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Anti-influenza Activity of Dihydroquercetin Against Lethal Influenza Virus Infection

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Background: Influenza virus infection represents a serious challenge for medical science and health protection activity over the world. Due to fast selection of drug-resistant strains, in addition to direct anti-viral compounds, other agents like anti-inflammatory agents, antioxidants should be used, especially in cases of severe influenza. The aim of the present study was investigation of the protective activity of dihydroquercetin (DHQ), the flavonoid from larch (*Larix sibirica* L.) wood, on lethal influenza virus infection in white mice.

Materials and methods: DHQ was extracted from larch heartwood and identified by HPLC assay. Mice were infected with influenza virus A/Aichi/2/68 (H3N2). DHQ was applied orally either after infection (therapeutic schedule), or both before and after infection (combined schedule). Each group was checked daily for dead animals for two weeks post inoculation. Based on the data received, percent of mortality and index of protection were calculated. On day 3, p.i. infectious activity of the virus was determined in lung tissue by titration in MDCK cells.

Results: Application of DHQ resulted in a dose-dependent decrease in mortality, up to 57.1 or 85.7% depending on the dose of compound, comparing to 68.8% for rimantadine. The mean day of death in the DHQ-treated animals was later than in control animals without drug treatment. The combined schedule of DHQ application appeared more effective for reducing the viral infection and morbidity (approximately 1.8-fold) compared to the therapeutic schedule that was started after virus inoculation. The infectious virus replicated in the lung tissue up to $10^{6.3}$ – $10^{6.7}$ EID₅₀/20 mg tissue. Application of rimantadine decreased the viral titer approximately 100-fold ($10^{4.3}$ – $10^{4.7}$ EID₅₀/20 mg tissue). Treatment of animals with DHQ led to a slight decrease in virus replication (maximum 10-fold). This effect correlated with the dose of the DHQ compound and its protective activity when applied by different schedules, although the effect did not exceed the activity of rimantadine.

Conclusion: Based on low toxicity and high protective activity of DHQ, it can be considered as a prospective tool to be used in complex prophylaxis and/or treatment of influenza, in particular in severe cases.

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Anti-viral Activity of Ingavirin (Imidazolyl Ethanamide Pentandioic Acid) Against Lethal Influenza Infection Caused by Pandemic Strain A/California/07/09 (H1N1)v in White Mice

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Background: In 2009 a new pandemic influenza virus appeared, that spread to more than 206 countries and caused more than 6250 deaths. In this regard, chemotherapy of influenza is one of the most important priorities for health protection. The main purpose of the

present study was to evaluate the activity of Ingavirin (pentadionic acid imidazolylethanamide) against pandemic influenza virus *in vivo*.

Materials and methods: Influenza virus A/California/07/09 (H1N1)v was passaged several times through mouse lungs. The 50% lethal dose (LD₅₀) of the final virus was determined. Mice were then inoculated with the virus and treated with Ingavirin either by prophylactic (five days prior to infecting) or therapeutic (five days beginning on day one after infection) schedule. Animals were monitored for mortality for 15 days. On day 3, p.i. virus titer in lung tissue was determined by titration in MDCK cells.

Results: Mice-adapted pandemic influenza virus A/California/07/09 (H1N1)v caused a lethal infection with ataxia, tremor and weight loss. In lungs of animals on day 3–4 p.i. hemorrhagic edema with intense cell infiltration was observed. Bronchial epithelium was destructed with denudation of basal membrane. Fatal cases started on day 3–4 p.i. depending on the dose of the virus. Ingavirin was the most effective when applied at 3–5 mg per kg body weight prior to inoculation. In this case it reduced mortality up to 82% comparing to control. Mean day of death in treated animals was 3.8 days later. When applied in therapeutic schedule, at dose 20 mg/kg it reduced mortality to 40% and prolonged a life of mice 3.1 days comparing to placebo-treated group. Virus titer in lung tissue of Ingavirin-treated mice was lower than in control group (3.4 and 5.5 log₁₀TCID₅₀/20 mg tissue, respectively). It correlated with mortality decrease depending on the dose of the compound and schedule of application.

Conclusion: Ingavirin is effective against current pandemic strains of influenza virus. Based on pattern of its activity *in vivo*, it has a mechanism of anti-viral action different from those of currently available antivirals and might be promising anti-influenza drug both as therapeutic and prophylactic agent.

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Activity of a Novel Fullerene-based Antiviral Against Influenza Virus Infection *In Vitro* and *In Vivo*

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Background: The main purpose of the present study was to evaluate a protective activity of newly synthesized water-soluble derivative of fullerene, fullerene-polyaminocaproic acid (FPAC), against influenza virus in cell culture and *in vivo* experiments.

Materials and methods: Toxicity of FPAC was determined by microtetrazolium test (MTT). Four strains of influenza virus, including A(H3N2), A(H5N2), A(H5N3) and B were cultivated in MDCK cells in presence of various concentrations of FPAC. Virus titer was determined for each concentration of FPAC based on the study of virus-induced cell destruction after 48 h of cultivation by MTT. Virus titer was then plotted against FPAC concentration, and EC₅₀ was calculated. For *in vivo* experiments mice were inoculated with influenza virus A/Aichi/2/68 (H3N2) and treated with FPAC intraperitoneally. Animals were monitored for 14 days. On day 3 post-inoculation their lungs were studied for virus titer in the tissue and virus-induced lesions by morphology analysis.

Results: CTD₅₀ and EC₅₀ of FPAC were estimated as >1000 and 300–500 µg per mL, respectively, depending on the strain of the virus, that gives a selectivity index 2–3, suggesting lack of antiviral activity. Reference compound Rimantadine appeared effective against all strains used, except flu B. Despite lack of activity in