EXPERIMENTAL INACTIVATED PURIFIED CONCENTRATED ADJUVANT RABIES VACCINE. EVALUATION OF ITS EFFICACY IN CATTLE

Z. BENÍŠEK[1], J. SÜLI[1], Š. ŠVRČEK[1], J. MOJŽIŠOVÁ[1], D. TAKÁČOVÁ[1], J. ZÁVADOVÁ[2], R. ONDREJKA[1], A. ONDREJKOVÁ[1]

[1]University of Veterinary Medicine, Košice, Slovak Republic
[2]Research Institute of Veterinary Medicine, Košice, Slovak Republic

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Abstract

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Efficacy of an experimental inactivated concentrated and purified rabies vaccine, potentiated with lipoid adjuvant was evaluated in comparative experiments performed in cattle by testing of specific cell-mediated immune response (CMI). Commercial inactivated rabies vaccines – Lyscelin (Czech Republic) and Rabisin (France) were used for comparison. Together with CMI evaluation by leukocytes migration inhibition test (LMI), postvaccination rabies antibodies were quantified by the ELISA method and by the rapid fluorescent focus inhibition test (RFFIT) on days 14, 28, 60 and 180 after immunization of the animals. The results of antigenic activity of tested vaccines show the best efficacy of the experimental vaccine from the point of view of humoral immunity, as well as cell-mediated immunity, even though all tested vaccines induced sufficient level of rabies antibodies (≥ 0.5 IU/cm^3) at all evaluated intervals.

Rabies vaccine, cell-mediated immunity, humoral immunity, cattle

In model experiments on laboratory animals efficacy of rabies vaccines can be evaluated on the basis of their immunogenic activity (e.g. NIH method – standard test recommended by WHO 1992; OIE 1996) or antigenic activity – determination of humoral but also cellular immune response after vaccination of target animals.

Almost an absolute correlation was proved between the titre of serum rabies antibodies and the level of rabies protection (Sikes et al. 1971; Blancou et al. 1983). Rabies antibodies can be quantified by more than 14 various techniques. At present, classic virus neutralization test on mice – VNT (Atanasiu 1973), rapid fluorescent focus inhibition test – RFFIT (Smith et al. 1973) and ELISA test (Atanasiu et al. 1977) are the most frequently used ones. Standardization of these tests is ensured by using the international standard reference serum with a declared value of international units in 1 cm^3 (IU/cm^3). Animals vaccinated against rabies must have a minimal level of rabies antibodies 0.5 IU/cm^3 one month after immunization (European Pharmacopoeia 1997).

Protective effect of rabies vaccination consists not only in the adequate specific antibody response, but it also depends on the defence mechanisms mediated by cells – CMI (Wiktor et al. 1977; Petermann et al. 1976; Murphy 1977). This fact led some authors to the opinion that the test for level determination of specific cell mediated immune response should be used in evaluation of post-vaccination immunity (in evaluation of rabies vaccines efficacy) (Kiss and Tuboly 1981; Pille and Matevosyan 1985).

Materials and Methods

Experiments aimed at comparing and evaluation of the antigenic activity of experimental and commercial rabies vaccines by determination of cellular and humoral immune response on one of the target farm animal species, cattle, were carried out.

Address for correspondence:
MVDr. Zdenek Beníšek, CSc.
Univerzita veterinárskeho lekárstva
Komenského 73
041 81 Košice, Slovenská republika

Phone: +421 95 62 29 924
Fax: +421 95 63 23 666
E-mail: benisek@VSVNOV. UVM.sk
http://www.vfu.cz/acta-vet/actavet.htm

Vaccines

In the experiments the following vaccines were used:

1. Experimental – inactivated concentrated purified rabies vaccine (Süliová et al. 1997; Beníšek et al. 1998), potentiated with lipoid adjuvant (Beníšek et al. 1999; Süliová et al. 1999);
2. Commercial inactivated rabies vaccine Lyscelin, op. No. 260594, produced by Bioveta Ltd. Ivanovice na Hané (Czech Republic);
3. Commercial inactivated rabies vaccine Rabisin, op. No. 60H301-0799, produced by Rhône-Mérieux (France).

