INTRADERMAL ANTI-RABIES IMMUNIZATION – POSSIBILITIES OF NEEDLELESS RABIES VACCINE ADMINISTRATION

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Abstract

The possibility to apply a needleless (by jet-injector) rabies vaccine was verified experimentally on target animal species, i.e. cattle and sheep. The results were compared with the data obtained by classic intradermal (i.d.) and intramuscular (i.m.) routes of vaccine administration. The post-vaccination humoral immunity level was evaluated by RFFIT and ELISA methods. Also the level of post-vaccination cell-mediated immunity by the test of a blastogenic response of blood lymphocytes to specific rabies antigen was determined. The results of experiments suggest the advantage of the intradermal administration of rabies vaccines for target animal species, as it is possible to use only one fifth dose of vaccine to provide sufficient anti-rabies protection. When needleless administration of vaccine in cattle was performed, a higher antibody response in comparison with i.m. vaccine administration was detected, although significantly higher titres were recorded only on day 35 after anti-rabies vaccination. No significant differences between classic i.d. and needleless routes of vaccine administration were recorded. Similar results were observed also in the case of cell-mediated immune response to rabies antigen in cattle. The mean titres of rabies antibodies in sheep on day 35 after anti-rabies vaccination suggest that the optimal route of rabies vaccine administration in this animal species is the i.d. needle or needleless vaccination on the dorsal part of the ear.

Key words: rabies, vaccine, vaccination route, needleless vaccination.

The anti-rabies immunoprotection has gone through some variations in immunization routes as well as in immunization schemas. Rabies vaccines intended for human and also for veterinary use are administered nowadays usually subcutaneously (s.c.) or intramuscularly (i.m.) in the amount of 1-2 ml per dose.

An alternative of the above mentioned administration of rabies vaccines is the intradermal (i.d.) administration, which was successfully applied in pre-exposure and also post-exposure anti-rabies immunization of humans as well as in pre-exposure experimental vaccination of animals (3, 4, 16). For simplification of i.d. vaccination of animals – especially in mass vaccination cases – it is possible to perform the i.d. vaccination by a needleless injector.

The skin plays important immune functions. The lymphoid tissue associated with the skin contains specialized cells that enhance immune responses. Intracellular space in the skin interstitium served as contact space of lymphatic capillaries and vessels which communicate with ancillary immune tissues or organs such as spleen and lymphatic nodes (20).

Several preliminary studies showed that i.d. administration of rabies vaccine could elicit a sufficient anti-rabies protection although lower vaccine doses were used in animals or in humans (1, 6, 8).

This study was aimed to verify the possibility to use an i.d. route of rabies vaccine administration by a needleless injector on target species of animals (cattle and sheep). In this order humoral and cell-mediated immune response were compared with those recorded following classical (by injection) routes of rabies vaccine administration.

Material and Methods

Animals. Thirty young bulls, weighing 250-300 kg, divided into 3 similar groups, and 20 sheep, two-year-old, divided randomly into 4 groups, were used.

Research was conducted according to the principles presented in the „Guide for Care and Use of Laboratory Animals“, published by the Government of Slovak Republic (10).

Preliminary (entrance) tests of non-specific immunocompetence evaluation. Preliminary tests of non-specific immunocompetence evaluation for uniform representation of different immunocompetent individuals in groups were carried out only in the more extensive experiment on cattle.
Determination of phagocyte activity (PhA) and phagocyte index (PhI) of blood neutrophil granulocytes and monocytes was carried out by the test of microspheric hydrophile particles phagocytosis – MSHP (26).

Non-specific humoral immunity was determined by quantification of whole immunoglobulin level blood serum according to Mc Ewan et al. (17) by zinc-sulphate test.

**Vaccine.** Commercial inactivated aluminium-adjuvanted rabies vaccine (Rabicell, Mekav Nitra a.s., Slovak Republic, lot No. 12) was used in the experiment.

**Routes of vaccine administration and inoculum volumes in cattle.**
- Group 1: intramuscular administration into the middle third of the neck; 1.0 ml;
- Group 2: intradermal administration into the middle third of the neck; 0.2 ml;
- Group 3: intradermal injection by needleless injector (jet-injector BAT – ZD Zlin, Czech Republic) into the middle third of the neck; 0.2 ml.

