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ORIGINAL ARTICLE

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Superiority of cyclosporin A over PSC-833 in enhancement of VP-16 efficacy in murine tumors in vivo

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Abstract PSC-833, a non immunosuppressive analogue of cyclosporin A, is an effective modulator of the multidrug-resistant tumor phenotype. Since both PSC-833 and cyclosporin A also enhance the cytotoxicity of VP-16 against drug sensitive L1210 leukemia cells in vitro we compared these agents as modulators of VP-16 efficacy in vivo. Compared to VP-16 treatment alone both PSC-833 and cyclosporin A significantly altered the survival of L1210 leukemia-bearing BDF/1 mice and Lewis lung carcinoma-bearing C57/Bl mice. Cyclosporin A enhanced VP-16 efficacy whereas PSC-833 impaired VP-16 efficacy against these murine tumors. Possible reasons for these disparate effects are discussed.

Key words Cyclosporin A · PSC-833 · VP-16

Introduction

Cyclosporin A (CsA) is a well-known modulator of multidrug resistance (MDR) [1, 2]. It also enhances the efficacy of VP-16 against drug-sensitive tumors [3]. PSC-833 is a nonimmunosuppressive analogue of CsA with MDR-modulating activity [4, 5]. Since we have recently observed that PSC-833 enhances the cytotoxicity of VP-16 against drug-sensitive L1210 leukemia cells in vitro [6], we compared its ability to enhance the efficacy of VP-16 in mice with L1210 leukemia and in mice with Lewis lung carcinoma (3LL) with that of CsA.

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Methods

Tumor lines

L1210 leukemia cells were maintained in vivo by sequential passage in host mice as previously described [3]. Lewis lung carcinoma cells (3LL) (kindly provided by Dr. V. Pollack, Pfizer, Groton, Ct.) were passaged by monolayer culture and harvested from exponentially growing cultures by detachment using light trypsinization.

In vivo treatment regimens

Experimental groups of ten or more female BDF/1 (L1210) or C57/Bl (3LL) mice were employed. Treatment regimens consisted of VP-16 at 5 mg/kg (L1210) intraperitoneally on days 1 and 3 or 30 mg/kg (3LL) intraperitoneally on day 1 either alone or with a chemotherapy modulator, after intraperitoneal inoculation of 10^5 L1210 leukemia cells or 10^6 3LL cells. In order to assess potential modulator toxicity, groups of additional control BDF/1 and C57/Bl mice without tumor were treated with VP-16 combined with PSC-833. The modulating agents CsA (Sandimmune, Sandoz Pharmaceutical Corp., East Hanover, N.J.; preparation of CsA used parenterally in humans) or PSC-833 (provided by Sandoz Pharmaceuticals) diluted in ethanol and corn oil) were given intraperitoneally at a dose of 10 mg/kg.

Statistical analysis

Data were analyzed using Kaplan-Meier methods for survival analysis. Differences in survivor functions obtained for groups treated with different agents were tested using the log-rank test. Three treatment regimens (VP-16 alone, VP-16/CsA, and VP-16/PSC) were compared with each other in both 3LL mice and mice bearing L1210 leukemia using pairwise tests. Adjustments for the multiple comparisons were made using the Bonferroni method. In order to have less than a 5% chance overall of finding a significant difference in survival between any pair of treatments when no true difference existed, the log-rank test for any pairwise comparison was interpreted as statistically significant for $P < 0.05/3 = 0.017$.

