuril is not recommended as a chemotherapeutic agent for rabbits in the dose of 1 mg/kg BW.

Keywords: Diclazuril, Cellular, Humoral, Immune, Serum Proteins

Introduction

Rabbits are important farm animals raised for a variety of purposes including meat, fur and wool production from some breeds. For many years, prophylactic use of anticoccidial drugs has been the primary means of controlling coccidiosis. Anticoccidial drugs such as diclazuril had a significant prophylactic effect on *Eimeria stiedai* infection in rabbits [1]. Diclazuril, Toltrazuril and Nicarbazin were introduced firstly and then followed by ionophores. Nowadays, these drugs are considered as a stone base component for controlling coccidiosis [2]. In order to overcome the drug resistance of *Eimeria* species, several researches had been done to seek alternative means of control through increased knowledge for understanding the immunomodulation and natural product feed additives [3].

Diclazuril is a chemical anticoccidial drug which is a nucleotide analogue for prevention of coccidiosis intended for use in broilers, chickens, turkeys and rabbits. Diclazuril is a benzeneacetonitrile that has potent activity against various stages of *Eimeria tenella* [4].

Few anticoccidials of this type have been developed [5]. Diclazuril is a very potent anticoccidial in rabbits and can be advocated for safe medication at 1 ppm for 7 days [6]. Many studies declared the potent activity and efficacy of diclazuril against various species of *Eimeria* [7,8]. Some immunologic studies have been performed to detect the effect of diclazuril on innate and humoral immune response. Several researches demonstrated that the therapeutic doses of diclazuril did not interfere with formation of protective immunity to *E. tenella* [9,10]. Diclazuril with a dose of 2 ppm did not affect the performance of rabbits and chickens but negative modulating effect on the humoral and cell-mediated immune response has been recorded [11,12]. Therefore, the aim of this study was to elucidate the potential adverse effects of diclazuril on rabbits at a dose of 1 mg/kg BW for 5 successive days.

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Animals and experimental design

Eighteen New Zealand white rabbits of 3 months old and weighing about 2 kg were used in this study. They were purchased from a private rabbitary without history of rabbit haemorrhagic viral disease (RHVD) outbreaks or RHVD vaccination. The rabbits were tested serologically and proved to be sero-negative for RHVD. They were housed in disinfected well ventilated metal cage and provided with *ad-libitum* commercial pellet ration and clean water and kept under observation for one week before being used.

Rabbits were classified into 3 groups (each of 6 rabbits) as the following; the first group was non vaccinated non treated and was kept as a negative control; the second group was vaccinated (0.5 mL RHVD S.C) non treated and was kept as a positive control. The 3rd group was vaccinated with RHVD (0.5 mL S.C) and treated by diclazuril 1 mg/kg BW (Diclosol[®] liquid each 1 mL contain diclazuril 10 mg, Pharma Swede Company) given orally for 5 successive days according to the company recommendation for lambs. Two blood samples were collected from the ear vein of 5 rabbits/group at 1st, 2nd, 3rd, 7th, 14th and 21st days post vaccination for studying both the innate and humoral immune responses. The first sample was 2-3 mL of blood collected in a sterile Wasserman tube containing heparin (50 IU/mL) to measure the phagocytic activity [13]. The second sample was 3-5 mL of blood collected in a sterile Wasserman tube without an anticoagulant.

These samples were allowed to clot, and the serum was separated by centrifugation at 3000 r.p.m. for 10 min and stored at -20°C in sterile Eppendorf tubes till the time of use. Estimation of lysozyme concentration [14], nitric oxide production [15], total serum proteins [16], globulins and serum gamma globulins was carried out [17,18].

Statistical analysis

Data in the present study were statistically analyzed using SPSS version 21, IBM Corp., Chicago, IL, USA. Analysis of variance (ANOVA) [19] was used to determine the effect of diclazuril on the immune parameters.

Results and Discussion

Rabbits are exposed to many negative modulating factors, such as bad hygiene, concomitant infection with other diseases, management errors and immune suppressive drugs [20]. Immune suppression is the main cause of many diseases such as cancer, autoimmune disorders and some chemotherapeutic drugs. Blood parameters serve as indicators for the effect of drugs on innate and adaptive immune response and other serum proteins. Treatment of the vaccinated (RHVD) rabbits with diclazuril (1 mg/kg BW) decreased the phagocytic activity (phagocytosis % and phagocytic index) significantly at the 3rd day post vaccination. While, lysozyme concentration and nitric oxide production were decreased at 1st, 2nd and 3rd day post vaccination compared with vaccinated non treated group.