**Routes of vaccine administration and inoculum volumes in sheep.**
- Group 1: intramuscular injection into the middle third of the neck; 1.0 ml;
- Group 2: intradermal injection on the dorsal part of the ear; 0.2 ml;
- Group 3: intradermal injection by needleless injector (jet-injector BAT – ZD Zlin, Czech Republic) on the dorsal part of the ear; 0.2 ml;
- Group 4: intradermal injection by needleless injector (jet-injector BAT – ZD Zlin, Czech Republic) on the medial side of thigh; 0.2 ml.

**Blood sampling time.**
- Cattle: before vaccination (day 0) and on days 14, 35, 90 and 180 after immunization;
- Sheep: before vaccination (day 0) and on day 35 after immunization.

**Antibody determination.** The following methods for the determination of rabies antibodies were used: rapid fluorescence focus inhibition test (RFFIT), according to Smith et al. (22) modified by Závadová et al. (29), in cattle and sheep and ELISA, developed in the Laboratory of Rabies Research of University of Veterinary Medicine in Košice, Slovak Republic (2, 23), in sheep.

**Blastogenic response of blood lymphocytes to rabies antigen in cattle:** lymphocyte blastogenic assay was performed according to Nagahata et al. (19). As specific antigen the rabies virus Vnukovo-32/107 was used (rabies vaccine strain).

**Statistical evaluation** of rabies antibody titres was carried out by means of the Student’s t-test.

**Results**

**Preliminary (tentative) tests of non-specific immunocompetence evaluation.** The results of the determinatation of phagocyte activity and phagocyte index of leukocytes as well as the amount of the whole immunoglobulins expressed in units of zinc-sulphate turbidity are summarized in Table 1. The animal groups were formed according to the non-specific immunocompetence – the mean values in groups were similar.

**Rabies antibodies in cattle.** Mean titres of rabies antibodies in cattle were the highest and they persisted the longest time after classic intradermal route of rabies vaccine administration, hence this route seems to be the optimal one. In comparison with i.m. route, significantly higher titres were achieved after this route already on day 14 after vaccination (i.m. 0.51 ± 0.11 and i.d. 0.73 ± 0.14 IU/cm³, P≤0.01). Significantly higher antibody titres were noticed also on days 35 and 90 after vaccination (P≤0.05). A considerable difference was recorded when i.m. and needless i.d. routes of administration were compared. The needleless application of the vaccine in cattle brought about a higher antibody response in comparison with i.m. vaccine administration, although significantly higher titres were recorded only on day 35 after the vaccination (i.d.-injector1.49 ± 0.57 and i.m. 1.07 ± 0.15 IU/cm³, P≤0.05). No significant differences between classic i.d. and needless routes of vaccine administration were recorded (Table 2). It is necessary to remark that sufficiently high titres of rabies antibodies were detected still on day 180 after vaccination by all the tested routes (required level > 0.5 IU/cm³ – according to WHO (28)). Total (100%) seroconversion was achieved almost in all animals on day 14 after i.d. vaccine administration, approximately in two thirds of animals after i.d. needleless administration and in 50% of animals after i.m. administration route (individual results are not shown).

**Rabies antibodies in sheep.** The mean titres of rabies antibodies in sheep on day 35 after anti-rabies vaccination (Table 3) suggest that the optimal route of rabies vaccine administration in this animal species is the i.d. vaccination on the dorsal part of the ear. The titres in this group of animals were higher in comparison with the group immunized i.m., although not significantly. Practically no differences were recorded in rabies antibody level when comparing the i.m. vaccinated group with that treated on the dorsal part of the ear by needless i.d. vaccination. The most disadvantageous seems to be the needless i.d. route on medial side of the thigh, because the mean titre of rabies antibodies was significantly lower in comparison with dorsal part of the ear (P<0.01) and did not achieve the required level for anti-rabies protection 0.5 IU/cm² (28).

The results of rabies antibody titres obtained by ELISA (Table 3) were identical and also suggested that the i.d. injection mode on the dorsal part of the ear (1.192 ± 0.222 UE/cm³) was the optimal route of rabies vaccine administration. The mean titre of rabies antibodies was significantly lower compared with i.m. administration (P<0.01) when the vaccine was administered on the medial side of thigh by i.d. needleless route.

**Blastogenic response of blood lymphocytes to rabies antigen in cattle.** The evaluation of cell-mediated immunity in cattle before vaccination (mean
values on day 0) showed stimulation index (SI) of lymphocyte values in all animals ranged from 0.53 to 1.6, i.e. in no animal there was achieved a regular value after stimulation with a non-specific mitogen (Table 4). The lymphocytes started to show the signs of stimulation. This was evident from the enhanced stimulation index of lymphocytes in all groups of animals, though most markedly after i.m. vaccine administration (Table 4). Compared i.d. administrations with i.m. route, a higher increase in SI values in group treated by needleless i.d. administration of vaccine on day 14 (P≤0.025) was recorded. The process of lymphocyte activation was still more intensive on day 35 (Tables 4 and 5). The changes of SI values in percentage (Table 5) in the group of i.d. needleless administration were practically identical with i.m. administration and they were more expressed than in the group of classic i.d. administration (Table 5). Regarding the dynamics of the process, it is evident that while the mean value of SI after the i.m. administration started to decrease, after the i.d. administrations it had still an increasing tendency (Tables 4 and 5). The changes in SI values are not significant because of the great intra-group dispersion.

Table 1
Distribution of cattle into groups according to the values of phagocyte activity, phagocyte index of leukocytes and to the values of the whole amount of immunoglobulins

<table>
<thead>
<tr>
<th>Group</th>
<th>PhA Le (%)</th>
<th>PhI Le</th>
<th>Whole Ig (UZST)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>44.8 ± 11.50</td>
<td>12.10 ± 1.50</td>
<td>25.59 ± 5.93</td>
</tr>
<tr>
<td>2</td>
<td>46.7 ± 14.27</td>
<td>11.86 ± 2.48</td>
<td>25.06 ± 5.74</td>
</tr>
<tr>
<td>3</td>
<td>46.0 ± 10.26</td>
<td>12.03 ± 1.36</td>
<td>25.36 ± 3.60</td>
</tr>
</tbody>
</table>

PhA Le - phagocyte activity of leukocytes
PhI Le - phagocyte index of leukocytes
UZST - units of zinc-sulphate turbidity

Table 2
Mean values of rabies antibody titres determined by rapid fluorescent focus inhibition test in cattle after different modes of anti-rabies vaccination

<table>
<thead>
<tr>
<th>Group</th>
<th>Administration route</th>
<th>Titre (IU/cm³)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>day 0</td>
<td>day 14</td>
</tr>
<tr>
<td>1</td>
<td>i.m.</td>
<td>0</td>
<td>0.51 ± 0.11</td>
</tr>
<tr>
<td>2</td>
<td>i.d.</td>
<td>0</td>
<td>0.73 ± 0.14 **</td>
</tr>
<tr>
<td>3</td>
<td>i.d. – needleless</td>
<td>0</td>
<td>0.66 ± 0.19</td>
</tr>
</tbody>
</table>

i.m. - intramuscular
i.d. - intradermal
IU/cm³ - international units in 1 ml
* - P ≤ 0.05; comparison with the i.m. administration
** - P ≤ 0.025; comparison with the i.m. administration

Table 3
Mean values of rabies antibody titres determined by rapid fluorescent focus inhibition test (RFFIT) and ELISA in sheep on day 35 after different modes of anti-rabies vaccination

<table>
<thead>
<tr>
<th>Group</th>
<th>Administration route</th>
<th>Site of administration</th>
<th>RFFIT (IU/cm³)</th>
<th>ELISA (UE/cm³)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>i.m.</td>
<td>middle third of neck</td>
<td>0.95 ± 0.15</td>
<td>1.063 ± 0.133</td>
</tr>
<tr>
<td>2</td>
<td>i.d. – by needle</td>
<td>dorsal part of ear</td>
<td>1.05 ± 0.20</td>
<td>1.192 ± 0.222</td>
</tr>
<tr>
<td>3</td>
<td>i.d. – needleless</td>
<td>dorsal part of ear</td>
<td>0.90 ± 0.18 **</td>
<td>0.981 ± 0.152 **</td>
</tr>
<tr>
<td>4</td>
<td>i.d. – needleless</td>
<td>medial side of thigh</td>
<td>0.49 ± 0.12 **</td>
<td>0.703 ± 0.124 **</td>
</tr>
</tbody>
</table>

Explanation as in Table 2.
** - P ≤ 0.01; comparison with i.m. administration
◉◉ - P ≤ 0.01; comparison of the i.d. - needleless administrations one with another
Table 4
Stimulation index of lymphocytes in cattle after anti-rabies vaccination by different routes of vaccine administration

<table>
<thead>
<tr>
<th>Group</th>
<th>Administration route</th>
<th>day 0</th>
<th>day 14</th>
<th>day 35</th>
<th>day 90</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>i.m.</td>
<td>1.08 ± 0.26</td>
<td>1.44 ± 0.21</td>
<td>1.93 ± 0.19</td>
<td>1.76 ± 0.21</td>
</tr>
<tr>
<td>2</td>
<td>i.d.</td>
<td>1.03 ± 0.12</td>
<td>1.20 ± 0.15*</td>
<td>1.70 ± 0.33</td>
<td>1.85 ± 0.39</td>
</tr>
<tr>
<td>3</td>
<td>i.d. – needleless</td>
<td>0.87 ± 0.15</td>
<td>1.33 ± 0.19</td>
<td>1.65 ± 0.27**</td>
<td>1.75 ± 0.22</td>
</tr>
</tbody>
</table>

Explanation as in Table 2.
* - P ≤ 0.025 (comparison with the i.m. administration)

Table 5
Mean values of stimulation index of lymphocytes in cattle expressed in percentage of values on day 0

<table>
<thead>
<tr>
<th>Group</th>
<th>Administration route</th>
<th>day 0 (%)</th>
<th>day 14 (%)</th>
<th>day 35 (%)</th>
<th>day 90 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>i.m.</td>
<td>100</td>
<td>142.0 ± 46.6</td>
<td>192.8 ± 66.8</td>
<td>175.6 ± 62.0</td>
</tr>
<tr>
<td>2</td>
<td>i.d.</td>
<td>100</td>
<td>117.8 ± 19.6</td>
<td>168.6 ± 45.1</td>
<td>183.6 ± 50.2</td>
</tr>
<tr>
<td>3</td>
<td>i.d. – needleless</td>
<td>100</td>
<td>159.3 ± 45.6*</td>
<td>191.2 ± 22.4</td>
<td>208.1 ± 47.9</td>
</tr>
</tbody>
</table>

Explanation as in Table 2.
* - P ≤ 0.025 (comparison with the classic i.d. administration by needle)

Discussion

The vaccination mode, use of adjuvant and vaccine dose are the factors which definitely influence both the cellular and the humoral immune response. Out of different vaccination routes, the most studied is the i.m. one; especially the depot effect is considered (18). The muscles are not considered as the site of antigen presentation, since they contain only a few dendritic cells (DC), macrophages, and lymphocytes. Subcutaneous (s.c.) route of administration has an advantage in activating depot sites for cytokines which can provide a longer contact with depot antigen (5). However, the skin is the only parenteral part of organism that can act as an immune organ by itself (27). Therefore its importance is accentuated in its utilization in immunostimulatory treatments.

Intradermal immunization is attractive also on the ground of easy access to a target organ, as well as by good representation of immune system elements in the skin (27). The lymphoid tissue associated with the skin contains specialized cells which increase the immune response. Keratinocytes produce interleukin-1 and a tumour-necrotic factor which can activate the lymphocytes, macrophages, and DC. When the vaccine is administered by i.d. route, DC growth factor is delivered directly into the skin where the DC are residing. DC are exposed to antigen and activated; they migrate into regional lymph nodes and activate the T-lymphocytes (7).

In possible prevention of various diseases of animals the advantage of i.d. administration of a vaccine or antigen was described. Samina et al. (21) administered the commercial leptospira vaccine (*L. hardjo*) in cattle by i.d. and s.c. routes. After the first vaccine dose there were no differences in immune response between the groups, however, after a booster dose the i.d. administration appeared to be markedly better. A successful use of i.d. administration of bovine herpesvirus-1 DNA vaccine in cattle was described by Littel van den Hurk et al. (13); the compared results of i.m. administration was less effective.

