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Use of Stepwise Linear Regression Combined with Analysis of Covariance to Analyze Drug Combination Interactions. S. Chiu, S. Michelson, V.R. Freitas, E.B. Fraser-Smith, and R.C. Schatzman, Syntex Research, Palo Alto, California, U.S.A.

A new technique has been developed to investigate drug combination interactions in in vitro anti-viral assays. This technique is able to examine whether a drug combination is acting additively, synergistically or antagonistically as defined by plaque reduction or cell toxicity. A stepwise linear regression is used for the additive model of drug-drug interaction; it is compared to a synergy model which also includes a term to account for combination effects. In addition, an analysis of covariance (ANCOVA) is performed to test for heterogeneity of slopes in the linear segment of the dose response curve. Examination of the changes in both the slopes and the y-intercepts of the fitted lines gives insight into the interaction of the drugs. This procedure offers a powerful tool for the analysis of drug interactions, and is more conservative than standard techniques, by first detecting consistent supra-additive or -antagonistic effects and then enabling examination of individual combinations as desired. These techniques provide a more accurate method of detecting the extent of drug interactions when compared with current technology. 3-D graphics and contour plots provide a graphic presentation that is easily interpretable. This procedure can be implemented on personal computers running SAS or other software containing stepwise linear regression and ANCOVA.

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In Vitro Efficacy of Ganciclovir Alone or in Combination with Zidovudine Against Human Cytomegalovirus. V.R. Freitas, E.B. Fraser-Smith, S. Chiu, S. Michelson, and R.C. Schatzman, Syntex Research, Palo Alto, California, U.S.A.

These studies were designed to determine the in vitro efficacy of ganciclovir (GCV) alone or in combination with zidovudine (AZT) against human cytomegalovirus (HCMV). In plaque assays, GCV alone (5-30μM) had good activity against HCMV (IC50 8-9μM), while AZT (25-800μM) was relatively inactive (IC50 508- >800μM). In combination, AZT and GCV had an additive effect on plaque reduction. In MTT cytotoxicity assays, both GCV and AZT alone were only slightly toxic to confluent uninfected MRC-5 cells ($\leq$18 and $\leq$28% reduction, respectively, at the highest drug concentrations tested). In proliferating cells, cytotoxicity of GCV remained low while AZT had an increased cytotoxicity ($\leq$16 and $\leq$46% reduction, respectively); in combination, additive effects were observed. When differences in antiviral activity and cell toxicity were compared using stepwise linear regression coupled with analysis of covariance, the results were biphasic. With GCV alone and in combination with 25-200μM AZT, a large increase in the slope of the difference curve was seen with increasing doses, indicating HCMV killing increased rapidly with minimal cell toxicity. With GCV in combination with 200-800μM AZT, a slight decrease in slope with a large offset was seen, indicating all virus had been killed with only small reductions in the cell metabolism of MTT. Comparison of plaque reduction with cell toxicity at all GCV and AZT combinations showed an additive rather than a synergistic or antagonistic effect on antiviral activity.