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Effect of Imidocarb dipropionate on the immune response to Foot and Mouth Disease vaccine in healthy and anaplasmosis-infected calves

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Abstract

Aim: This work was performed to investigate the effect of a potent anti-protozoan drug, Imidocarb on the cell mediated and humoral immune response to foot and mouth disease vaccine (FMDV, O_1 strain) in normal and Anaplasmosis-infected calves.

Materials and Methods: A total of 55 male mixed bred calves were used and divided into two main groups of 25 calves each. The first group was healthy and the second was Anaplasma - infected calves. FMDV was administered in both groups. Calves of the first and second groups were subdivided into equal five subgroups of 5 calves each. The first subgroup was vaccinated control. The treated subgroups were each given 3 mg / kg body weight Imidocarb dipropionate in a single intramuscular dose at one week before vaccination, at time of vaccination, one week and two weeks post vaccination with FMDV (O_1), respectively. The cellular immune response in the different groups was evaluated weekly, however antibody titers were measured by ELISA and serum neutralization test

Results: Imidocarb increased rate of erythrocyte rosette forming lymphocytes when it was administered one week before vaccination, at time of vaccination and one week post vaccination. Imidocarb increased antibody titre of FMDV in both normal and anaplasmosis-infected calves. The protection rate due to challenge with virulent FMDV was high in treated calves as compared with the vaccinated control.

Conclusion: The best immunopotentiating effect of Imidocarb is achieved by dosing one week before vaccinating calves with FMD vaccine.

Key words: anaplasmosis, calves, imidocarb, immune response, Foot and Mouth Disease, vaccine.

Introduction

Anaplasmosis is one of the most important tick-born disease (TBD) of cattle, worldwide [1,2,3]. Bovine anaplasmosis is caused by cattle infection with the tick-borne bacterium, *Anaplasma marginale* [4]. Anaplasmosis, caused by the intraerythrocytic rickettsia *Anaplasma marginale* (*Rickettsiales: Anaplasmataceae*), is an economically important disease of cattle which is endemic in tropical and subtropical regions of the world [4-7]. Infection of cattle with *A. marginale* causes bovine anaplasmosis, a mild to severe hemolytic disease that results in considerable economic loss to both dairy and beef industries [8] .This obligate intracellular pathogen can be transmitted biologically by ticks, mechanically by transfer of infective blood on the mouthparts of biting insects [4, 9], and, less commonly, by transplacental transmission from dams to their calves [1]. Acute anaplasmosis can be prevented by combining tick control and vaccination. However this method of control has numerous limitations and improved approaches are needed [10]. Vaccine use is not widely available in several countries; consequently treatment of cattle with babesiacides plays a central role in management of this disease [11]. A wide range of antibacterial and antiprotozoal drugs have been used for the treatment of bovine anaplasmosis [12-15]. Imidocarb dipropionate at the dose rate of 3mg/kg body weight is effective in prevention and treatment of anaplasmosis in cattle [16]. The infected animals were treated with a single injection and carrier status was successfully eliminated with two intramuscular injection of Imidocarb. Imidocarb is one of the most effective and successful in chemosterilization of persistant *Anaplasma marginale* infection in cattle [17]. Moreover, the intraperitoneal administration of Imidocarb significantly increased serum IL-10 levels and lowered TNF-alpha levels in mice [18], suggesting that a novel anti-inflammatory effect of Imidocarb could be utilized to treat inflammatory conditions. The cell mediated immune response to FMD vaccine in cattle study showed expression for only IL-2and IFN [19]. No information is available about the immunopharmacological effect of Imidocarb in calves.

Therefore this study was aimed to investigate the effect of Imidocarb on the cell-mediated and humoral immune response following foot and mouth disease vaccine in healthy and anaplasmosis - infected calves.

Materials and Methods

Ethical approval: The experiment was carried out

according to the National regulations on animal welfare and Institutional Animal Ethical Committee.

Drugs: Imidocarb dipropionate (Imizol®) was obtained from Pitman-Moore Limited, Harefield, England. Imizol is available in the form of a sterile clear aqueous injectable solution 12%W/V. It was given at a single dose of 3mg/ kg body weight by intramuscular route [16].