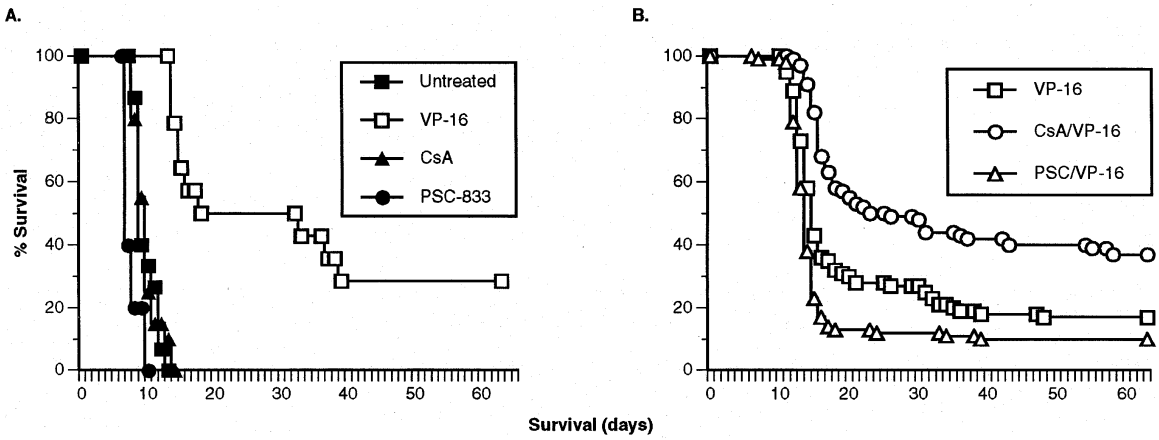


Fig. 1 A, B Survival curves of groups of BDF/1 mice bearing L1210 leukemia (**A**) untreated or treated with VP-16, CsA or PSC-833 and (**B**) treated with VP-16 alone or with CsA or PSC-833

