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Benzimidazole Ribonucleosides: *In Vitro* Immune Function Effects of TCRB. M.R. Nassiri, D.E. Lopatin, N. Van Poperin, L.B. Townsend, and J.C. Drach. University of Michigan, Ann Arbor, Michigan 48109, U.S.A.

An assessment of the effects on immunological parameters is important when evaluating drugs which may be used to treat immunosuppressed patients. Evaluation of the effects on lymphocyte transformation or on natural killer cell (NK) activity provides a means of examining such compounds *in vitro*. Lymphocyte transformation is a process whereby new DNA synthesis and cell proliferation occurs in lymphocytes which have been stimulated with appropriate mitogen. NK activity is an innate cytotoxic activity, mediated by cells characterized as large granular lymphocytes, and appears to play an important role in immunosurveillance against neoplastic and infectious diseases. In this study, we examined the inhibitory effects of TCRB on lymphocyte transformation and on the cytolytic activity of NK cells in order to reveal potential detrimental effects of this compound. The two plant lectins, concanavalin A and pokeweed mitogen were used to stimulate human peripheral blood mononuclear cells (PBMC) in the absence or presence of TCRB and ganciclovir. [^{3}H]Thymidine was added to the cultures prior to harvest to measure the biosynthesis of cellular DNA. The concentration of compound that resulted in 50% inhibition of [3H]thymidine was identified (IC_{50}). For both compounds and both lectins, the IC_{50} values exceeded 100 μM. NK assays were performed by adding human PBMC (effector cells) to ^{51}Cr-labeled K-562 human erythroleukemic cells (target cells) at effector to target ratios ranging from 5:1 to 25:1. The activity of effector cells was assessed by adding TCRB and ganciclovir to the cell mixtures prior to incubation for 4 hours at 37°C. Cytotoxicity was determined by comparing the chromium released by the treated cell categories to the control (target and effector cells only). As found with lymphocyte transformation, the IC_{50} for both TCRB and ganciclovir were in excess of 100 μM. These findings show that TCRB is relatively non-inhibitory to both lymphocyte transformation and NK function *in vitro*, and suggest that it may not adversely affect immune functions *in vivo*. This study was supported by contracts N01-AI42554, N01-AI72641 and grant U01-AI31718 from N.I.A.I.D.

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Benzimidazole Ribonucleosides: Antiviral Activity and Cytotoxicity of Combinations of TCRB with Ganciclovir, Acyclovir, and Zidovudine. S.R. Turk, M.S. Ludwig, E.D. Kreske, M.N. Prichard, M.R. Nassiri, C. Shipman, Jr., L.B. Townsend, and J.C. Drach. Departments of Biologic & Materials Sciences, School of Dentistry and Medicinal Chemistry, College of Pharmacy, University of Michigan, Ann Arbor, MI 48109, U.S.A.

Certain ribosyl benzimidazole nucleosides have been identified by us as possessing potent and preferential antiviral activity against human cytomegalovirus (HCMV). We report herein the antiviral activity and cytotoxicity of combinations of one of these compounds, TCRB [2,5,6-trichloro-1-(β-D-ribofuranosyl)benzimidazole], with acyclovir (ACV), ganciclovir (GCV), and zidovudine (AZT). Cytotoxicity was measured in uninfected KB cells using a microtiter dye binding assay for proliferating cells. Antiviral activity was evaluated using a microtiter antibody capture assay performed on fixed, HCMV-infected human foreskin fibroblasts. The three-dimensional analytical methodology of Prichard and Shipman (*Antiviral Res.* **14**: 181-206, 1990) was utilized to evaluate drug-drug interactions. This type of analysis graphically identifies peaks of synergy or antagonism and the particular drug concentrations at which these phenomena occur. Synergistic antiviral activity was observed when TCRB was combined with either ACV, GCV, or AZT. The TCRB:GCV combination exhibited the most significant peak of synergy, centered at 0.1 μM TCRB and 1 μM GCV. Surprisingly, this combination also revealed a smaller peak of antagonism at 30 μM TCRB and 30 μM GCV. The synergy observed with the TCRB:AZT pairing occurred only at relatively high (>100 μM) concentrations of AZT. In contrast, synergistic cytotoxicity was seldom noted with any of these combinations. In summary, these results indicate potential benefits of these drug combinations when used in the treatment of HCMV infections. This work was supported by contracts N01-AI42554, N01-AI72641 and grant U01-AI31718 from N.I.A.I.D.