Foot and Mouth Disease vaccine (FMDV): FMDV was obtained from the Serum and Vaccine Research Institute, Abbassia, Egypt. Each dose contained 80-100 x 10^6 virus particles of O_1/93 Egypt. The prepared vaccine was stored at 0°C and checked for sterility, safety and potency test. Each dose was 2ml given subcutaneously.

Animals and husbandry: 55 male mixed bred calves, 6 -9 months old and weighing 150- 200kg were used. Calves were kept under 12 h light/dark cycle and fed good quality concentrates and hay, with free access to water.

Experimental design: The animals were allocated to two groups of 25 calves each. Calves of the first group were free from Anaplasmosis and vaccinated with FMDV. The calves of the second group were Anaplasma-infected animals and vaccinated with FMDV. After that, each group were subdivided into equal five subgroups of 5 calves each. The calves in subgroup 1 were vaccinated with FMDV (O1 strain) and kept as nontreated control. The calves in subgroups 2, 3, 4, and 5 were given each 3 mg / kg body weight Imidocarb dipropionate in a single intramuscular dose at one week before vaccination, at the time of vaccination, one week post vaccination and two weeks post vaccination, respectively. The immune cellular response in the different groups against FMDV was evaluated weekly in normal calves for 8 weeks post vaccination. Humoral immunity was measured weekly for 9weeks then every 2 weeks up to the 17th week post vaccination. Blood Samples from the all groups (5 ml each) were taken in sterilized heparinized centrifuge tube to be used for cell-mediated immune response; Erythrocytes rosette per cent (ER %). Ten ml of blood were collected in sterilized macartany bottle from all vaccinated animals for separation of serum to determine the humoral immune response by ELISA and serum neutralization test.

Assay for immune response to FMDV: Effect of Imidocarb dipropionate on the cellular immune response of calves was determined by measurement of percent of Erythrocytes' Rosette forming lymphocytes (ER %). However, effect of Imidocarb on humoral immune response of vaccinated calves with FMDV was evaluated by estimation of the antibody titre using serum neutralization test (SN T), and ELISA test as well as challenge test.

Determination of percent of Erythrocytes Rosette forming lymphocytes (ER%): The affinity of T-lymphocytes to form a rosette with sheep RBCs was done according to the method of Kaupp et al. [20]. Firstly Amino ethyl thiourinium bromide (AET) treated sheep erythrocytes was prepared by mixing one volume of packed sheep RBCs to 4volumes of fresh AET, then treated sheep RBCs were washed three times with PBS (pH7.2) and resuspended in fetal calf serum and adjusted to 0.5%. 100 ul of separated lymphocytes suspension containing $2x10^6$ cells /ml were mixed with 100 ul of AET treated sheep RBCs, and then incubated at $4C^o$ for 18hours. After that the cell pellet was resuspended in a drop of 1% acridine orange and then counted. At least 300 lymphocytes were evaluated and all binding more than 3sheep RBCs were considered positive. The percent of lymphocytes forming rosette (X) was determined according to the following equation:

X% = (No. of Erythrocyte Rosettes (ER))/(Total No. of counted lymphocytes) x 100

Humoral immune response: FMDV-specific total antibody titers were determined by SNT and ELISA.

Serum neutralization test: Neutralizing antibodies were detected by a microplate virus neutralization assay modified by Pega et al. [21]. Antibody tires were expressed as the reciprocal of the final dilution of serum in the serum/virus mixture.

ELISA test: The method of ELISA was performed according Ma et al. [22].

Challenge test: All calves at 21 days post vaccination were challenged by FMDV; virulent strain and kept for observation for 7 days. The protection percent was calculated and recorded in all calves plus five susceptible control calves.

Foot and Mouth Disease Virus (FMDV) "virulent strain": The calves were challenged with a FMDV, O_1 / 93 Aga virus (a stock glycerinated filtrate of bovine tongue epithelium stored at -70°C). The virus was diluted to contain 10^4 mice protection ID50 per ml and each calf was given 1ml by intradermolingual inoculation of 0.1 ml at ten sites.

