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Antiviral activity of polyhydrated fullerenes against influenza A virus H1N1

Polina Zaremba [1,*], Krystyna Naumenko [1], Svitlana Zahorodnia [1] and Mykhailo Yelipashev [2]

[1] Zabolotny Institute of Microbiology and Virology of NASU, 154 Acad. Zabolotny Str., Kyiv, 03143, Ukraine;
[2] Private Research Laboratory Yelipashev, Kyiv, Ukraine.

* Corresponding author: polinakarpets@gmail.com

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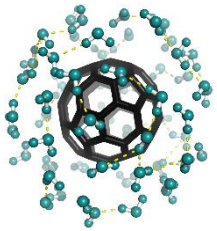
Graphical Abstract

K050

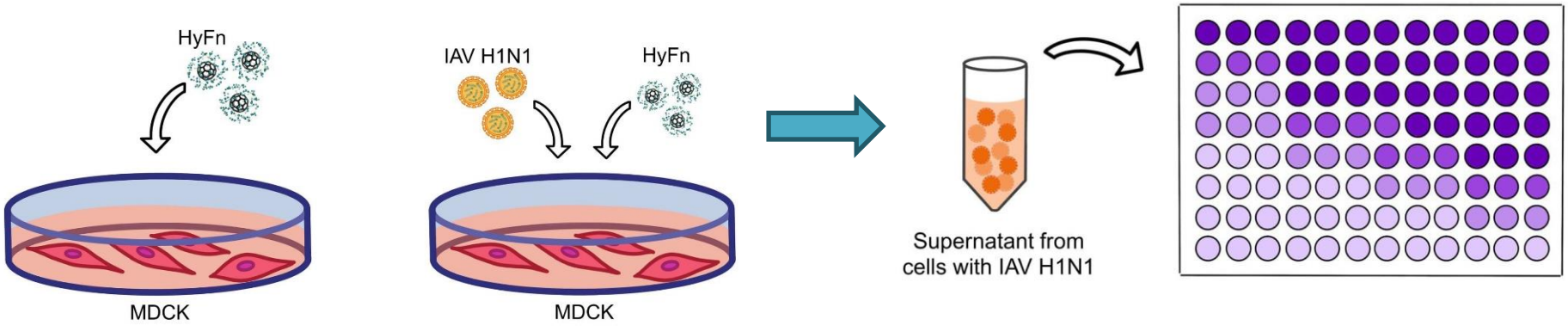


(C60/C70/C76/C78/C84)(ONa)20(OH)40x12H2O

Cytotoxicity

Antiviral activity

Titration and calculation of TCID50



Abstract:

Today, the development of drugs against influenza viruses remains an urgent problem. Hydrated fullerenes have high stability and relatively low toxicity, which undoubtedly makes them attractive candidates for the role of antiviral agents.

Therefore, the aim of this work was to study the cytotoxicity and antiviral activity of the drug K050 (manufactured in Ukraine) - an aqueous colloidal solution of a polyhydrated mixture of fullerenes - HyFn5 and HyFn6 against influenza A virus H1N1. Cell culture MDCK was used in the experiments. Cytotoxicity was determined using the MTT assay. *De novo* infectivity of influenza virus was determined by Median Tissue Culture Infectious Dose assay, TCID50 titers were calculated by the Reed-Muench method.

The cytotoxicity of HyFn5 and HyFn6 was 11.79 mg/ml and 16.03 mg/ml, respectively. Oseltamivir at a concentration of 0.15 mg/ml was used as a reference drug and showed 22.33% of inhibition of viral activity (or AVA). Hydrated fullerenes showed a high dose-dependent effect, with the highest rates of AVA by 96.48% for HyFn5 and 96.54% for HyFn6. A study of *de novo* virus infectivity showed a decrease in titer, compared with the virus control with the largest reduction by 4 orders of magnitude for both HyFn5 and HyFn6.

