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In Vitro Investigation of Cytotoxic Action of Hemocyanins on Cell Cultures

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Molluscan hemocyanins have particular interest due to their significant immunostimulatory properties. Besides, anti HSV-1 activity of these preparations have been shown recently (Pagano and Gershburg, 2005). Epstein Barr virus (family *Herpesveridae*) is essential for different medicine spheres, but the drugs that have antiEBV activity are limited by Gancyclovir and Acyclovir (Velkova et al., 2009). The purpose of our work is study of the native hemocyanins from *Rapana venosa* (RvH) and *Helix vulgaris* (HvH) and their isoforms as substances with feasible antiEBV activity. Cytotoxic action of hemocyanins (HvH, RvH) on cell cultures was investigated in vitro. The following cultures of cells have been used in the work: B95-8 – leukocytes of monkeys-marmaset, transformed by EBV, Raji – human B-lymphocytes, which produce only separate early antigenes, but not virus particles, Namalwa – human B-lymphocytes. Influence of hemocyanins on viability and proliferative activity of lymphoblastoid cells was characterized by cytomorphological and colorimetric methods. Viability of cell cultures was determined by staining of them with 0.4% tripan blue ("Sigma", USA) which was used for revealing dead cells. Proliferative activity of lymphoblastoid Raji cells was studied with use of MTT-assay ("Sigma", USA). Analysis was carried out within concentrations from 2000 μg/ml to100 μg/ml of RvH, HvH and structural subunits RvH1, RvH2, HvH1 and HvH2. Concentrations of the investigated substances which caused 50% inhibition of viability of cells that are CC_{50} are submitted in the table. Table—Cytotoxic concentration of tested hemocyanins in different cultures of lymphoblastoid cells.

Cells	CC_{50} (μg/ml)			
	RvH1	RvH2	HvH1	HvH2
Raji	720	700	260	50
Namalwa	1358	1000	185	355
B95-8	1352	709	255	705

Thus, cytotoxicity of hemocyanins in several cell cultures of B-phenotype was defined. Low toxic hemocyanins are selected for investigation of anti EBV activity.

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The Antienteroviral Effect of Oxoglaucine and Phenotypic Characterization of the Oxoglaucine Resistant Mutant of Coxsackievirus B1

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The medicinal and economic impact of enteroviral infections imposes the search of safe and specific inhibitors of enterovirus replication. Oxoglaucine (OG), an aporphinoid alkaloid isolated from Glaucium flavum Crantz, which can also be obtained synthetically, possesses a promising antiviral effect against the replication of a panel of 15 tested enteroviruses. The selectivity index (SI), defined as the ratio between the 50% inhibitory concentration (IC_{50}) and the 50% cytotoxic concentration (CC_{50}), both determined in the CPE-inhibition test, ranges from 20 to above 200 depending on the virus. Time of addition study in the one-step virus growth cycle set-up reveals strong inhibition during the early periods of virus replication. Since growth of resistant virus is considered as an indicator of specific antiviral activity, OG-resistant progeny was developed *in vitro* for poliovirus 1 and the six coxsackieviruses B. Viruses develop rapidly phenotypic signs of resistance. A correlation is established between the sensitivity to OG and the necessary number of serial passages for the selection of resistant mutants. The more sensitive the virus to the antiviral effect, the faster the selection of resistant progeny. The phenotypic characteristics of the OG-resistant mutant of coxsackievirus B1 (CV-B1), selected after 20 consecutive passages in increasing concentrations of OG, are determined. The resistant CV-B1 possesses a lower infectious titer in comparison to the ancestral strain. The resistance index, defined as the ratio between the 90% effective concentration (EC_{90}) of OG for the resistant virus and EC_{90} for the ancestral strain, both determined in the virus yield reduction essay, as well as the sensitivity index, which is the titer in the presence of drug divided by that in its absence, and the IC_{50} for the virus from each consecutive passage are determined and the gradual increase of the rate of the relative resistance is established. The selected OG-resistant CV-B1 progeny will further serve as a tool for understanding the mechanism of antiviral action of OG.

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The Disease Course and Host's Response to Mousepox is Dependent on Inoculation Route

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The pathogenesis of an infectious agent is greatly affected by its route of infection. Variola virus (VARV) causes a systemic, fulminant disease following a respiratory tract infection with a case-fatality rate of 10–30%. In contrast, infection through the skin results in a systemic infection, but with a milder disease course and case-fatality rate of <1%. Therefore, it is important that the route of infection and the challenge virus used to evaluate antivirals reca-