Acta Veterinaria (Beograd), Vol. 48. No.1, 27–36, 1998.

UDK 619:615.371

EXAMINATION OF IMMUNOGENICITY OF EXPERIMENTAL SUBUNIT VACCINES AGAINST PI3 VIRUS IN CALVES

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(Received, 24. December 1997.)

The objective of our study was to check the immunogenicity of experimental subunit vaccines against PI3 virus in a biological assay on 30 experimental calves of the Yugoslav Simmental breed, aged 5-6 months, of body weight 200-230 kg. One experimental vaccine was prepared from purified glycoprotein subunits, isolated from the outer envelope of the SD2 strain of the PI3 virus; wherease the other subunit vaccine was prepared from purified glycoprotein antigens, isolated from the external envelope of the Yugoslav strain of the virus. Antigens of these vaccines with haeagglutinating titres of 128 HJ/0,1 ml, were adsorbed on the adjuvant - Al(OH)3, dissolved at 100 mmol/l PBS. The final concentration of the adjuvant in the vaccines was 5 mg/ml. One group of 10 experimental calves was immunised with 2 ml ofsubunit vaccine from strain SD2 (s/c per animal) and revaccinated on day 21 of the assay. The second group of 10 experimental calvres was vaccinated with 2 ml of the other subunit vaccine, prepared from the Yugoslav strain ofthe virus (s/c per animal) and revaccinated after 21 days. The third group of 10 nonvaccinated experimental calves served as the control in the assay. The geometric mean titres of HI antibodies against Pi3 virus(GMT log 2/25 μl) in the sera of the first group of 10 vaccinated calves were as follows: on day 7 after vaccination: 3,8; on day 21; 4,9, and on day 56: 3,5 and in the sera of the second group of vaccinated calves on day 7: 3,5; on day 21: 4,4 and on day 56: 3,3. The geometric mean titre of HI antibodies against the PI3 virus (GMT log 2/25 μl) present in the sera of 10 nonvaccinated, control animals on day 56 of the assay was only 1,3. These experimental subunit vaccines against the PI3 virus with low concentrations of the glycoprotein antigens, i. e. 0.30 mg per dose, induced a satisfactory humoral immune response in the organisms of all vaccinated animals.

Key words: PI3 virus, glycoprotein antigens, subunit vaccine, imunization, calves.

INTRODUCTION

In immunoprophylaxis of acute respiratory infections of calves caused by the PI3 virus, classical viral vaccines are usually used as inactivated or live

attenuated vaccines, prepared from completely inactivated or attenuated virus particles.

One defect of inactivated vaccines is the long period from the administration of the vaccine till the induction of an immunological reaction (i. e. 10 - 14 days) during which animals can be infected with PI3 viruses. Some other disadvantages of vaccines against the PI3 virus prepared fro whole inactivated viral particles (especially of polyvalent inactivated vaccines against the PI3 virus, IBR virus, Pasteurella multocida, Diplococcus and Staphylococcus) are their weaker immunogenic effect due to the high level polycional reaction in imunized organisms and the damage inflicted to immunologically important viral antigens during the process of inactivation (Mihajlović et al., 1979)

However, an important disadvantage of live - attenuated vaccines is the possibility of reactivation of the vaccine viruses, as, under certain conditions (passage through susceptible organisms), a virus that had lost virulence could regain it. Among other defects are the appearance of pyrogenic and general or local allergic reactions in the vaccinated organisms.

Significant efforts to correct the above mentioned defects have made in this field. The isolation of imunologically important glycoprotein antigens from the outer envelopes of PI3 viruses in a biologically active and purified form represents a prerequisite for the preparation of specific subunit vaccines against the PI3 virus, as described in the papers of Ray et al., (1985.) Morein et al., (1983.) Milić (1993.) and Milić et al. (1994).

We decided to examine in paralell the immunogenicity of our two experimental subunit vaccines against the PI3 virus in a biological assay on calves.

MATERIAL AND METHODS

I Vaccines

The experimental subunit PI3 vaccines were sterile liquid suspensions of purified glycoprotein antigens (haemagglutinin - neuraminidase and fusion proteins with Mr of 78, 40 and 35 kD) isolated from the outer envelopes of PI3 viruses according to the method of Milić (1993) and Milić et al., (1994).