Statistical analysis: The statistical analysis was performed using the SPSS® 10.0 software package (SAS, Cary, NC, USA). Results are presented as arithmetic mean ± standard errors (SE).

Results

The effect of Imidocarb dipropionate on cell mediated immunity of calves vaccinated with FMDV was evaluated by measuring the affinity of T-lymphocytes to form a rosette with sheep RBCs (Table-1).There was a highly significant increase in the rate of ER% in normal calves given Imidocarb before and at the time of vaccination as compared with the vaccinated control. However, the rate of ER% in anaplasmiod-infected calves and administered

Table-1. Effect of a single intramuscular injection of Imidocarb dipropionate (3mg/Kg b.wt.) on the erythrocyte rosette % in blood of calves vaccinated with FMD vaccine (±S.D, n=5).

Weeks	Control	Treatment groups			
post vaccination	vaccinated	1 week before	At time of vaccination	1 week post vaccination	2 weeks post vaccination
1	41.2± 0.37	46.2± 0.37***	48.8± 0.20***	41.1± 0.24	40.8± 0.37
2	44.8± 0.0.20	51.6± 0.40***	54.8± 0.20***	45.8± 0.49	44.6± 0.40
3	49.6± 0.24	58.0±0.55***	61.6± 0.24***	50.8± 0.49	50.0± 0.00
4	53.4± 0.24	63.8±0.20***	68.0± 0.32***	57.2 ± 0.49***	56.4± 0.40***
5	54.6± 0.24	66.2± 0.20***	69.2± 0.20***	58.2 ± 0.37***	57.0± 0.32***
6	54.6± 0.24	64.4± 0.20***	67.2± 0.37***	57.2 ± 0.20***	55.2± 0.20
7	50.6±0.24	61.0± 0.00***	62.8± 0.37***	54.2± 0.24***	51.2 ± 0.20
8	46.2±0.37	58.0± 0.55***	60.4± 0.24***	49.2± 0.20***	46.2± 0.37

* P < 0.05; **P < 0.01; ***P < 0.001

Table-2. Effect of a single intramuscular injection of Imidocarb dipropionate (3mg/Kg b.wt.) on the erythrocyte rosette % in anaplasmosis-infected calves vaccinated with FMD vaccine (±S.D, n=5).

Weeks	Control	Treatment groups			
post vaccination	vaccinated	1 week before	At time of vaccination	1 week post vaccination	2 weeks post vaccination
0	25.0± 2.02	21.4± 1.96	11.2± 1.80	16.4± 1.72	20.4± 3.50
1	28.0 ± 2.49	15.0 ± 1.34*	7.8± 1.43	11.8± 1.16	13.0± 2,70
2	29.0± 2.95	7.2 ± 0.58***	4.8 ± 0.49**	6.0± 0.77***	7.6± 1.50**
3	32.4± 2.70	4.8±0.37***	1.8 ± 0.20***	3.0± 0.32***	3.8± 1.07**
4	34.2± 3.80	4.0 ± 0.45***	0.6 ± 0.24***	1.0 ± 0.00***	1.6± 0.40***
5	36.6± 5.53	1.0± 0.0***	0.2± 0.20***	0.4± 0.24***	0.8± 0.20***
6	39.8± 6.13	0.6 ± 0.24***	0.0 ± 0.00	0.0 ± 0.00	0.2± 0.20***
7	41.8 ±5.80	0.0± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00
8	44.2± 5.46	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00

* P < 0.05; **P < 0.01; ***P < 0.001

Table-3. Effect of intramuscular injection of Imidocarb dipropionate (3mg/Kg b.wt.) on the Log10of antibody titre using ELISA test in the normal calves vaccinated with FMD vaccine (±S.D.,n=5).

