

XXIV RITA Canada - Rabies in the Americas Meeting Abstracts

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Resumos dos trabalhos apresentados no encontro pelo Instituto Pasteur, unidade da Secretaria de Saúde do Estado de São Paulo referência em controle da raiva animal e humana.

Comparison of *in vivo* and *in vitro* transfection of antibodies against rabies

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This study describes the comparison of *in vivo* and *in vitro* transfection of antibodies to use as a new mechanism for antiviral therapy against rabies. The transfection was made *in vitro* with neuroblastoma murine cell line (Neuro-2a) and *in vivo* with 21 years old mice. Both cells and mice were infected with different infectious doses (0.1; 1.0; 10 and 100 ID₅₀) of the rabies virus. After 24 hours the infected cells and mice were transfected with polyclonal antibodies against rabies produced in equines (unpurified hyperimmune serum - total antibody) using a cationic reagent, the lipofectamine 2000 previously diluted 1:20, for the negative transfection control, only minimum essential medium was used. The ability of the antibodies to neutralize the rabies virus *in vitro* was determined by counting the number of fluorescent foci for each of the infectious doses and comparing the results with the controlling group to give the percentage inhibition. The ability of the antibodies to neutralize the rabies virus *in vivo* was determined by counting the number of mice that survived or died 35 days after the transfection in compare the controlling group. The results of neutralization showed that for lower viral concentrations (0.1 and 1.0 ID₅₀), viral inhibition was 100% for the *in vitro* and *in vivo* transfection. When were used 10 and 100 ID₅₀, the viral inhibition was 89,2 % and 90,3% respectively in the *in vitro* transfection, all mice died when these doses are used in the *in vivo* transfection. In conclusion, when higher viral concentrations were used the results of *in vitro* transfection do not are reflected *in vivo*. The viral load of the patient that was exposed is an important point to decide the most efficient mechanism for use in antiviral therapy against rabies.

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Solubilization of the rabies virus glycoprotein using different detergents

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Introduction: Rabies virus glycoprotein (RVG) is important in the biology and pathogenesis of rabies virus infection. This transmembrane RVG is highly immunogenic, inducing the production of neutralizing antibodies (VNA), the activation of helper T and cytotoxic T lymphocytes. The RVG may have several applications for both laboratory diagnosis and research of rabies disease. **Aim:** The production of rabies virus to the concentration and solubilization of glycoprotein. **Methods:** The challenge virus standard-11 strain (CVS) was replicated on BHK-21 cells. Supernatants from virus-infected cells were harvested at 72 hours

post infection, and the virions were concentrated by centrifugal filter (Amicon Ultra). The evaluations of viral suspensions with highest dilution with 100% of the BHK-21 cells infected were performed by direct immunofluorescence. Three detergents (0,6% CHAPS, 2% Octyl β-(+)-glucopyranoside (OPG) and 0,1% Triton X-100) in buffer (50 mM Tris-HCl, 150 mM NaCl, 10% DMSO, 4 mM EDTA) were used to solubilize RVG from CVS. RVG was quantified using neutralization test and demonstrated by photos. **Results and Discussion:** The highest dilution, viral suspensions with 100% of BHK-21 cells infected, corresponded 1:512, increasing the viral dilution in 256-fold using Amicon column. The experimental data showed that the detergents Triton X-100 were not as good as CHAPS and OPG in the solubilization of RVG. The results suggested that the technique of enhanced the concentration of viral particles was effective in solubilization of glycoprotein, thereby paving the way for purification this membrane protein. Thus, new methods may be developed for the diagnosis of rabies using G protein.

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Eastern equine encephalitis virus isolation *in vitro*, BHK-21 and N2A cell cultures

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The Laboratory of Rabies Diagnosis at the Pasteur Institute of Sao Paulo routinely receives equine brain samples for rabies diagnosis. Between the years 2000-2010, 2.122 samples were received and only 24.6% were positive for rabies. These data confirm the importance of the differential diagnosis for equine encephalitis caused by other infectious diseases like alphaviruses. Viral isolation of Eastern equine encephalitis (EEE) is the most definitive method and can be performed in both mice and cell culture. VERO and BHK-21 cell cultures are recommended by OIE for EEEV and Western equine encephalitis virus (WEEV) isolation. However, VERO cell culture is the most commonly used. One sample diagnosed negative for rabies and positive for EEEV by isolation in mice and confirmed by hemi-nested RT-PCR was used for evaluate the isolation in cell culture. EEEV and WEEV were used as positive controls and Minimum Essential Medium as negative control. The sample was diluted at 20% (w/v) and 1mL of this suspension was inoculated in VERO, BHK-21 and N2A cell cultures. The presence of cytopathic effects (CE) was observed at 48 hours in VERO and N2A. In BHK-21 the CE was observed at 24 hours. The viral isolation was confirmed by hemi-nested RT-PCR in all cells culture. These results show the ability of N2A cells in replicate the EEEV. In conclusion, the N2A and BHK cells, lineages commonly used in rabies diagnosis, were able for viral isolation of the EEEV.

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