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Use of Drug Combinations for Selection of Human Immunodeficiency Virus Type 1 (HIV-1) Variants with Decreased Sensitivity to Hydroxyethylurea Isostere Containing Protease Inhibitors *in vitro*.

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G.D. Searle's unique hydroxyethylurea isostere protease inhibitors SC-52151 and SC-55389A were used to select drug resistant HIV-1 variants *in vitro*. Clinical and laboratory HIV-1 strains were passaged in T cell lines or peripheral blood mononuclear cells (PBMCs) in the presence of single drugs or drug combinations. Combination treatments were made either simultaneously or sequentially. Resistant variants were defined by EC_{50} values at least 10-fold higher than control virus passaged for an identical period. Proviral DNA was amplified by the PCR and the nucleotide sequence of the protease gene was analyzed from multiple subclones. In all cases, sequential drug combinations selected for resistance to both drugs. HIV_{RF} selected for resistant to SC-52151 consistantly had amino acid substitutions G48V and V82A, and with the sequential addition of SC-55389A, a majority of clones also exhibited L63P, and in some cases I54T. A clinical isolate grown in the presence of SC-55389A had one amino acid substitution N88S, and with the sequential addition of SC-52151, gained T31I. Modelling studies based on templates derived from high resolution x-ray structures of prototypical hydroxyethylurea inhibitors bound to recombinant HIV-1 protease suggest that the amino acid at position 88 can form essential H-bond interactions with the amino acids at positions 31 and 29 which form the S2/S2' inhibitor binding subsites. Clinical and laboratory strains that were resistant to either single drug did not revert to wild type when grown in the absence of the drug for at least 8 weeks, suggesting that these variants may become a stable component of the population. The observed changes in the protease gene were recreated in proviral clones, and evaluated for growth characteristics and cross resistance to other asymmetric protease inhibitors. SC-52151 and SC-55389A were also used simultaneously to treat wild-type clinical and laboratory strains. Although multiple simultaneous treatment selections were performed under various conditions, no resistant variants were isolated.

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COMBINED ANTIVIRAL EFFECT OF FLAVONOID AND ACYCLOVIR

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The combined antiviral activity of flavonoids and acyclovir was studied on the multiplication of herpes simplex virus types 1 and 2 in HEp-2 cells and on pseudorabies (Aujeszky) virus in chick embryo fibroblasts by the yield reduction method. Antiviral effects of flavonoids have been described both **in vitro** and **in vivo** against herpesviruses. Additive and synergistic antiviral effects have been observed with the combination of quercetin and 5-ethyl-2'-deoxyuridine in cell cultures. The present study deals with the **in vitro** antiviral effect of the combination of flavonoids and acyclovir. Simultaneous application of flavonoids (quercetin, quercitrin, apigenin) with acyclovir resulted in synergistic antiviral activity against herpesviruses. Quercetin did not influence the development of resistance of herpes simplex virus type 1 to acyclovir.