Weeks	Control	Treatment groups			
post vaccination	vaccinated	1 week before	At time of vaccination	1 week post vaccination	2 weeks post vaccination
1	1.02± 0.06	1.38± 0.03***	0.90± 0.00	1.08±0.03	1.14± 0.04
2	1.83± 0.03	2.10± 0.000***	1.95± 0.00**	1.89±0.04	1.71± 0.06
3	2.01± 0.04	2.49± 0.04***	2.13± 0.03*	2.01±0.04	1.89±0.04
4	1.89 ± 0.04	2.34±0.04***	2.10± 0.00***	1.98±0.04	1.89±0.04
5	2.13± 0.03	2.46 ± 0.04***	2.34± 0.04**	2.13±0.03	2.01±0.04
6	2.13± 0.03	2.49± 0.04***	2.40± 0.00***	2.40±0.04***	1.95±0.00
7	2.07± 0.03	2.40± 0.0***	2.31± 0.04**	2.31±0.04**	1.95±0.00
8	1.92± 0.03	2.34 ± 0.04***	2.10 ± 0.00***	2.10±0.000***	1.89±0.04
9	1.77± 0.03	2.10± 0.00***	1.92 ± 0.03**	1.98±0.03**	1.77±0.03
11	1.62±0.03	2.0 4± 0.04***	1.77 ± 0.04*	1.74±0.04*	1.65±0.03
13	1.47± 0.03	1.95± 0.07***	1.68± 0.06*	1.62±0.06	1.53±0.03
15	1.38±0.03	1.68± 0.03**	1.53± 0.04*	1.44±0.06	1.32±0.03
17	1.17± 0.03	1.38± 0.03**	1.32± 0.06	1.26±0.06	1.20± 0.00

* P < 0.05; **P < 0.01; ***P < 0.001

Imidocarb was increased from the 2nd week after treatment (Table-2).

Administration of Imidocarb at one week before vaccination increased the $\log_{10}$ titre of antibody by using ELISA test in normal and anaplasmiod - infected calves post vaccination with FMDV (Tables-3 and 4). When Imidocarb was given simultaneously with vaccination and/or one week after vaccination, a significant increase in antibody titre was obtained from the second week post vaccination. Furthermore, Imidocarb administration at the time of and/ or one week post vaccination increased the antibody titre in anaplasmiod- infected calves. The effect of Imidocarb on serum neutralization antibody titre in healthy and anaplasmiod –infected calves and vaccinated with FMDV are shown in Tables-5 and 6. Intramuscular administration of Imidocarb dipropionate in a single dose of 3 mg/kg body weight increased the SN antibody titre when the drug was given one week before vaccination, and at the time of vaccination with FMDV. Moreover, the effect of Imidocarb dipropionate on the virus challenge test in normal and Anaplasmosis- infected calves vaccinated with FMDV (O_1 strain), with 5 susceptible calves as a control for challenge. All the vaccinated calves were protected; this was indicated by absence of tongue and foot lesions but the control calves showed over 4 tongue lesions and lesions on the 4 feet which indicate satisfactory challenge.

Discussion

Foot and Mouth Disease is an acute feverish and contagious viral infection that can be seen in all ruminating double nailed animals [23,24]. FMD has been known to be present in Egypt for more than 40 years causing drastic loses in milk and meat with deaths among young animals [25]. Theileriosis is a disease of

Table-4. Effect of intramuscular injection of Imidocarb dipropionate (3mg/Kg b.wt.) on the Log10 of antibody titre using ELISA test in anaplasmiod- infected calves vaccinated with FMD vaccine (±S.D.,n=5).

