The evaluation of neurovirulence of mumps virus strains with alternative newborn rat-based safety test

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INTRODUCTION
Because of neurotropic and neurovirulent properties of mumps virus, neurovirulence testing of live attenuated vaccine is required by most national regulatory organisations. Such testing is performed in monkeys (Ph.Eu. 01/2008:20618-Test for neurovirulence of live virus vaccines). But results obtained from these tests do not necessarily distinguish among the neurovirulent strain from those that are not. As a part of an international collaborative study we investigated the neurovirulence of three vaccine strains (JL5, Urabe AM9 and L-Zagreb) and two wild-type mumps viruses isolated in Croatia (9218/Zg98 and MuVi/Zagreb.HRV/28.12) by the neurovirulence assay in newborn rats.

MATERIALS AND METHODS

1- Mumps viruses
Three vaccine strains (JL5, Urabe AM9 and L-Zagreb) and two wild-type mumps viruses isolated in Croatia (9218/Zg98 and MuVi/Zagreb.HRV/28.12) were used in the present study. All were divided into 0.5 ml aliquots and kept at -80°C and shipped on dry ice. Virus titre was determined by plaque test as described in Forčić et al (2010).

2- Inoculation of rats
The test was performed essentially as described in Rubin et al (2005) with minor modifications in the brain processing. LEW /SsNHsd rats (Harlan Sprague Dawley) bred at the Institute of Immunology were used. Each sample was inoculated in 30 to 40 animals (4-5 litters) except for the mock inoculation where 10 animals were inoculated. Litters of 1-day-old rats were inoculated with 100 pfu of virus in a 10 μL volume of MEM by use of a 27-gauge needle. The inoculation site was in the left parietal area of the skull, ~2 mm left of midline and midway between the bregma and lambda. On day 30 after inoculation, all rats were euthanized by CO2 asphyxiation. Brains were removed and were fixed in 10% neutral buffered formalin for 1 week. Institutional guidelines for the care and use of laboratory animals were strictly followed.

3- Brain processing
Fixed brains were cut in half in the sagittal plane along the anatomical midline. To each brain hemisphere, a second cut was made in the same plane 3–5 mm from the first cut. The brain sections were immersed in the Cryofix gel (Biognost) and frozen in liquid nitrogen. Frozen tissue was cut with the cryostat into 15-μm-thick sections which were placed on glass slides and dried. A single 10-mm-thick section was obtained from each hemisphere at a depth of ~0.5–1.0 mm from the surface. Tissue sections were placed on glass slides, warmed overnight, rehydrated, stained with hematoxylin-eosin (H-E), and dehydrated, and cover slips were attached with Permount (Fisher Scientific). Stained slides were placed on a scanner, and the scanned image was transferred to a computer. ImageJ image analysis software was used to measure (in pixel units) the cross-sectional area of the entire brain (excluding the cerebellum) and the cross-sectional area of the lateral ventricle.

4- Neurovirulence assessment
The rat neurovirulence test (RNVT) score was calculated as the ratio/percentage of the area of the ventricle and the area of the whole brain. The data were analyzed with descriptive statistics using Statistica 6.0 software (StatSoft Inc.) and shown as Box and Whiskers plots.

RESULTS

Figure 1. pictures of brain on day 30 after inoculation and brain processing

CONCLUSION

- The results obtained by alternative assay on two vaccine strains (JL5 and Urabe AM9) correspond to the results obtained by the National Institute for Biological Standards (NIBSC) in the United Kingdom and the Food and Drug Administration in the United States.
- Strain L-Zagreb also showed reduced neurovirulent properties as expected because it is a vaccine strain.
- Wild-type mumps virus isolates showed high neurovirulence.
- These results indicate that the test in newborn rats is suitable for assessing the neurovirulence of mumps viruses.
- The test is robust, reproducible and follows the 3R principles.

REFERENCES: