ORIGINAL ARTICLE

IN VIVO AND *IN VITRO* CHARACTERIZATION OF A VACCINE RABIES STRAIN ISOLATED FROM FIELD

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Abstract

Rabies is a fatal acute viral zoonosis, causing 62,000 human deaths every year. The oral rabies vaccination (ORV) programs of foxes, the main reservoir of rabies in Europe, are the most effective tools to control and finally to eradicate the disease. In order to characterize by *in vivo* and *in vitro* assays a vaccine rabies strain isolated from a rabid cattle following the ORV program, it has been used BHK21, N2a cell line and mice inoculation test. The results showed that the vaccine strain is able to grow on BHK21 cells, like an attenuated live vaccine, as well as on N2a cell line, usually used for diagnosis, while wild rabies strains from naturally infection were isolated on N2a cells only. The intra-cerebrally inoculated mice with vaccine strain developed disease signs and died in 12 days, one day later than inoculated mice with a local strain virus from natural infection.

Rezumat

Rabia este o boală virală acută şi fatală ce produce anual 62.000 de morţi în rândul populaţiei umane. Programele de vaccinare antirabică a vulpilor, rezervorul de rabie în Europa, sunt cele mai eficiente instrumente în controlul şi eradicarea bolii. Pentru a caracteriza *in vivo* şi *in vitro* o tulpină vaccinală de virus rabic izolată de la o vacă turbată în urma programului de vaccinare orală a vulpilor au fost folosite liniile celulare BHK21 şi N2a, precum şi testul de izolare al virusului rabic prin inoculare intracerebrală a şoarecilor. Rezultatele au arătat că tulpina vaccinală îşi păstrează proprietatea de a cultiva pe linia BHK21, specific tuturor vaccinurilor vii atenuate de virus rabic, dar se izolează şi pe linia celulară N2a, linie celulară folosită uzual în diagnosticul de laborator al bolii. Şoarecii inoculaţi intracerebral cu tulpina vaccinală au dezvoltat boala şi au murit la 12 zile, o zi mai târziu decât şoarecii inoculaţi cu tulpini locale de virus rabic izolate în urma infecţiei naturale.

Keywords: rabies vaccine, BHK21 cells, N2a cells, intra-cerebrally mice inoculation

Introduction

Rabies is a Central Nervous System zoonotic disease, with the causative agent rabies virus, the negative-sense single stranded RNA viruses of the genus *Lyssavirus* within the family *Rhabdoviridae*, which causes between 37,000 and 87,000 human deaths annually, worldwide [4, 5]. This seems to be an acute underestimation, since a study in Tanzania has been suggested that only 3% of human cases of rabies virus are recorded by central authorities [11, 16]. In Europe, canine rabies has been eradicated from developed countries by control measures, such as dog movement restriction and mass vaccination and now the major reservoir of rabies was replaced by wild animals, especially red fox (*Vulpes vulpes*) [6]. Extensive oral vaccination programs (ORV) with baits for red foxes have reduced the incidence of rabies in many Western European countries. Therefore, for this purpose there have been developed attenuated live vaccines in suitable cell culture lines [1]. The attenuated live vaccines may have a low residual pathogenicity and could occasionally induce rabies in target or non-target species such as foxes, raccoons, cattle, dogs and cats [7, 8, 18].
In this paper we aimed to investigate *in vivo* and *in vitro* a vaccine rabies virus strain isolated from a rabid cattle, following the oral vaccination program of foxes.

Materials and Methods

For this study there have been used 11 brain samples, 3 negative as negative controls and 9 positive, of which 8 wild strains and one vaccine strain, diagnosed using standard methods including the fluorescent anti-body test (FAT), the rabies tissue culture infection test (RTCIT), PCR and sequencing [10, 17]. As positive control, we used viral suspension from the vaccine, used during the oral vaccination program.

Cell lines. Murine neuroblastoma (N2a) and baby hamster kidney (BHK21) cell lines have been used for incubation with centrifuged 10% brain suspensions diluted 1:5 in cell culture medium for 24 - 72 hours as previously described [11,12].

Animals. The mouse inoculation test was performed according to the Romanian law 205/2004 and directive number 2010/63/EU. Rabies virus was isolated after its intracerebral inoculation into 2 weeks old mice as previously described [3, 15]. Mice were euthanized at the appearance of disease signs, and infection was confirmed by FAT.

FAT results for RTCIT was estimated based on the percentage of positive (fluorescent) cells.

Tissue culture slides and smears of mouse brain slides were examined at a magnification of200x - 400x by Leica UV microscope. The captures were performed by Nikon D40.

Results and Discussion

Virus isolation on cell culture results are shown in Table I.

Table I

Comparative RTCIT results on BHK21 and N2a cell lines to different time points (24, 48 and 72 hours)

	BHK21 % cells positive				N2a % cells positive			
	Negative control	Positive control	Vaccine strain	Wild strains	Negative control	Positive control	Vaccine strain	Wild strains
24 hours	-	+ (83)	+ (2)	-	-	+ (86)	+ (8)	-
48 hours	-	+ (92)	+ (29)	-	-	+ (96)	+ (50)	-
72 hours	-	+ (100)	+ (83)	-	-	+ (100)	+ (88)	+ (1)

Rabies virus from vaccine strain isolated from the field was identified on BHK cell after 24 hours of inoculation. After 72 hours approximately 83% of cells were infected. There were no fluorescence cells on negative controls and on wild rabies virus strains. The percentage of the infected cells was almost 100% after 24 hours of incubation on positive control. This demonstrates that our vaccine strain isolated from a rabid animal is adapted on cell culture like most animal rabies vaccines. The BHK21 cell line is used for obtaining biological products, because its origin is very well documented and it has been proven to be safe and efficacious over decades [9].

