DEVELOPMENT OF MUMPS VIRUS PREPARATION FOR THE ONCOLYTIC VIROTHERAPY APPLICATON IN ANIMALS

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INTRO

The mumps virus (MV) is an RNA virus from the *Paramyxoviridae* family. MV primarily causes respiratory infections with painful swelling of the parotid glands in its only natural host – humans. Attenuated MV-based vaccines have been in use for decades and have significantly reduced the mumps incidence in human population. MV has been documented as a potent oncolytic agent in different preclinical but also early clinical studies. Due to its stringent host specificity, MV seems as an ideal candidate for use in veterinary medicine since it does not cause disease in animals, but successfully replicates in different animal cell cultures, tissues and organs.

OBJECTIVES

We aim to develop an appropriate methodology for reliable and precise characterization of MV preparations, produced from supernatants of MV-infected Vero cell cultures. Another goal is to find the longest time frame and storage conditions in which properties of stabilizer-free MV preparations do not substantially change.

METHODS

Five independent batches of MV (MV1-MV5) were prepared at the laboratory scale. Samples prepared completely equally from cultures of non-infected cells served as controls (KV1-KV5). Compositional properties were monitored immediately upon preparation and after the 14 and 21-day storage at 4 °C and at -70 °C or lower: infective virus quantity ($CCID_{50}$ assay); total particle (viruses and extracellular vesicles) quantity and size (NTA); host cell DNA (qPCR) and protein (ELISA) content; chromatographic fingerprint in the form of SEC chromatograms generated by the usage of UV, refractive index and light scattering detectors (HPLC), genomic composition of viral population (RNA seq) and microbial purity.

For more details go to:

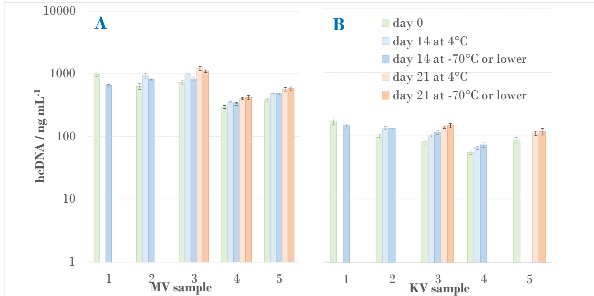

Figure 1. Host cell DNA (hcDNA) content changes in MV (A) and KV samples (B) during the storage at two temperatures presented as the mean concentration (column height) and 95% confidence interval (vertical bars) from at least 6 independent measurements for each sample.

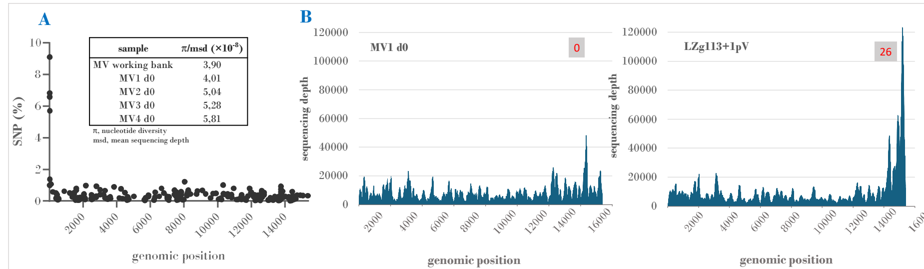

Figure 4. Genomic characterization of MV samples. SNPs' positions and percentages in sample MV1 d0, chosen as a representative figure for all oncolytic MV samples; insert table shows population diversity statistics (A). Genome coverage distribution of sample MV1 d0 with low amount of defective viral genomes (DVGs) and DVG-enriched sample (LZg113+1pV); number of DVGs supported with >100 counts with ViReMa-a and DI-tector algorithms is shown in red (B).

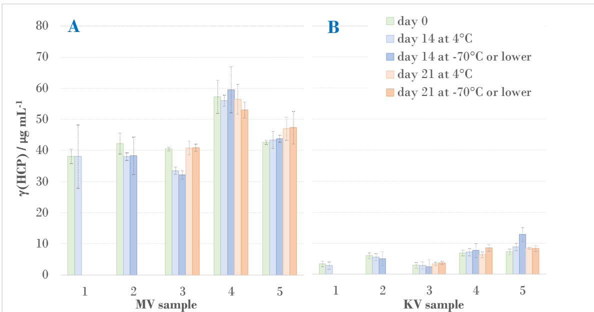

Figure 2. Host cell protein (HCP) content changes in MV (A) and KV samples (B) during the storage at two temperatures presented as the mean concentration (column height) and 95% confidence interval (vertical bars) from at least six independent measurements for each sample.