Tested animals

Young fattened cattle of 250-300 kg were used in the experiments. A total of 25 animals were tested on the basis of non-specific cellular immunity level, determined by a dermatological test with dinitrofluorinebenzene – DNFB (Paulík and Vrzgula 1989; Paulík et al. 1991). Fifteen animals with the highest cellular immunity level (with the highest reactive number) were selected into 3 groups and used in the experiments; and the individual groups were averaged. The number of animals was 5 in each group.

Immunization scheme

Group 1: experimental vaccine with lipoid adjuvant was administered to the animals intramuscularly (i.m.) at a dose of 1.0 cm^3.

Group 2: vaccine Lyscelin was applied i.m. at a dose of 2.0 cm^3.

Group 3: vaccine Rabisin was applied i.m. at a dose of 1.0 cm^3.

Blood sampling from the tested animals was performed on day 14, 28, 60 and 180 after immunization.

CMI examination

For determination of specific cell-mediated immune response on immunization by rabies vaccines, leukocyte migration inhibition test (LMI) (Bendixen 1977) performed by a capillary method was used in the above mentioned intervals. Purified rabies vaccine strain Vnukovo-32/107 as a specific antigen was used in the LMI test. Its optimal concentration was determined before the experiment. This fact is important in the prevention of inducement of non-specific inhibiton of leukocyte migration by the antigen. Undiluted antigen with migration index MI = 1.04 was applied in the experiment. The migration index was calculated as the quotient of leukocyte migration area in the presence of antigen and the leukocyte migration area without antigen. MI < 0.9 indicates positive antigen dependent inhibition of migration; MI = 0.9-1.1 is allowed to be a reaction without response; when MI > 1.1 probable leukocyte migration stimulated by antigen should be considered.

Serological examination

Rabies antibodies in the serum of experimental animals were quantified in parallel by two methods:

ELISA test was carried out employing a kit developed in our laboratory (Süliová et al. 1988; Beníšek et al. 1989). An antispecies conjugate – peroxidase labelled rabbit antibodies to bovine's IgG – RAB/IgG Px (Sigma, USA) was used.

RFFIT test was carried out by the method of Smith et al. (1973) in modification according to Závadová et al. (1996).

An international standard serum – 30 IU/cm^3 (Statens Seruminstitut, Copenhagen, Denmark) was used in both tests. Rabies antibodies values in sera of tested animals were expressed in IU/cm^3.

Statistical evaluation of results

Statistical evaluation of CMI results as well as of rabies antibodies quantification by the methods ELISA and RFFIT was carried out by means of the Student's *t*-test. The results obtained from the Group 1 immunized by experimental vaccine served as the basis for comparison.

Results

Evaluation of specific cell-mediated immune response to vaccination with the above mentioned vaccines (LMI test) by determination of leukocyte migration index (MI) is shown in Table 1. All tested vaccines can be considered sufficiently effective. There were not significant differences in the MI values of individual vaccines on days 14, 28 and 60. The vaccine Lyscelin was not effective enough from the viewpoint of CMI on day 14, when the average migration index exceeded the limiting value for positive result, i.e. 0.9. Considering that the positive antigen-dependent migration inhibition is suggested by MI value lower than 0.9, then the best results were found in the experimental vaccine and also in vaccine Rabisin. Significant difference in MI values was notable in case of vaccine Lyscelin compared to the experimental vaccine on day 180 ($p < 0.01$). The average MI

value of the experimental vaccine in this interval was also the lowest, yielding the best result of all tested vaccines.

Table 1
Values of leukocyte migration index (MI) in cattle in various intervals after vaccination with experimental and commercial vaccines

Vaccine	Day 14	Day 28	Day 60	Day 180
Experimental	0.88 ± 0.02	0.87 ± 0.01	0.85 ± 0.02	0.83 ± 0.02
Lyscelin	0.92 ± 0.03	0.89 ± 0.02	0.88 ± 0.03	0.88 ± 0.03^{xx}
Rabisin	0.87 ± 0.02	0.86 ± 0.03	0.88 ± 0.02	0.87 ± 0.03

xx – $p < 0.01$

The results of all serological examinations also indicate the advantages of the experimental vaccine. Already on day 28, evaluation results of antigenic activity of tested vaccines by ELISA method (Table 2) show significant differences in potency of the experimental vaccine and vaccine Lyscelin ($p < 0.05$). On days 60 and 180, the difference was even more expressive ($p < 0.001$). Significant difference in efficacy in favour of the experimental vaccine was determined on day 180, even in comparison with the vaccine Rabisin ($p < 0.05$).