Therefore, the obtained results show that polyhydrated fullerenes show not only high activity in inhibiting the reproduction of influenza virus type H1N1, but also a decrease in its infectious titer. This gives a good start for further research and determination of the mechanisms of action of these fullerenes.

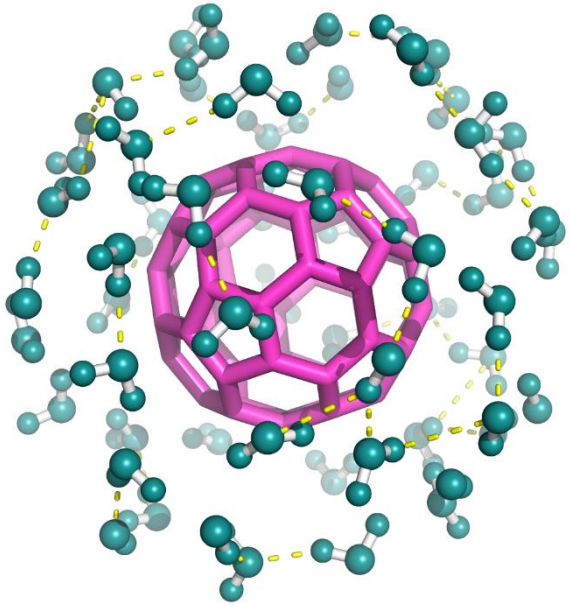
Keywords: antiviral agents; hydrated fullerenes; influenza virus; nanomaterials.

Introduction

Fullerenes and their derivatives show inhibition of the activity of many viruses, including RNA-containing ones[1]. These nanostructures attract the attention of many researchers, in particular because of their properties.

K050 - an aqueous colloidal solution of a polyhydrated mixture of fullerenes with a mass ratio of 78,1% C60/C70 and 21,9% fractions of higher fullerenes C76/C78/C84 (according to patent UAN№124328).

In this study we investigated the antiviral activity of two pools of the drug K050: HyFn5 and HyFn6.



[1] Innocenzi P, Stagi L. Chem Sci. 2020;11(26):6606-6622.

Results and discussion

The cytotoxicity of hydrated fullerenes was determined using the MTT method on cell culture MDCK, the total incubation period was 48 hours.

Table 1. The cytoxicity of substances

Compound	CC50, mg/ml
HyFn5	11,79±0,58
HyFn6	16,03±0,8
Oseltamivir phosphate	0,29±0,01

Based on the obtained results, the CC50 indexes were calculated.

Oseltamivir phosphate (further – oseltamivir) was used as a reference drug active against influenza A virus H1N1.

Results and discussion

The determination of antiviral activity was carried out according to the post-expository scheme: the substances were added after the previous cell incubation with the virus, the visualization was carried out using gentian violet.

Fullerenes demonstrate a high percentage of inhibition of the viral reproduction, as well as the dose-dependent effect.