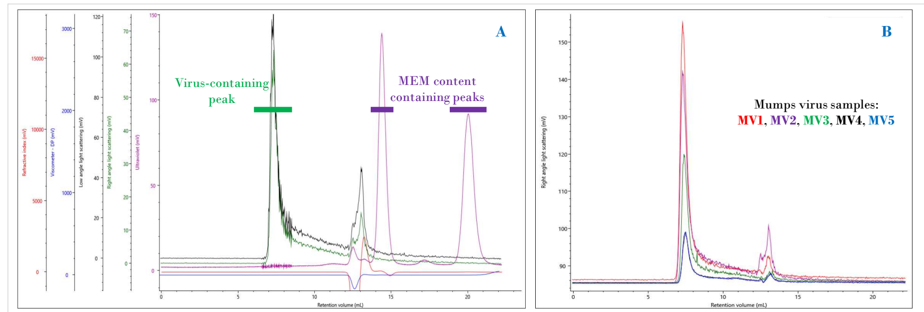

Figure 5. Representative multi-detector SEC chromatogram of MV2 sample. SEC was performed on TSKGel G4000PW column (7.5 × 300 mm; Tosoh Bioscience, Japan) in 0.1 M phosphate–sulphate running buffer, pH 6.6 at a flow rate of 0.8 mL/min and at RT on a HPLC system (Shimadzu, Japan). Advanced detection was obtained on Omnisec Reveal (Malvern Panalytical Ltd., United Kingdom) multi-detector module (refractive index, right angle light scattering, low angle light scattering, viscometer, ultra violet detectors) (A). RALS-detected virus peak variability in five MV samples (B).

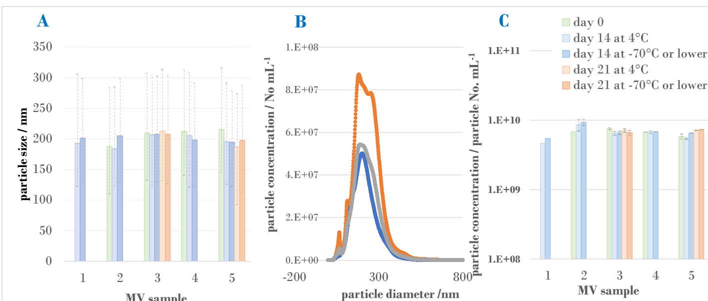

Figure 3. Total particle size and concentration. Average particle size (column height) and the size distribution range (vertical bars) in MV samples during the storage at two temperatures (A); typical particle size distribution chart for three MV samples (B); average particle concentration in MV samples during the storage at two temperatures (C).

Figure 6. Infective virus quantity decrease in individual MV samples during the storage at two temperatures for 21 day presented as the decrease in virus titre ($logCCID_{50}$ mL^{-1}) (A) and as the titre share in initial titre (%) (B). Average decrease of infective virus titre (column height) and 95% confidence interval (vertical bars) calculated from data from 5 individual experiments (C).

RESULTS

The established laboratory-scale production was reproducible in consecutive preparation of MV samples, free from bacterial or fungal contaminants. Infective virus content was 7.63±0.22 $logCCID_{50}$ mL^{-1}, while total particle content was $6.7 \times 10^9 \pm 1.1 \times 10^9$ mL^{-1}. Eighty percent of the particles was within the diameter range 132-306 nm, with the dominant population of 206 nm in diameter. The MV samples contained 40.9±3.3 μg mL^{-1} HCP and 602±338 ng mL^{-1} hcDNA. The non-infected control cultures also contained particles but in significantly lower quantity (2.5×10^8 mL^{-1}) and of smaller size (87% of the size of the most abundant particle population in MV samples). Control samples contained up to 8-times lower quantity of HCP and hcDNA – 4.4 μg mL^{-1} and 97 ng mL^{-1}, respectively. The chromatogram in size exclusion chromatography revealed the virus peak in the void volume of the column which was visible only under light-scattering detectors, while UV detector did not detect any change in chromatogram in comparison to the pure culture medium or control samples. Differences in virus peak area size have been noticed between different samples, but their full meaning has yet to be resolved. RNA sequencing data have demonstrated genomic stability of viral populations during the production process. Identical consensus sequence was obtained for all MV samples, their variability was low and comparable. Although DVGs were predicted bioinformatically in the NGS datasets of MV1-MV4, their presence was supported with low count numbers. The storage for 21 days induced a loss in virus infectivity, less pronounced at 4 °C (50%) than at lower temperature (65%), while other parameters were not changed.

CONCLUSION

The methodology developed and data generated provide a deep insight into the composition of oncolytic MV preparation required for the discussion with the regulatory authorities on its appropriateness for the use in veterinary medicine to treat the tumours. The data may contribute to the decision-making process of the medical regulatory agencies to determine future quality requirements for these new and fast-developing novel therapeutics.

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