The first subunit vaccine was prepared from glycoprotein antigens isolated from the peoplos of SD_2 strain of PI3 virus. The other experimental vaccine was made fromglycoprotein subunits isolated from the outer envelopes of the Yugoslav strain of the PI3 virus. These abovementioned isolated subunits with a haemagglutinating titre of 128 HJ/ 0,1 ml were adsorbed on an adjuvant of Al (OH_3), suspended at 100 mmol/l in phosphate buffered saline (PBS,pH 7,0). The total virus protein concentration in these vaccines was 0,15 mg/ml i. e. 0,30 mg per dose of 2 ml. The adjuvant concentration in the vaccines was 5 mg/ml.

The isolation and purification of glycoprotein antigens from the outer envelopes of the above mentioned viruses were done by preparative ultracentrifugation in linear K-Na-tartrate gradients with Triton X-100,as described by Milić (1993).

The method of specific marking of surface glycoprotein antigens of the PI3 virus with D-(6-3H) glucosamine (Amersham) was used throughout the whole process ofpurification and separation of glycoprotein subunits from outer viral envelopes (Milić et al., 1991).

The immunogen in the vaccine was identified by SDS-PAGE electrophoresis in a discontinuous buffer system (Laemmili), 1970) with selective staining of virus proteins (PAS-staining method, according to Gordon, 1983) during which the glycoprotein fractions of the isolated subunits stained purple; as well as with the HI test (Mihajlović, 1984, Clarke and Casals, 1958).

The total protein concentrations in the samples of purified glycoprotein subunits were determined by the methodof Lowry et al., (1951). Hemagglutinating activities of the isolated glycoprotein subunits were examined by the method of direct hemagglutination (Clarke and Casals, 1958; Mihajlović 1984.).

The biological activities of the fusional glycoprotein antigens, important immunogenic components of the isolated glycoprotein subunits of the PI3 virus, were assessed in vitro in reactions of cell fusion of bovine turbinate ceels - BT, according to the method described by Scheid and Choppin (1974) and Milić, (1993).

The pyrogenic test on experimental rabbits was performed according to the Pharmacopoeia Yugoslavica IV and Pharmacopoela Britanica (Vet.) (1985. Add 1992.) Itshowed that the vaccine was apyrogenic, as it did not cause any rise in the body temperature in the immunized animals. The virus strains for the production of the vaccine were individually propagated in continuous cell lines. (MDBK-for SD2 strain and PK-15 for the Yugoslav strain) for 96^h at 36^0C. The propagated PI3 viruses has a titre of $LD_{50} = 10^{-3,5}$ $TCID_{50}$) for the SD2 strain and $LD_{50} = 10^{-3,38}$ ($\log = 10^{-3,38}$ $TCID_{50}$) for the Yugoslav strain, with haemagglutinating titre 64HJ/0,1 ml.

II Biological assay on experimental calves

The immunogenicity of the subunit vaccines against the PI3 virus, prepared from both the SD2 strain, (128 HJ/0,1 ml,) as well as from the Yugoslav strain of the virus, was examined in two experimental groups of 10 calves of the Yugoslav strain of the virus, was examined in two experimetal groups of 10 calves of the Yugoslav Simmental breed, aged 5-6 months, of body weight 200-230 kg; whilst a third group of 10 similar animals served as the control.

One group of 10 calves was immunized with 2 ml of the subunit vaccine,prepared from the SD2 strain of the PI3 virus, subcutaneously (s/c per animal) and revaccinated with the same dose 21 days after the first vaccination.

The second group of 10 calve was vaccinated with the same dose of the subunit vaccine, prepared from the Yugoslav strain of the virus (s/c per animal) and revaccinated on day 21 of the assay.

Prior to the vaccination, the sera of the experimental calves were examined for the presence of HI antibodies against the PI3 virus by the haemagglutination inhibition test. The results were negative in all the calves prior to the vaccination, except for three calves from the control group in which low HI antibody titres were found. The immune response of the vaccinated calves was examined by the haemagglutination inhibition test 7,21 and 56 days after the administration of the vaccine.