Virus isolation on murine neuroblastoma cells showed approximately the same results with BHK21 cells. The difference was that after 72 hours all wild strains infected N2a cells (Figure 1) and on the other hand the percentages of the N2a fluorescent cells inoculated with vaccine strain were consistently higher, compared with BHK infected cells. After 24 hours of incubation on N2a cells, there were 4 times more infected cells than on BHK21 inoculated with vaccine strain. After 48 hours, the positive cells doubled and after 72 hours there were at the same level (Figure 2, Figure 3). This vaccine strain finding suggested that even for strain adapted to grow on BHK21 cell line, neuronal cell lines like N2a proved more susceptible for rabies routine virus isolation methods [14, 15]. According to previous research, Rudd and co-workers explained these features based on the similarity of the mouse neuroblastoma cell surface receptors, identical to that in normal central nervous system [13].

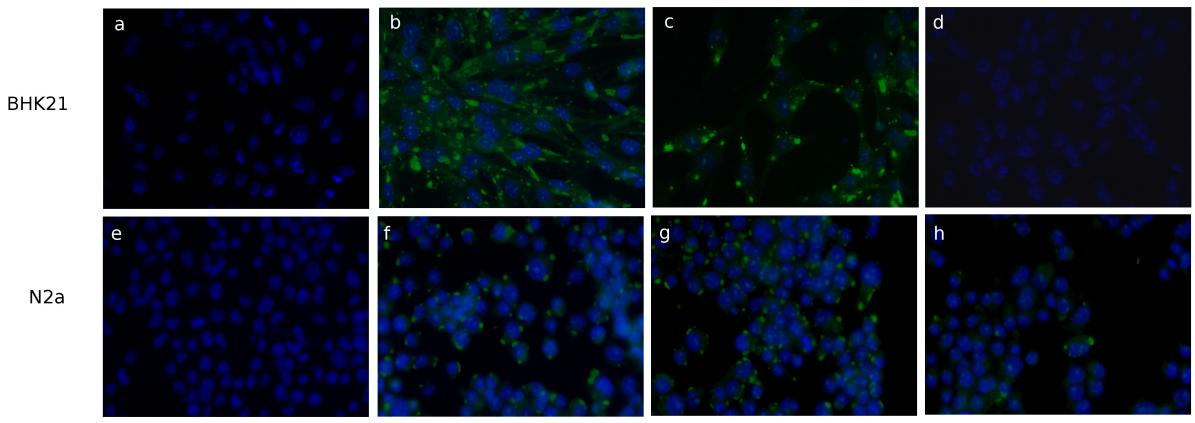

Figure 1.

Comparative RTCIT results on BHK21 and N2a after 72 hours incubation: a, e - negative control; b, f - positive control; c, g - vaccine strain; d, h - wild local strain. Green fluorescence means rabies viral particles stain with commercial fluorescence antibodies against rabies. Blue fluorescence - nucleus stein with DAPI. Magnification 400x.

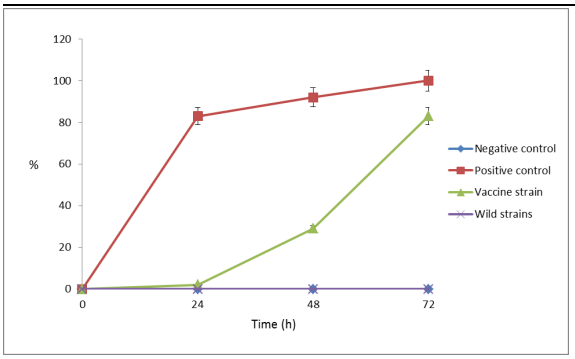

Figure 2.
Percentages of BHK21 positive cells according to incubation time

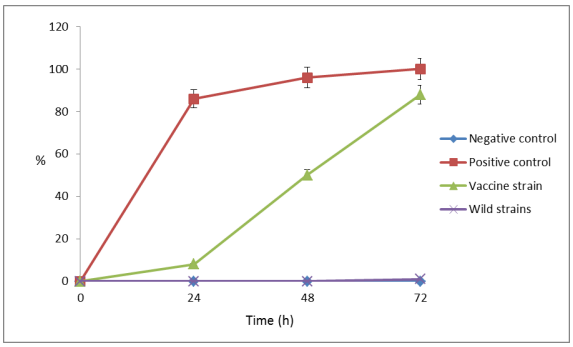

Figure 3.
Percentages of N2a positive cells according to incubation time

Mice intra-cerebrally inoculated with vaccine and wild strains developed rabies signs, and died in 11, respectively 12 days and they were FAT positive. Intracerebral mouse inoculation test showed that the vaccine strain isolated from field had similar pathogenicity with wild local strains, and might be possible to produce diseases at the certain conditions as it has been previously shown [7, 18].
In European countries, rabies vaccines for veterinary use are approved by the competent state authorities and should be in line with international standards for vaccines [1, 3, 5]. Since April 2013, the target animal batch safety test is no longer required. Relevant requirements adopted among different countries [2] foresee adequate quality assurance systems (good manufacturer practices-GMP), seed lot systems to control consistent batch production (hence increasing vaccine quality and safety) and pharmacovigilance, based on post-marketing surveillance of immunological products in order to detect and report safety problems in the field. Nowadays, WHO [5] recommends the use of rabies vaccines that induce no adverse signs in target or non-target species.

Conclusions

According to the study results we concluded that, on one hand, the vaccine strain isolated from rabid cattle still kept the properties in order to be cultivated on BHK 21 cell line, like all animal vaccines against rabies. On the other hand, the isolated vaccine strain is as photogenic as a wild strain, mice which were intra-cerebrally inoculated developed rabies signs as fast as the mice inoculated with the wild strain.

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