Table 2
Evaluation results of the antigenic activity of vaccines tested by ELISA method in cattle after vaccination with the experimental and commercial vaccines (level of rabies antibodies expressed in IU/cm^3).

Vaccine	Day 14	Day 28	Day 60	Day 180
Experimental	0.782 ± 0.091	2.241 ± 0.205	2.024 ± 0.163	1.266 ± 0.159
Lyscelin	0.614 ± 0.115	1.636 ± 0.307^{x}	1.112 ± 0.218^{xx}	0.638 ± 0.193^{xx}
Rabisin	0.662 ± 0.086	1.911 ± 0.201	1.885 ± 0.194	0.826 ± 0.113^{x}

x – $p < 0.05$
xx – $p < 0.01$

Similar results were obtained using RFFIT method (Table 3). Differences in antigenic activity values between the experimental vaccine and commercial vaccines were more notable, while they were significant already on day 14 – vaccine Lyscelin ($p < 0.05$) and on day 28 – vaccine Rabisin ($p < 0.05$).

Table 3
Evaluation results of the antigenic activity of vaccines tested by RFFIT method in cattle after vaccination with the experimental and commercial vaccines (level of rabies antibodies expressed in IU/cm^3)

Vaccine	Day 14	Day 28	Day 60	Day 180
Experimental	1.16 ± 0.24	2.58 ± 0.56	2.42 ± 0.60	2.18 ± 0.43
Lyscelin	0.68 ± 0.22^{x}	0.99 ± 0.20^{xx}	1.14 ± 0.27^{xx}	0.82 ± 0.18^{xx}
Rabisin	0.82 ± 0.19	1.33 ± 0.58^{x}	1.68 ± 0.19^{x}	1.44 ± 0.32^{x}

x – $p < 0.05$
xx – $p < 0.01$

It is necessary to mention that seroconversion with protective titres (titres of rabies antibodies ≥ 0.5 IU/cm^3) was induced by all tested vaccines in all intervals except the vaccine Lyscelin on 14th day of rabies antibodies quantification.

In evaluation of the harmlessness of the experimental rabies vaccine there were found no immediate (anaphylactoid) reactions in any experimental animals immunized by the

experimental adjuvant vaccine. The values of body temperature, heart rate, respiratory rate and food intake did not show abnormalities when compared with the physiological values.

Discussion

For the complex efficacy evaluation of experimental inactivated concentrated purified rabies vaccine potentiated with lipoid adjuvant, intended for domestic animals (Beníšek et al. 1999; Süliová et al. 1999) it was necessary to perform comparative experiments using an important target animal species – cattle. Comparative experiments for the verification of the efficacy of this experimental vaccine on the most important species, domestic dogs, especially with different methods of vaccine administration, were the subjects of our previous work (Beníšek et al. 1998). The method of determination of specific cell-mediated immune response, leukocyte migration inhibition test (LMI), was also used for evaluation of antigenic activity of the experimental and also commercial vaccines on cattle. Several authors have used specific CMI tests for evaluation of antigenic activity of rabies vaccines. Kiss et Tuboly (1981) verified the efficacy of fenolized brain rabies vaccine (Fermi type) and live cell vaccine prepared from strain Vnukovo-32. They studied the correlation between the levels of virus neutralizing antibodies and values of cellular immunity tests (*in vitro* tests for evaluation of lymphocyte activity). They recognised that virus neutralizing antibodies of vaccinated sheep considerably decreased after 6 months, but the intensity of specific cellular response was almost unchanged even after 12 months. Pille and Matevosyan (1985) have obtained similar results.