In our previous study (3) we obtained similar results following i.d. immunization of dogs with an inactivated rabies vaccine; the dose of 0.2 ml provided the anti-rabies protection of dogs for 15 months, while the 1 ml dose of i.m. administered vaccine did not arrange for it for so long time. Hunasker and Perino (11) informed about the examination of immune response of various i.d. administered antigens – viral, parasitic, bacterial and fungal. According to positive results of immune response evaluation they considered the i.d. route of administration as a promising alternative in comparison with the conventional immunization routes – i.m. and s.c. Administration of DNA rabies vaccines by classic mode did not give rise to rapid humoral response, therefore an experiment on monkeys was carried out when the vaccine was administered by i.d. route into the auricle. Even though the s.c. administration generated little higher titres of virus neutralizing antibodies, the result of experiment was encouraging (14). The same authors described an experiment, when one dose of i.d. administered rabies DNA vaccine into the auricle of dogs had induced the production of adequate and persistent level of virus neutralizing rabies antibodies (15). Also Taracha et al. (2003) obtained positive results in experiments when applied DNA vaccine and an i.d. administered booster dose of a recombinant vaccine prepared from an antigen 85A *Mycobacterium tuberculosis*. Therefore it is evident that i.d. administration also of DNA vaccines can induce an adequate anti-rabies protection in vaccinated individuals.
The needleless administration of a rabies vaccine was described for the first time by Toma et al. (25) and Koutchoukali et al. (12). The jet-injector „Dermojet“ was used to apply rabies vaccine on the inner side of the ear of dogs (eminent target animal species). The rabies antibody titres were sufficiently high for one year after the i.d. needleless vaccination (the vaccine dose was 0.1 ml into the both ears) in comparison with s.c. vaccination route, when the rabies vaccine was administered on the both sides of thorax in similar dose, i.e. twice 0.1 ml. The s.c. administration of vaccine was carried out also with the dose of 1.0 ml divided and applied twice in time of 15 d. The experiment demonstrated the same rabies antibody levels after i.d. needleless administration as after s.c. administration of rabies vaccine, but it was used in five times lower vaccine dose (25). Similar results were obtained with the jet-injector „Dermojet“ also by Koutchoukali et al. (12).

Intradermal route of rabies vaccine administration was approved also in human medicine, especially in post-exposure vaccination but always by the route of injection. The vaccine was administered mostly on several sites (minimum two) and doses (4). A combined mode of rabies vaccine administration was also verified. Intradermally vaccinated humans received a booster dose administered i.m.; one i.m. administered booster dose after i.d. primovaccination induced adequate anti-rabies protection (9).

In our experiments the possibility of needleless rabies vaccine administration was tested. The advantage of this vaccination mode is the possibility of its using in mass vaccinations of target animals (dogs or cattle). The results of our experiments suggest the advantage of i.d. administration of rabies vaccines for target animals, because a one fifth of vaccine dose provides for adequate anti-rabies protection. However, the needleless administration route of rabies vaccine is not absolutely perfect. The jet injector used in our experiments dosed 0.2 ml of the vaccine volume, therefore, only one dose on one site was applied. The jet-injector BAT is adapted to the character of cattle skin; its original function is tuberculinization of cattle. We used the jet-injector for needleless i.d. administration of rabies vaccine and we recorded better immune response in cattle in comparison with i.m. administration, even though significantly higher titres were recorded only on day 35 after anti-rabies vaccination. No significant differences between classic i.d. and needleless routes of vaccine administration were recorded.

Similar results were obtained also in the evaluation of cell-mediated immune response to rabies antigen. The differences between SI of lymphocytes were not significant at different vaccine administration routes, but it is necessary to remember the lower start SI values in the group of i.d. needleless treated animals and the faster increase of these values already on the day 14 in comparison with the needle modes of vaccine administration (i.d. and also i.m.). It is confirmed also by percentage expression of results of SI in individual groups. The SI of lymphocytes indicated their activation grade, i.e. their ability to react to stimulation with mitogen or specific antigen by proliferation.

Different modes of rabies vaccine administration were aimed to induce adequate anti-rabies protection also in sheep. The mean titres of rabies antibodies detected on day 35 suggested that in sheep the most optimal route of rabies vaccine administration was the i.d. one on the dorsal part of the ear, by needle and needleless, respectively. The differences of rabies antibody levels – immune response to vaccination – in sheep after i.d. vaccination on dorsal part of the ear were not significant. The most inefficient seems to be the i.d. needleless route on the medial side of thigh. This route of rabies vaccine administration did not induce at least the minimum titres of rabies antibodies necessary to provide anti-rabies protection on day 35. It is caused probably by the character of skin, which does not allow the penetration of adequate vaccine volume (optimally 0.2 ml) in this part of the skin.

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References