Table 1: Effect of oral administration of diclazuril (1 mg/kg BW) for 5 successive days on phagocytosis % and phagocytic index in vaccinated rabbits (Mean±S.E), n=5

Groups	Phagocytic percent %	Phagocytic index
Control	71.40± 0.60 ^c	4.20± 0.05 ^c
Vaccinated only	85.80± 1.24 ^a	6.86± 0.04 ^a
Vaccinated and treated	75.00± 0.70 ^b	4.84± 0.09 ^b

Means within the same column carrying different letters are significant at $P \leq 0.05$

Phagocytes act as storage for lysozyme, myeloperoxidase, acid hydrolysis, and complement activators [21]. The decrease in phagocytic percent and index (Table 1) express the adverse effect of diclazuril (1 mg/kg BW). These results were in agreement with Hassan *et al.* [11] who reported that administration of 2 ppm diclazuril given for 7 weeks with diet for New Zealand rabbits lead

to leukopenia and neutrophilia. Lysozyme is part of the innate immune system which is a major secretory product of macrophages with antimicrobial and immunomodulating actions which reflect on the activities of macrophages [21]. The reduction of lysozyme concentration was significantly noticed as a side effect of diclazuril administration (Table 2).

Table 2: Effect of oral administration of diclazuril (1 mg/kg BW) for 5 successive days on lysozyme concentration ($\mu\text{g/mL}$) in vaccinated rabbits (Mean \pm S.E), n=5

Groups	Serum lysozyme conc. ($\mu\text{g/mL}$)		
	1 st day	2 nd day	3 rd day
Control	120.0 \pm 0.70 ^c	130.0 \pm 0.31 ^c	137.0 \pm 0.32 ^b
Vaccinated only	190.60 \pm 0.40 ^a	197.0 \pm 0.32 ^a	191.80 \pm 0.48 ^a
Vaccinated and treated	130.40 \pm 0.50 ^b	140.6 \pm 0.40 ^b	135.60 \pm 0.40 ^c

Means within the same column carrying different letters are significant at $P \leq 0.05$

Table 3: Effect of oral administration of diclazuril (1 mg/kg BW) for 5 successive days on serum nitric oxide production ($\mu\text{g/mL}$) in vaccinated rabbits, (Mean \pm S.E) n= 5

Groups	Serum nitric oxide production ($\mu\text{g/mL}$)		
	1 st day	2 nd day	3 rd day
Control	18.82 \pm 0.32 ^b	19.10 \pm 0.35 ^b	18.88 \pm 0.46 ^b
Vaccinated only	27.70 \pm 1.11 ^a	28.28 \pm 1.53 ^a	27.60 \pm 0.92 ^a
Vaccinated and treated	21.36 \pm 1.92 ^b	21.02 \pm 1.33 ^b	22.30 \pm 1.68 ^b

Means within the same column carrying different letters are significant at $P \leq 0.05$

Concerning nitric oxide (NO) which is considered a product of immune system cells such as macrophages is a potent biological effector regulating blood vessels dilatation, serving as a neural messenger and playing a complex role in inflammatory response. It is activated by cytokine microbial compounds which are derived from amino acid L-arginine by the enzymatic activity of inducible nitric oxide synthase (iNOS) and functions as a tumoricidal and antimicrobial molecule *in vitro* [22]. The current investigation revealed significant reduction of nitric oxide production

which was supported by Mohammed [12] who stated that 2 ppm diclazuril administration resulted in a significant reduction in the percent of monocytes, basophils and heterophils which play an important role in phagocytosis. It was found in the present investigation that the production of nitric oxide was significantly decreased. Consequently, it could be concluded that these cells are responsible for nitric oxide release. The results demonstrated the positive correlation between nitric oxide production and lysozyme concentration.

Table 4: Effect of oral administration of diclazuril (1 mg/kg BW) for 5 successive days on total serum protein level (g/dl) in vaccinated rabbits, (Mean±S.E) n= 5

Groups	Total protein g/dl		
	1 st week	2 nd week	3 rd week
Control	5.08± 0.10 ^b	5.00± 0.07 ^b	5.40± 0.12 ^b
Vaccinated only	7.32± 0.06 ^a	7.10± 0.31 ^a	7.78 ± 0.07 ^a
Vaccinated and treated	5.24± 0.05 ^b	6.42± 0.14 ^a	7.28 ± 0.14 ^a

Means within the same column carrying different letters are significant at $P \leq 0.05$.