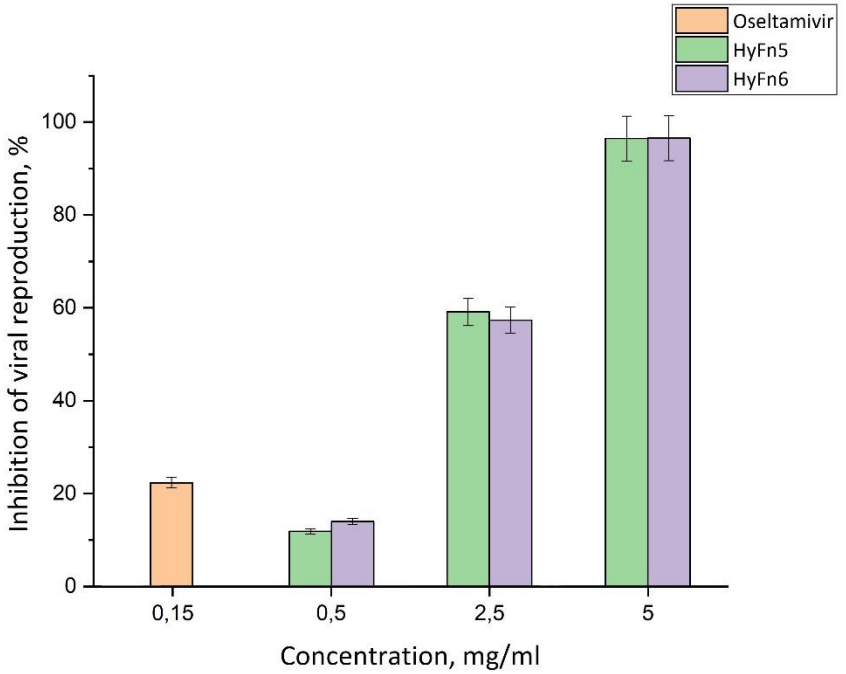


Fig. 1. Antiviral activity of HyFn5 and HyFn6 against Influenza A virus H1N1

Results and discussion

Study of the infectivity of the virus *de novo* after antiviral activity showed a decrease in titer, compared with the control of the virus (TCID50/50 µl - $4{,}1x10^{10}$):
for HyFn5 titers were from $3.88x10^{6}$ to $3{,}36x10^{8}$, and for HyFn6 - from $3{,}45x10^{6}$ to $2{,}53x10^{8}$. For Ozeltamir value TCID50/50 µl was $2.98x10^{7}$.

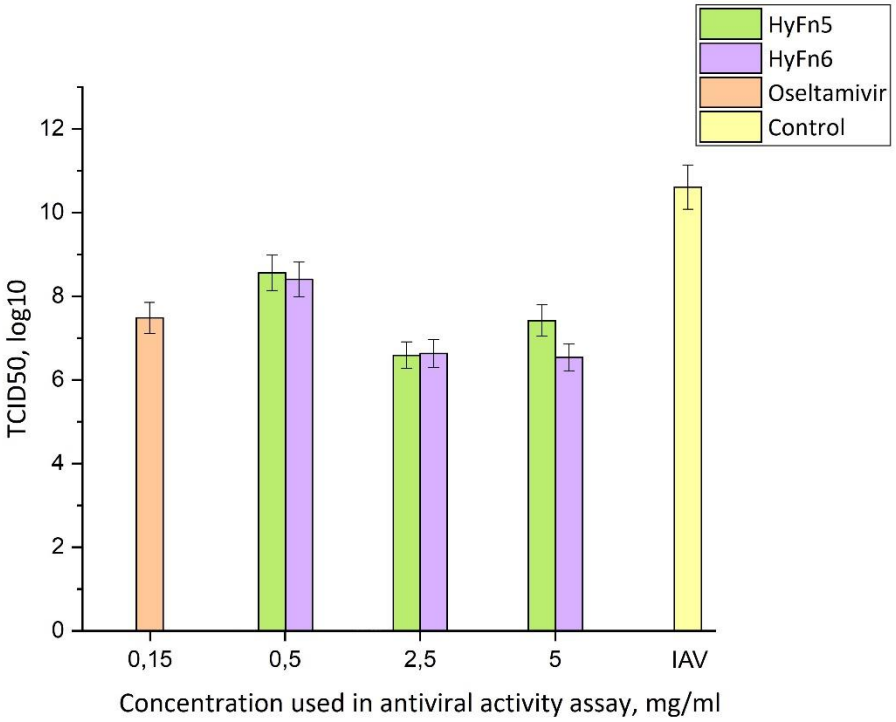


Fig. 2. Infectious titers of the virus *de novo*

Conclusions

- Pools of hydrated fullerenes HyFn5 and HyFn6 show low toxicity and high dose-dependent antiviral effect against influenza virus, compared to oseltamivir phosphate.

- They also reduce the infectious titer of the virus up to 4 orders of magnitude.

- Drug K050 is promising for further research as an antiviral agent against influenza A virus.