III The HI test was carried out accurding to the standard in Limbro microplates with 0,5% suspension of guinea pig erythrocytes (Mihajlović, 1984, and Clarke and Casals, 1958.). The titres of the virus neutralizing HI antibodis against the PI3 virus in the blood sera of the examined animals were expressed in mean geometric titres (Gmt log 2/25 μl), according to Sjurin et al., (1984).

RESULTS

In the first group of experimental calves, vaccinated with the subunit vaccine against the PI3 virus, prepared from SD2 strain, the HI antibody titres in the sera of the immunized animals reached the values of 1: 16 and 1:32 already after 7 days post vaccination; whereas, on day 21 they increased to 1:32 and 1:32 and 1:64. After 56 days, most of the calves had HI titres of 1: 16 (-Table 1).

Table 1. HI antibody titres for PI3 virus in the sera of the first group of vaccinated calves

	Days after vaccination (revaccination)		
No	7	21	56
1.	1 : 16	1 : 32	1 : 16
2.	1 : 16	1 : 32	1 : 16
3.	1 : 8	1 : 8	1 : 8
4.	1 : 16	1 : 32	1 : 8
5.	1 : 16	1 : 32	1 : 4
6.	1 : 32	1 : 64	1 : 16
7.	1 : 8	1 : 32	1 : 16
8.	1 : 8	1 : 16	1 : 16
9.	1 : 16	1 : 32	1 : 16
10.	1 : 32	1 : 64	1 : 16

The HI titre values, found in the sera of the calves vaccinated with the subunit vaccine, strain SD2, are given in mean geometric antibody titres against the PI3 virus (GMT log 2/25 μl), which on day 7 after the vaccination were 3.8; on day 21 after the vaccination 4.9, and on day 56 they were 3.5 -(Table 2).

Table 2. Geometric HI antibody titres for PI3 virus in the sera of the first group of vaccinated calves (GMT log 2/25 μl)

	Days after vaccination (revaccination)		
No	7	21	56
1.	4	5	4
2.	4	5	4
3.	3	3	3
4.	4	5	3
5.	4	5	2
6.	4	6	3
7.	3	5	4
8.	3	4	4
9.	4	5	4
10.	5	6	4
Mean geometric titres	3.8	4.9	3.5

The second group of 10 calves, vaccinated with the subunit vaccine prepared from the Yugoslav strain also had satisfactory titres of HI antibodies against the PI3 virus. At 7 days after vaccination they, were 1: 16 in 7 calves; whilst in 3 animals they weree lower (1:4 and 1:8 in one of the calves). On day 21 after vaccination, the HI antibody titres were increased or remained unchanged in comparison to those obtained on day 7. On day 56 after vaccination the HI titres in the sera of the immunized animals were satisfactory (-Table 3).

Table 3. HI antibody titres for PI3 virus in the sera of the second group of vaccinated calves

	Days after vaccination (revaccination)		
No	7	21	56
1.	1 : 16	1 : 16	1 : 8
2.	1 : 16	1 : 64	1 : 16
3.	1 : 16	1 : 32	1 : 16
4.	1 : 16	1 : 32	1 : 4
5.	1 : 8	1 : 16	1 : 8
6.	1 : 4	1 : 4	1 : 4
7.	1 : 16	1 : 32	1 : 32
8.	1 : 16	1 : 32	1 : 16
9.	1 : 16	1 : 32	1 : 16
10.	1 : 4	1 : 8	1 : 4

The titres of the HI antibodies against the PI3 virus of the second group of experimental calves, in mean geometric titre values (GMT log 2/25 μl) were 3.5 on day 7; 4.4 on day 21, and 3.3 on day 56 after vaccination (Table 4).

Table 4. Geometric HI antibody for PI3 virus in the sera of the second group of vaccinated calves (GMT log 2/25 μl)

	Days after vaccination (revaccination)		
No	7	21	56
1.	4	4	3
2.	4	6	4
3.	4	5	4
4.	4	5	2
5.	3	4	3
6.	2	2	2
7.	4	5	5
8.	4	5	4
9.	4	5	4
10.	2	3	2
Mean geometric titres	3.5	4.4	3.3

The course of immunobiological reaction in the vaccinated calves was similar to that in the first group of experimental animals. The highest HI antibody level against the PI3 virus occurred on day 21 after vaccination. The titres of HI antibodies found against the PI3 virus were not significantly lower than the values obtained for the first group.