Weeks	Control	Treatment groups			
post vaccination	vaccinated	1 week before	At time of vaccination	1 week post vaccination	2 weeks post vaccination
1	0.78± 0.06	0.9 6± 0.04*	0.96± 0.04*	0.72 ±0.06	0.72 ± 0.06
2	1.05± 0.05	1.38 ± 0.07**	1.23 ± 0.03*	1.14 ±0.04	1.02 ± 0.03
3	1.50± 0.04	1.71 ± 0.04**	1.68 ± 0.03**	1.56 ±0.06	1.56 ±0.03
4	1.29 ± 0.06	1.50 ±0.00**	1.47 ± 0.03*	1.44 ±0.04	1.44±0.04
5	1.35± 0.04	1.74 ± 0.06***	1.65± 0.05**	1.65 ±0.00***	1.59 ±0.04**
6	1.44± 0.06	1.89 ± 0.04***	1.83 ± 0.03***	1.71 ±0.04**	1.77 ±0.03**
7	1.71± 0.04	1.95± 0.05**	1.98 ± 0.03***	1.92±0.06*	1.89 ±0.04*
8	1.71± 0.04	1.86 ± 0.04*	1.83 ± 0.03*	1.95 ±0.07*	1.92 ±0.03**
9	1.56± 0.03	1.65± 0.00*	1.68 ± 0.03*	1.71 ±0.04*	1.74 ±0.06*
11	1.32 ±0.06	1.59 ± 0.04**	1.56 ± 0.06*	1.68±0.06**	1.56 ±0.04*
13	1.23 ± 0.03	1.44± 0.04**	1.38 ± 0.04*	1.44±0.06**	1.41±0.04*
15	1.11 ±0.04	1.26 ± 0.04*	1.35 ± 0.04**	1.38±0.06**	1.26 ±0.04*
17	0.93 ± 0.03	0.99 ± 0.04	1.14 ± 0.04**	1.17±0.03**	1.05± 0.06

* P < 0.05; **P < 0.01; ***P < 0.001

Table-5. Effect of intramuscular injection of Imidocarb dipropionate (3mg/Kg b.wt.) on the serum neutralization antibody titre expressed by Log10 in the normal calves vaccinated with FMD vaccine (±S.D. n=5).

Weeks	Control	Treatment groups			
post vaccination	vaccinated	1 week before	At time of vaccination	1 week post vaccination	2 weeks post vaccination
1	0.81± 0.04	1.08 ± 0.03***	0.90± 0.00	0.81 ±0.04	0.90 ± 0.00
2	1.56± 0.04	1.83 ± 0.03***	1.71 ± 0.04*	1.62±0.03	1.41 ± 0.04
3	1.86± 0.04	2.16 ± 0.04***	2.01 ± 0.04*	1.89 ±0.04	1.71 ±0.04
4	1.56 ± 0.04	1.89 ±0.04***	1.80 ± 0.00***	1.62±0.03	1.62 ±0.03
5	1.77± 0.03	2.22 ± 0.03***	2.13± 0.03***	1.89±0.04*	1.77 ±0.03
6	1.98± 0.03	2.34 ± 0.04***	2.25 ± 0.00***	2.01 ±0.03	1.92 ±0.03
7	1.92± 0.03	2.25 ± 0.03***	2.10 ± 0.00***	2.04 ±0.04*	1.83 ±0.03
8	1.71± 0.04	2.04± 0.04***	1.92± 0.03**	1.92 ±0.03**	1.68 ±0.03
9	1.68± 0.03	1.89 ± 0.04**	1.77 ± 0.03	1.71 ±0.04	1.68 ±0.03
11	1.44 ±0.04	1.65± 0.00**	1.53 ± 0.03	1.5±0.00	1.53 ±0.00
13	1.23 ± 0.03	1.50 ± 0.05**	1.32 ± 0.03	1.32±0.06	1.23±0.03
15	1.14 ±0.04	1.32 ± 0.03**	1.23 ± 0.03	1.23±0.03	1.11 ±0.00
17	0.02 ± 0.03	1.17 ± 0.03**	1.11 ± 0.04	1.11±0.04	1.08± 0.03

* P < 0.05; **P < 0.01; ***P < 0.001

Table-6. Effect of intramuscular injection of Imidocarb dipropionate (3mg/Kg b.wt.) on the serum neutralization antibody titre expressed by Log10 in anaplasmiod- infected calves vaccinated with FMD vaccine (±S.D.,n=5).

