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In Vitro Inhibitory Effects of Combinations of Picornavirus Replication Inhibitors

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To assess the possible interactions among picornavirus replication inhibitors dual combinations of enviroxime, disoxaril, arildone, S-7, guanidine hydrochloride, PTU-23 and HBB were tested against poliovirus type 1 (Mahoney) replication in FL cells. Beforehand, the individual 50% inhibitory concentration (IC_{50}) in the plaque inhibition test has been determined for each compound. Each of the dual combinations with enviroxime or HBB being one of the partners, showed synergistic or additive effects. Combining disoxaril with enviroxime, HBB or PTU-23 resulted in synergistic effects, while combining it with guanidine hydrochloride, S-7 or arildone revealed antagonistic ones. Arildone showed additive and synergistic combined effects with enviroxime, HBB and PTU-23 and antagonistic ones with disoxaril, S-7 and guanidine. All dual combinations of PTU-23 were synergistic with the exception of the pair PTU-23 and guanidine that showed an antagonistic effect. Guanidine revealed additive to synergistic interactions with HBB and enviroxime but antagonistic ones with disoxaril, arildone and PTU-23. Guanidine hydrochloride or PTU-23 when combined with S-7 showed unusual combined effects – synergistic with an antagonistic zone. The combinations of S-7 with enviroxime or HBB were synergistic but those with disoxaril or arildone were antagonistic ones. Research on combined interactions of picornavirus replication inhibitors could possibly contribute to the development of efficient chemotherapy of infectious diseases caused by picornaviruses, as well as to the better understanding of the mode of action of those inhibitors.

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Development of Resistance to Disoxaril in Coxsackievirus B1 Infected Newborn Mice

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In accordance with our previous data [L. Nikolaeva, A. S. Galabov, Acta Virologica, in press], disoxaril, administered s.c. in a course of 10 days post virus inoculation at a daily dose of 25 mg/kg in newborn albino mice (ICR line),, infected with coxsackievirus B1 (3-5 LD_{50}/mouse s.c.), manifested a marked antiviral effect. A lengthening of the mean survival time and some decrease of the mortality rate were found. Measurment of viral infectious titer in the mouse brain samples taken every day since the 2nd day post virus inoculation revealed a strong decrease (>2 $\log_{10}$ $CCID_{50}$) of the virus titer as compared to the control (placebo) group till the 7th day included. Then, a sharp increase of the infectious virus content on days 8th and 9th was recorded. The latter coincided chronologically with a presence of a low-titer virus or a lack of infectious virus in brain samples of mice from the placebo group consisted at that time of convalescent or healthy animals. Analysis carried out on susceptibility to disoxaril of viral isolates from mouse brain taken on days 8th and 9th post infection showed a presence of resistant progeny. These data could explain the moderate protective effect of disoxaril *in vivo* in contrast to its strong antiviral activity when tested in cell culture experiments.

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Effect of 6-Azacytidine to Experimental Adenoviral Infection.

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OBJECTIVE. The adenoviral infection is widely distributed human pathology causing respiratory and ocular disorders. There is no specific antiviral chemopreparate for treatment of it. We tested the nucleoside analog 6-azacytidine (6-AC) for its` ability to restrict the adenoviral infection in cell culture and in newborn syrian hamsters. METHODS. Hep-2 cells were infected with adenovirus type 5 (strain Ad 75) and the percentage of cells with viral inclusions was evaluated in presence of 6-AC. Immunoperoxydase staining was performed on these cells to reveal viral hexon protein. Newborn syrian hamsters were infected subcutaneously with 0,1 ml (10^6 inclusion-forming units) of the virus. 6-AC (50 mg/kg) was applied subcutaneously once a day for 3 days after infection. Virus titers were determinated in liver, spleen, kidney, heart, thymus and lungs of animals. Histological analysis of these organs was carried out for signs of viral lesions. RESULTS. The use of 6-AC (8 micrograms/ml) led to significant decrease both of cytotoxic effect of the adenovirus and hexon- positive cells percentage (80 and 88 % of inhibition, respectively). Infective virus was found in the liver, kidney and heart of syrian hamsters (up to 13, 7 and 7 days p.i., respectively). The application of 6-AC resulted in the shortened time of virus presence (7 days in the liver and 4 days in kidney and heart) and lowered virus titers on day 3 p.i. (liver - 2,7 and 4,1, heart - 0 and 3,2, kidney - 0 and 2.4 lg CTD_{50}, in presence and abcence of 6-AC, respectively). CONCLUSIONS. 6-AC or 6-AC-based substances are highly perspective for antiadenoviral drugs development.

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Activity of Zn-finger inhibitors against the arenaviruses Junin, Tacaribe and Pichinde. E.B. Damonte, C.C. García, N.A. Candurra. Laboratorio de Virología, Dpto. Química Biológica, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, 1428 Buenos Aires, Argentina.

Fifteen antiretroviral Zn-finger active compounds with diverse chemical structures, including azoic compounds, hydrazide derivatives, disulfide-based reagents and others, provided by the National Cancer Institute (Frederick, USA) were screened in vitro against Junin virus (JUNV), the etiological agent of Argentine hemorrhagic fever, by a virus yield inhibition assay in Vero cells. Cytotoxicity was evaluated simultaneously by the MTT method. From the total of compounds, three were totally inactive as antivirals, nine presented moderate anti JUNV-activity and three were truly active with EC_{50} (effective concentration 50%) values in the range 6.5-9.3 μM. The most active inhibitors demonstrated similar or higher efficacy and selectivity in vitro than ribavirin, the only drug known to be of any benefit in the treatment of patients with arenavirus infection. These agents, named NSC20625, 3-7 and 2-71, demonstrated a broad range of action against arenaviruses, including several attenuated and pathogenic strains of JUNV as well as the antigenically related Tacaribe virus (TACV) and Pichinde virus (PICV). The direct treatment of JUNV and TACV virions with the compounds has shown two types of behavior, with a lack of direct correlation between antiviral effect in infected cells and virus inactivating ability of these agents: the aromatic disulfide NSC20625 was a very potent virucidal agent, with IC_{50} (inactivating concentration 50%) values greatly exceeding the EC_{50}, whereas the other two compounds exhibited moderate or negligible virus inactivating properties.