In the third (control) group of 10 nonvaccinated calves, the HI test prior to the experiment showed that 3 animals were positive for the presence of HI antibodies against the PI3 virus, with titres of 1:2; whereas in the remaining control calves the HI titres were negative. At the end of the experiment, i. e. after 56 days, all the calves had positive HI antibody titres, ranging from 1:2 to 1:4 (Table 5).

Table 5. HI antibody titres for PI3 virus in the sera of the control group of experimental calves

	Days from the begining of the experiment	
No	0	56
1.	0	1 : 2
2.	1 : 2	1 : 2
3.	0	1 : 4
4.	0	1 : 2
5.	0	1 : 2
6.	1 : 2	1 : 2
7.	0	1 : 2
8.	0	1 : 4
9.	0	1 : 4
10.	1 : 2	1 : 2

The results of the HI test in the control calves are given in mean geometric titres against PI3 virus (GMT log 2/25 μl). (Table 6).

Tabela 6. Geometric HI antibody titres for PI3 virus in the sera of the control group of experimental calves

	Days from the begining of the experiment	
No	0	56
1.	0	1
2.	1	1
3.	0	2
4.	0	1
5.	0	1
6.	1	1
7.	0	1
8.	0	2
9.	0	2
10.	1	1
Mean geometric titres	0.3	1.3

On the basis of the values for the HI antibodies against the PI3 virus, obtained after vaccination of the experimental calves with the tested subunit vaccine, during a period of 56 days, it can be concluded that the vaccination was satisfactory. The highest titres of HI antibodies against the PI3 virus were obtained in the first group of calves, vaccinated with the subunit vaccine, prepared from strain SD2 with 128 HJ/0.1 ml (Table 1 and 2). The titres of HI antibodies recovered from the second group of calves, vaccinated with the subunit vaccine of 128 HJ/01 ml, prepared from the Yugoslav strain of the virus the PI3, were slightly lower than the HI titres in the first group of calves (Table 3 and 4).

Throughout the immunogenicity examination of the subunit vaccines against the PI3 virus all experimental calves were under the constant care of veterinarians. None of the animals from the two vaccinated groups become affected with the respiratory syndrome caused by parainfluenza 3 paramixovirus, nor did any of the nonvaccinated animals from the control group. Low HI antibody titres aggainst the PI3 virus (1:2) were obtained only in 3 nonvaccinated calves at the outset of the experiment; whereas on day 56, HI antibodies were present in the sera of all the animals from the control group (titre: 1:2 to 1:4) (Tables 5 and 6).

The low values of the geometric mean titre of the HI antibodies for the PI3 virus (GMT log 2/25 μl) in the sera of 10 nonvaccinated, control calves on day 56 of the assay (of 1.3), showed that the animals had come into contact with the virus. (Table 6). However, these calves did not develop the respiratory syndrome because of the absence of the predisposition stress factors.

DISCUSSION

The results of the studies of immunogenicity of the polyvalent inactivated vaccine against the PI3 virus, Pasteurella multocida, Diplococcus pneumoniae and Staphylococus, in a biological asaay on the experimental calves (Mihajlović et al. 1979.), showed that this vaccine had weaker immunogenic properties; whilst our experimental subunit vaccines prepared only from purified glycoprotein antigens isolated from the PI3 viruses, induced a stronger immune response in the vaccinated animals.

The immunogenic properties of glycoprotein antigens (haemagglutinine-neuraminidase and fusion proteins - HN and F), isolated from the outer envelopes of the PI3 viruses were confirmed in the biological assay on the experimental animals described by Morein et al.,(1983); Ray et al., (1985) Milić et al., (1994) etc.

The results pertaining to the individual role of HN and F proteins of the PI3 virus in the induction of a protective immune response in hamsters demonstrated that these antigens represent weak immunogens to experimental animals; whilst applied together as a mixture of the purified HN and F proteins, the induce the synthesis of virus - neutralising antibodies with a higher titre (Ray et al., 1988).

The investigations of immunogenicity of isolated glycoprotein subunits of the PI3 virus have shown that the size and shape of the specific glycoprotein antigen molecules have an essential role in the induction of the body's immune reaction.