Weeks	Control	Treatment groups			
post vaccination	vaccinated	1 week before	At time of vaccination	1 week post vaccination	2 weeks post vaccination
1	0.42± 0.07	0.69 ± 0.04*	0.60± 0.00*	0.42 ±0.07	0.42 ± 0.07
2	0.90± 0.00	1.44 ± 0.04***	1.23 ± 0.03***	0.99±0.04	0.87 ± 0.03
3	1.29± 0.04	1.65± 0.00***	1.50 ± 0.00***	1.38±0.03	1.38 ±0.03
4	1.08± 0.03	1.35 ±0.00***	1.32 ± 0.03***	1.26±0.04**	1.20 ±0.06
5	1.23± 0.03	1.56 ± 0.04***	1.38± 0.03**	1.50±0.00***	1.38 ±0.03**
6	1.23± 0.03	1.68 ± 0.07***	1.56 ± 0.06**	1.59 ±0.04***	1.62 ±0.03***
7	1.41± 0.04	1.71 ± 0.06**	1.56 ± 0.04*	1.65±0.07*	1.71 ±0.04***
8	1.50± 0.00	1.86± 0.04***	1.77± 0.04***	1.68 ±0.07*	1.77 ±0.03***
9	1.20± 0.00	1.53 ± 0.03***	1.44 ± 0.06**	1.56 ±0.06***	1.68 ±0.03***
11	1.11 ±0.04	1.29± 0.04*	1.23 ± 0.03*	1.29±0.06*	1.50 ±0.00***
13	1.05 ± 0.00	1.17 ± 0.03**	1.17 ± 0.03**	1.17±0.03**	1.38±0.03***
15	0.93 ±0.03	1.14 ± 0.04**	1.11 ± 0.04**	1.14±0.04**	1.20 ±0.00***
17	0.78 ± 0.07	1.02 ± 0.03*	0.99 ± 0.04	0.99±0.04*	1.02± 0.03*

* P < 0.05; **P < 0.01; ***P < 0.001

lymphoid organs of cattle resulting in profound leucopenia, a high lymphoid cell parasiyosis and lymphocytes lyses and thus reduction in the immunocompetence of infected calves [17]. The present study results show that Imidocarb dipropionate in a dose of 3mg/kg b.wt. when injected intramuscularly in normal and anaplasmiod-infected calves caused a significant increase in the rate of ER % in calves vaccinated with FMDV. These results suggested that the drug may stimulate the cellular immune response [2]. A significant increase in the antibody titre against FMDV was recorded in the treated groups injected before, simultaneously and/or one week after vaccination, with a considerable increase in infected groups injected two weeks post vaccination. These results indicate that the drug may stimulate the humoral immune response against FMDV in both techniques (ELIZA and serum neutralization tests). Moreover, the results of serological tests indicate that vaccinated calves (normal and anaplasmiod-infected) treated with Imidocarb gave rise to a high level of antibodies which protected calves against subsequent challenge. Similar results were described by

Bengelsdorff [26] who stated that vaccinated calves were protected when SNT > 1.2 $\log_{10}$. The drug allowed the animal to develop protective immunity against at least homologous challenge of FMDV [27]. This achieved through stimulation of humoral and cellular immunity. It is also suggested that the immunostimulating effects of the tested drug attribute to the release of a mixture of cytokines which promote antibody formation, increased number of both T and B lymphocytes and enhanced interferon production. The significant increase in the antibody titre against FMDV in normal calves treated with Imidocarb dipropionate explained the ability of the drug to optimize the effective uses of the vaccine [28].

The drug injected before or at the time of vaccination, may have a role in the stimulation of antigen presenting cells, T and B cells, so as to generate a large number of memory cells. Administration of Imidocarb after vaccination may have similar effects but it decreased with the time as the antigen by the time is fully presented and already recognized by memory cells.

Conclusion

Administration of Imidocarb dipropionate (3mg/kg b.wt) either at the time of vaccination or post vaccination against FMDV in calves stimulates the immune response with increased level of antibody titre and prolonged duration of protection. It is suggested that the best time of Imidocarb administration is one week before vaccination. Furthermore, improvement in the immune response to FMD vaccine occurred after Imidocarb therapy in anaplasmiod- infected calves.

Authors' contributions

The present study was part of IMI's MVSc thesis programme in collaboration with advisory members and guide (NAA and IMS). NAA designed and approved the experimental protocol, provided guidance during entire experiment and revised the manuscript. HYE performed statistical analysis. All authors read and approved the final manuscript.

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Competing interests

The authors declare that they have no competing interests.

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