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Development of methods for production of new molecular anti-viral vaccines and suppression of non-wished virus genes by nucleotide fragments transfer and nucleotide modifications

Background

Taking in consideration the risk of thrombs by SARS-CoV-2 Spike (S) protein (because of its binding to cellular ACE2) and of amyloid brain plaques by its nucleocapsid (N) protein, it is necessary molecular (DNA, RNA and/or protein) vaccines be designed against other viral proteins, and boosting by siRNAs against virus genes, coding proteins S and N.

Materials and Methods

***In vitro*-incubated mammalian cells were inoculated with vaccine avipoxviral strains (*Poxviridae* family) FK (fowl) and Dessau (pigeon) with initial infectious titers 10^4CCID_{50}/ml and $10^{6.5}CCID_{50}$/ml, respectively (in which were observed 50% cytopathological changes in the infected cells). Separate subpopulations of the so inoculated mammalian cells were freezed in the presence of cryoprotector Dimethylsulfoxide (DMSO), and others - with different concentrations of the methylxantine/purine analogs (Aminophylin and 61-Tartrate), 24 hours after the viral inoculation, for 24 and 48 hours. After thawing and reincubation, as source of extracellular virus served the cultural fluids, and of intracellular – the scraped cellular monolayers.**

Results

The titers of the intracellular forms were significantly higher that these of the extracellular (Fig. 1).

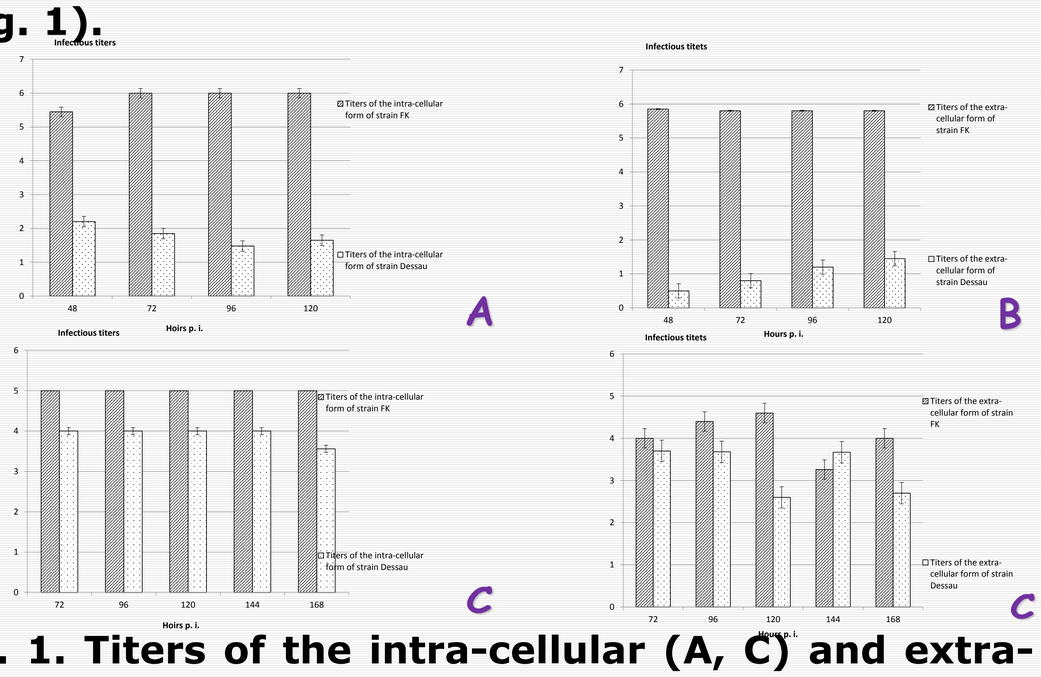

Fig. 1. Titers of the intra-cellular (A, C) and extra-cellular (B, D) forms of the vaccine avipoxviral strains FK and Dessau, incubated in avian embryonic cells from DEC 99 cell line (A, B) mammalian embryonic cells from EBTr cell line (C, D).

Transfer of nucleotide (DNA and/or RNA) fragments between virus and cellular genomes due to activated fusion on the influence of DMSO plus drastic temperature changes was proposed, which suggests possibilities for production of molecular vaccines and suppression of nonwished genes. 50% cell viability on the influence of both methylxantine derivatives on *in vitro*-incubated cells was reached at dilution values between 10^{-4} M/ml in the cells, inoculated with strain FK, and 10^{-3} M/ml in the cells, inoculated with strain Dessau, at the 24-th hour after the virus inoculation. These results show the purine analogs antiviral activity, by taking in consideration the observed higher virulency of strain FK (in higher dilutions of the viral suspension) compared with strain Dessau.

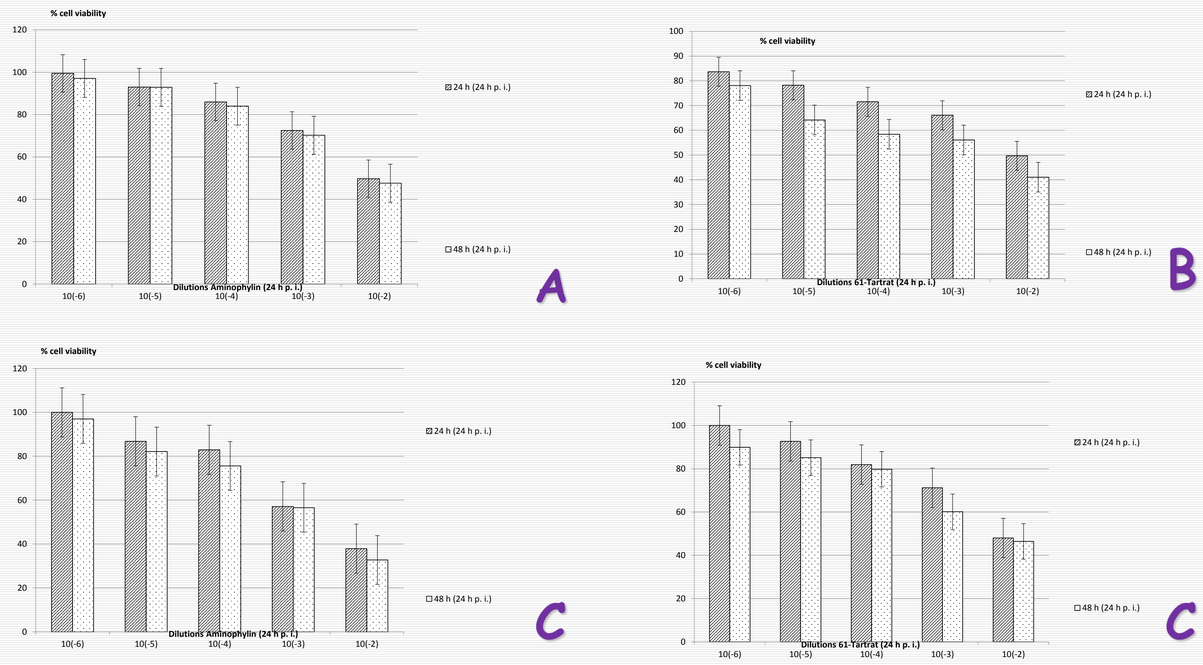

Fig. 2. Cytotoxic and anti-viral activity of methylxantine/purine derivatives at the 24-th and 48-th hours of the treatment of cell cultures, 24 hours after virus inoculation of the *in vitro*-incubated cells: A, B from the mammalian embryonic cell line EBTr (A, B); from the avian embryonic cell line DEC 99 (C); with Aminophylin (A, C); with 61-Tartrat (B, D).