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SHORT-INTERFERING RNA EVALUATION IN EXPERIMENTAL MICE RABIES VIRUS INFECTION CAUSED BY VARIANT 2 AND VARIANT 3

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Objective: Rabies is an ancient disease and until now no effective treatment is available. Treatment using short-interfering RNA (siRNA) to inhibit rabies virus (RABV) replication showed promising results in vitro. Our purpose was to evaluate the efficacy of siRNA in treating mice experimentally infected with different street RABV strains. **Materials and Methods:** 3 groups of 20 C57/BL6 mice, SPF, 4-6 weeks-old were inoculated in gastrocnemius muscle with 3 different RABV strains. A variant 2 isolated from a dog [dv2 (LD50 10-3.39/0.03 mL)], a variant 2 isolated from a human [hv2 (LD50 10-6.66/0.03 mL)] and a variant 3 isolated from a human [hv3 (LD50 10- 6.66/0.03 mL)] . For each group, 10 mice remained untreated and 10 mice were treated with a mix of 3 different siRNA sequences (3.3 µM each) associated with lipofectamine (Brandão et al. 2007) based on rabies virus N gene as a target. Animals received a single dose of siRNA mixture, via intraperitoneal route, 24h post RABV inoculation (p.i) and were observed during 30 days. Cox Proportional Hazards models were used to estimate lethality rates and Hazard Ratios (HR) between groups. **Results:** For dv2, lethality was 37.5% in the inoculated group and 50% in the siRNA group (P= 0.71; HR= 0.75); For hv2, lethality was 100% in the inoculated group and 70% in the treated group (P= 0. 27; HR=0.57); For hv3, lethality was 60% in the inoculated group and 80% in the treated group (P= 0. 21; HR= 1.97). **Conclusion:** The efficacy of siRNA seems to be associated to the RABV strain once the results of survival was variable in the groups submitted to siRNA and infected with different RABV strain . The siRNAs used were designed based on Pasteur virus N gene sequence, a fixed strain while in our study street RABV strains were used. Even considered as a conserved gene, studies showed significant genetic variability. A nearly perfect complementary sequence between siRNA molecule and the viral RNA target is necessary for mRNA cleavage. Our RABV N gene sequences showed 85.7% – 95.2% of homology between v3 and siRNAs sequences and 95.2 % – 100% to v2, confirming this natural variability and the better results obtained with the variant 2. In this study, a nonbiological delivery system was used and an important point is the difficulty of siRNAs delivery within CNS being this a major problem in practical therapeutic.

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CANINE AND FELINE RABIES IN THE STATE OF SÃO PAULO – DATA FROM THE PASTEUR INSTITUTE OF SÃO PAULO, 1999-2012

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The São Paulo State Rabies Control Program, coordinated by the Pasteur Institute of São Paulo, has been involved in endeavors to improve the epidemiological surveillance of rabies in dogs, cats and bats, particularly in urban areas. Since 1998, when the last case of canine rabies due to antigenic variant 2 occurred in the state, the Institute has received 66,352 canine central nervous system samples, 17,796 samples from cats and 38,133 from bat specimens. Using conventional techniques for rabies diagnosis, 11 dogs, 8 cats and 554 bats were found to be positive for this disease. When molecular techniques, such as antigenic typing and genetic sequencing, were used, antigenic variant 3, which is associated with *Desmodus rotundus*, was detected in 11 dogs and 7 cats; this variant has also been identified in bat species from the genus *Artibeus* spp., which has synanthropic habits. Only one cat was positive for variant 4, which is associated with *Tadarida brasiliensis*. It should be noted that the signs and symptoms of cats and dogs infected by rabies variants associated with bats were quite different from those in dogs infected with variants associated with dogs (variants 1 and 2). In general, symptoms were atypical, the most common clinical picture being that of paralysis and hemorrhagic gastroenteritis, and cases were nearly always isolated. Positive samples from cats and dogs frequently came from animals that had been run over and died. These findings show the importance of maintaining active epidemiological surveillance of cats and dogs and of sending samples for rabies diagnosis, particularly in regions where rabies is known to occur in urban areas.

PT.056
EVALUATION OF HUMORAL IMMUNE RESPONSE CONFERRED BY PURIFIED CHICK EMBRYO CELL VACCINE (PCECV) AND PURIFIED VERO CELL VACCINE (PVCV) USED IN COMPLETE AND MIXED PREEXPOSURE SCHEMES AT PASTEUR INSTITUTE OF SÃO PAULO

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The most widely used vaccines in the Western world for human rabies prophylaxis are Purified Chick Embryo Cell Vaccine (PCECV) and Purified Vero Cell Vaccine (PVCV), which is used at Pasteur Institute of São Paulo since 2000. Whereas in Brazil there are no reports of the use of PCECV in replacement or complementation to PVCV, the aim of this study was to evaluate humoral immune response of people vaccinated against rabies in complete and mixed pre-exposure schemes using PCECV and PVCV with different routes of application, intramuscular (IM) and intradermal (ID). According to the vaccination scheme received, 115 serum samples from different individuals were distributed in seven groups: three containing serum samples from patients who received complete schemes with one vaccine and one route of application – 3 PVCV (IM), 3 PVCV (ID) e 3 PCECV (IM) -, used as reference; and four containing serum samples from patients who received both vaccines and two routes of application, featuring a mixed scheme – 2 PVCV (ID) + 1 PCECV (IM), 1 PVCV (ID) + 2 PCECV (IM), 2 PCECV (IM) + 1 PVCV (ID) e 1 PCECV (IM) + 2 PVCV (ID). The humoral immune response was analyzed based on the levels of rabies virus neutralizing antibodies (RVNA) obtained by rapid fluorescent focus inhibition test (RFFIT); through calculation of its maximum and minimum values, median, standard deviation, 1st and 3rd quartiles, interquartile range and Kruskal-Wallis test. Differences statistically significant (p-value < 0.05) were observed when the group 2 PVCV (ID) + 1 PCECV (IM) was compared with the groups 3 PVCV (IM) and 1 PCECV (IM) + 2 PVCV (ID). The group containing serum samples from patients who received 2 PVCV (ID) + 1 PCECV (IM) is the one responsible for statistical differences observed, because it is the group in which higher RVNA titers were detected, ie, the group with the best humoral immune response. Despite the differences observed, in all serum samples, RVNA titers obtained can be considered adequate (≥0.5 UI/mL), regardless of the vaccine and routes of application used. The use of PCECV for rabies vaccination, in complete or mixed pre-exposure scheme, showed satisfactory results in the induction of humoral immune response and its use may be adopted when it is necessary to complement or replace PVCV. Financial Support: Instituto Pasteur, São Paulo/SP, Brasil