The current study illustrated a proteinogram analysis (Tables 4). A significant decrease in total serum protein (hypoproteinemia) at 1st week post vaccination was recorded in comparison with vaccinated non treated. A

high significant decrease in gamma globulins (%) all over the experimental period was obvious when compared with vaccinated non treated group (Table 5).

Table 5: Effect of oral administration of diclazuril (1 mg/kg BW) for 5 successive days on serum gamma-globulins (%) in vaccinated rabbits, (Mean±S.E) n= 3

Groups	protein fraction (gamma %)		
	1 st week	2 nd week	3 rd week
Control	16.34± 0.10 ^c	16.40± 0.01 ^c	16.80± 0.01 ^c
Vaccinated only	18.64± 0.01 ^a	18.51± 0.01 ^a	18.64 ± 0.01 ^a
Vaccinated and treated	15.47± 0.02 ^b	16.21± 0.02 ^b	17.30 ± 0.01 ^b

Means within the same column carrying different letters are significant at $P \leq 0.05$.

The decrease in total serum proteins after the first week of the experiment is going with the main stream of the adverse effect of the diclazuril on the immune system. The decrease in serum globulins level is attributed to the reduction in antibodies produced by B-cells, the reconstruction, activation and hyperplasia of lympho-reticular cells at the beginning of immunogenesis. The reduction of globulins synthesis is one of the factors which lead to immune deficiency which resulted from malfunction of lymphocytes. These results were in agreement with those previously reported by El-Kahkey [23] who measured the effect of two doses of semduramicin (25 and 50 ppm) on the cellular and humoral immune response on broiler chickens vaccinated against Newcastle viral disease. The author reported that 50 ppm had non-significant changes in albumins but a significant decrease in serum gamma globulins at the 1st and 3rd week post vaccination at both dose (25 and 50 ppm) of semduramicin.

Our results were also in accordance with Abdel-Hafez [24] who reported that 2 ppm of diclazuril decreased the total serum proteins and globulins level (gamma-globulins). The observed decrease in gamma-globulins at 1st and 2nd week post vaccination may be due to the negative effect of diclazuril on the lymphoid organs which is considered the main part in synthesis of gamma globulins as reported by Cooper *et al.* [25].

Conclusion

In conclusion, particular chemotherapeutics such as diclazuril lead to immune deficiency” secondary immune deficiency”, which refers to augment disease risk. Therefore, diclazuril is not recommended in the current dose for treating coccidia in rabbits.

Conflict of interest

The authors declare no conflict of interest.

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الملخص العربي

بعض التأثيرات المناعية للدايكلازوريل في الأرانب النيوزلاندييه

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تم إجراء هذه الدراسة لبيان التأثير المناعي للدايكلازوريل (1 مجم/كجم من وزن الجسم) عند إعطائه لمدة خمسة أيام متتالية كمضاد للكوكسيديا في الأرانب المحصنة بلقاح النزف الدموى الفيروسي. تم اخذ عينتان من الدم من كل أرنب (عينة باستخدام مضاد التخثر والعينة الأخرى بدون مضاد التخثر) في اليوم الأول والثاني والثالث و في الأسبوع الأول و الثاني والثالث من إنتهاء العلاج والتحصين. لقد أظهرت النتائج أن إستخدام الديكلازوريل بهذه الجرعة أدى إلى حدوث نقص في الإستجابة المناعية الخلوية المتمثلة في نقص في القدرة على الإلتهاام ونشاط إنزيم الليزوزيم و كذلك مستوى اكسيد النتريك وفي الإستجابة المناعية السائلة المتمثلة في تأثير الديكلازوريل على مستوى الأجسام المضادة حيث أدى إستخدام الديكلازوريل بهذه الجرعة الى حدوث نقص في مستوى الأجسام المضادة وكذلك مستوى البروتينات الكلية في الأسبوع الاول والجلوبيولينات من نوع جاما وأجزاء البروتينات المختلفة. لذلك لا يفضل إستخدام الديكلازوريل بهذه الجرعة لعلاج الأرانب .