Ray and Compans (1987) incorporated the glycoproteins (HN and F), isolated from the PI3 virus into phospholipid vesicles of 40 to 45 nm diameter in order to improve their immunogenicity for the preparation the subunit vaccine.

In order to improve immunogenic properties of HN and F glycoproteins with Mr of 78, 40 and 35 kD, isolated from the peplosof thepurified PI3 viruses, we adsorbed them on an adjuvant ($Al(OH)_3$ dissolved at 100 mmol/l in PBS. This method was used in the preparation of our experimental subunit PI3 vaccines with 128 HJ/0,1 ml.

Applying these subunit vaccines to calves, gave satisfactory HI antibody levels in the sera of the vaccinated animals, already on the seventh day post vaccination (the geometric mean titres of the HI antibodies against the PI3 virus were 3.8 and 3.5).

The highest levels of the Hi antibodies against the PI3 virus, expressed as mean geometric titres, were determined 21 days after vaccination in the sera of both groups of vaccinated calves and were 4.9 in the first group, and 4.4 in the second group of immunized animals.

The mean geometric titres of the HI antibodies against the PI3 virus in the sera of the vaccinated calves on day 56 after vaccination were 3.5 in the first experimental group and 3.3 in the second group of animals.

The results obtained confirmed that our experimental subunit vaccines against the PI3 virus with very low viral glycoprotein of 0.15 mg/ml i.e. 0.30 mg

per dose of vaccine and haemagglutinating activities of 128 HJ/0.1 ml, induced the synthesis of virus - neutralizing HI antibodies with a satisfactory titre in all vaccinated calves.

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ISPITIVANJE IMUNOGENOSTI EKSPERIMENTALNIH SUBJEDINIČNIH VAKCINA PROTIV VIRUSA PI3 TELADI

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SADRŽAJ

Cilj naših istraživanja bilo je ispitivanje imunogenosti eksperimentalnih subjediničnih vakcina protiv virusa PI3 u biološkom ogledu na 30 eksperimentalnih teladi, rase Jugoslovenski simentalac, starih 5 - 6 meseci, telesne težine 200 -230 kg. Jedna eksperimentalna vakcina pripremljena je od prečišćenih glikoptoteinskih subjedinica izolovanih iz spoljašnjeg omotača SD2 soja virusa PI3, dok je druga subjedinična vakcina pripremljena od prečišćenih antigena izolovanih iz spoljnjeg omotača Jugoslovenskog soja virusa. Antigen ovih vakcina sa hemaglutinacionim titrom od 128 HJ/0,1 ml, adsorbovani su na adjuvans - Al (OH) 3, rastvoren u 100 mmol/l PBS-a. Krajnja koncentracija adjuvansa u vakcinama iznosila je 5 mg/ml. Jedna grupa od 10 eksperimentalnih teladi imunizovana je sa po 2 ml subjedinične vakcine (s/c po životinji) i revakcinisana 21-og dana ogleda. Druga grupa od 10 eksperimentalnih teladi vakcinisana je sa po 2 ml subjedinične vakcine pripremljene od Jugoslovenskog soja virusa (s/c po životinji) i revakcinisana posle 21 dana. Treća grupa od 10 nevakcinisanih eksperimentalnih teladi služila je kao kontrola u ogledu. Srednji geometrijski titri HI antitela protiv virusa PI3 (GMT log 2/25 μl) u serumima prve grupe od 10 vakcinisanih teladi bili su 7-og dana vakcinacije 3,8; 21-og dana 4,9 i 56-tog dana 3,5, a userumima druge grupe vakcinisanih teladi iznosile su 7-og dana od vakcinacije 3,5; 21-og dana 4,4 i 56-og dana 3,3. Srednji geometrijski titar HI antitela protiv virusa PI3 (GMT log 2/25 μl), prisutan u serumima 10 nevakcinisanih, kontrolnih životinja, iznosio je samo 1,3. Ove eksperimentalne subjedinične vakcine protiv PI3 virusa, sa niskim koncentracijama glikoproteinskih antigena odnosno od 0,30 mg po dozi, indukovale su zadovoljavajući humoralni imunološki odgovor u organizmima svih vakcinisanih životinja.