In our experiments, the dynamics of specific rabies antibodies production and the indicators of specific cellular immunity only until day 180 after immunization was evaluated. It was not our aim to determine the maximal lasting of relevant levels of virus neutralizing antibodies or particular significant values of specific cellular immunity after vaccination with the experimental vaccine. From the point of view of epizootiological situation in rabies in Slovakia and also because of rabies vaccination of cattle taking place only in emergency form of focus rise (fox rabies) in cattle pastured in an endangered area – the rate of immunity onset after vaccination and preservation of sufficient levels of specific antibodies or cellular immunity for a minimum of 6 months is important. Regarding the correlation of virus neutralizing antibodies levels and specific cellular immunity, our results also confirm conclusions of the above-mentioned authors. Whereas values of virus neutralizing antibodies levels in case of all tested vaccines showed a decreasing tendency on days 60 and 180, values of specific CMI indicators, on the contrary, had a rising tendency in all tested vaccines; the best results, however, were yielded by the experimental vaccine on day 180 (average MI = 0.83). In spite of the above-mentioned facts we recommend to use LMI test for evaluation of rabies vaccines antigenic activity on target animals only as a semi-quantitative method (suitable vaccine: MI < 0.9, unsuitable vaccine: MI ≥ 0.9).

Significantly higher efficacy of the experimental vaccine (especially from the point of view of humoral immunity indicators – day 180) is not only due to concentrated and purified antigen in the vaccine, but also due to the use of areactogenic metabolisable as well as highly effective lipoid adjuvant (Beníšek et al. 1999; Süliová et al. 1999) for the support of the vaccine effect. The possibility of adequate rabies immunity induction in cattle administration of a dose of only 1 cm^3 of experimental vaccine is not a negligible fact. Also, areactogenicity of the used lipoid adjuvant is important because of absence of local as well as total (anaphylactoid) reactions, that were not rare in the past (Krejčí et al. 1988; Toman et al. 1992). Sihvonen et al. (1994) described the experiences with the use of 3 kinds of inactivated adjuvant (aluminium hydroxide) rabies vaccines (Rabdomun, Rabisin, Madibovin) in cattle. They detected rabies virus neutralizing titres ≥ 0.5 IU/cm^3 in 80 % of

animals a month after the first vaccination. According to their findings, the first vaccination with one dose of the above-mentioned intramuscularly administered rabies vaccines induces short-time seroconversion in 80 % of experimental animals. There were no significant differences in the efficacy of used vaccines. They detected adequate titres of rabies antibodies (≥ 0.5 IU/cm^3) after 9 months in only 35 % of vaccinated animals. For guarantee of adequate immunity the authors recommend to apply a booster dose of these vaccines 1 month after the first vaccination. They obtained similar results also in experiments on domestic dogs (Sihvonen et al. 1995). In our previous work (Beníšek et al. 1998) we tested the experimental vaccine on domestic dogs. Seroconversion with protective titres of rabies antibodies was detected in all experimental animals, also on day 450 after immunization without using a booster dose of this vaccine.

The evaluation results of antigenic activity of experimental rabies vaccine on cattle unambiguously suggest (although only in certain time intervals) its high efficacy also in this important target animal species.

Experimentálna koncentrovaná purifikovaná adjuvantná antirabická vakcína. Hodnotenie účinnosti na hovädzom dobytku.

V práci bola hodnotená účinnosť experimentálnej inaktivovanej koncentrovanej a purifikovanej antirabickej vakcíny potencovanej lipoidným adjuvans v pokusoch na hovädzom dobytku; testovaním bunkami sprostredkovanej špecifickej imunitnej odpovede (CMI). Pre porovnanie boli použité aj komerčné antirabické inaktivované vakcíny Lyscelin (Česká republika) a Rabisin (Francúzsko). Súčasne s hodnotením CMI pomocou testu inhibície migrácie leukocytov (LMI), boli kvantifikované postvakcinačné antirabické protilátky metódou ELISA a rýchlym fluorescenčným fokus inhibičným testom (RFFIT) na 14., 28., 60. a 180. deň po imunizácii zvierat. Výsledky hodnotenia antigénnej aktivity testovaných vakcín poukazujú na najlepšiu účinnosť experimentálnej vakcíny tak z hľadiska humorálnej, ako aj bunkami sprostredkovanej imunity, pričom všetky testované vakcíny indukovali dostatočnú hladinu antirabických protilátok (≥ 0.5 UI/cm^3) vo všetkých hodnotených časových intervaloch.

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