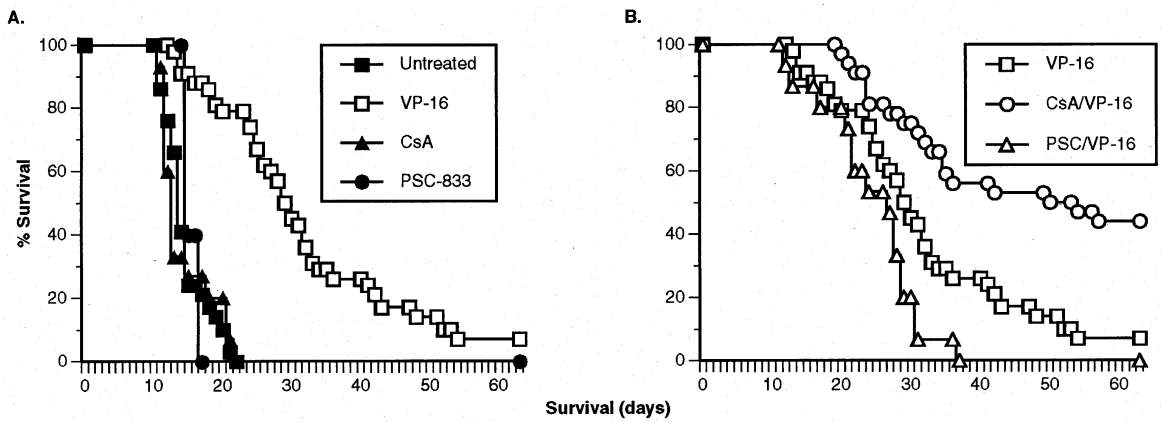
Results

Figures 1 A and 2 A show that CsA and PSC-833 failed to alter the survival of host mice bearing either L1210 leukemia or 3LL respectively. Figure 1 A shows the results of simultaneously performed experiments with the modulator controls, whereas Fig. 1 B and Fig. 2 show the pooled results of multiple experiments. In Fig. 1 B the survival of VP-16-treated L1210 leukemia-bearing mice is compared with the survival of mice treated with an equivalent dose of VP-16 with the addition of either CsA or PSC-833. Analysis of these survival curves reveals that the efficacy of VP-16 was significantly improved by the addition of CsA ($P < 0.0001$) and was significantly impaired by the addition of PSC-833 ($P < 0.004$). An additional control experiment showed that treatment with VP-16 combined with the corn oil-based vehicle used in the PSC-833 formulation failed to alter the survival of leukemic mice from that seen with VP-16 treatment alone ($P = 0.69$, data not shown). We have previously shown that the addition of cremaphor EL, the vehicle used in CsA solubilization fails to alter the effect of VP-16 in L1210 leukemia-bearing BDF/1 mice [6]. Figure 2 B shows the results of the treatment of mice bearing 3LL. The addition of CsA to VP-16 significantly improved the efficacy of VP-16 ($P < 0.0002$) and the addition of PSC-833 to VP-16 significantly impaired the efficacy of VP-16 ($P < 0.01$). The survival of animals bearing either L1210 leukemia or 3LL treated with VP-16/CsA was significantly higher than the survival of those treated with VP-16/PSC-833 ($P < 0.0001$). All pairwise comparisons were statistically significant at less than the 5% level after adjustments for multiple comparisons. All control BDF/1 and C57/Bl mice that were treated with combined VP-16 and PSC-833 in the absence of tumor survived for more than 60 days.

Discussion

There has been considerable interest in the potential clinical use of CsA and PSC-833 as modulators of MDR. Preliminary trials with CsA have produced encouraging results in the treatment of MDR myeloma and refractory acute

Fig. 2 A, B Survival curves of groups of C57/Bl mice bearing 3LL (**A**) untreated or treated with VP-16, CsA or PSC-833 and (**B**) treated with VP-16 alone or with CsA or PSC-833



myeloid leukemias [7, 8]. Although the use of CsA in these studies has been limited to short courses over a few days rather than for prolonged periods, PSC-833 has the theoretic advantage of being nonimmunosuppressive. Clinical trials with this agent are now underway.

Since chemotherapeutic cure of patients with malignant tumors occurs more frequently with initial therapy rather than upon salvage treatment after disease relapse, we have felt it important to investigate potential chemotherapeutic enhancement in drug-sensitive tumors. We have previously shown that CsA enhances the efficacy of daunorubicin in murine hepatoma and Ehrlich ascites carcinoma as well as the efficacy of VP-16 in P388 and L1210 leukemias in vitro and in vivo [3, 9]. Chan et al. have reported encouraging results in patients with retinoblastoma upon the addition of CsA to initial chemotherapy before disease relapse [10]. Keller et al. have reported that PSC-833 enhances the effect of VP-16 against xenotransplanted nonglycoprotein-expressing human colon adenocarcinoma in vivo [11], and we have recently reported that PSC-833 enhances in vitro toxicity of VP-16 in parental drug-sensitive L1210 leukemia [6].

Our current results showed that CsA and PSC-833 were inactive against murine L1210 leukemia and 3LL when used alone but that they significantly altered the survival of tumor-bearing mice when combined with VP-16. The addition of CsA to VP-16 enhanced survival whereas, surprisingly, the addition of PSC-833 to VP-16 shortened host survival compared with VP-16 therapy alone.

Both CsA and PSC-833 alter the pharmacokinetics of VP-16. This effect probably relates to the ability of these compounds to bind to P-glycoprotein which results in impaired hepatobiliary clearance of VP-16 thereby increasing the area of VP-16 under the plasma concentration versus time curve [11, 12]. Mechanisms beyond pharmacokinetic alteration of VP-16 must also be operative since we have noted that both CsA and PSC-833 enhance VP-16 cytotoxicity in vitro [3, 6]. When directly compared at equimolar concentrations, CsA shows significantly greater potentiation of VP-16 cytotoxicity than PSC-833 against L1210 leukemia [6]. CsA also shows interesting immunemodulating effects when combined with VP-16 producing specific antitumor immunity in a significant percentage of L1210 leukemia-bearing mice [13, 14]. These observations would predict superiority of CsA to PSC-833 as an in vivo enhancing agent of VP-16 but they fail to explain the significant impairment of VP-16 efficacy we observed with PSC-833. Although L1210 leukemia- and 3LL-bearing mice in our current study died with tumor, we cannot exclude the possible enhancement of systemic VP-16 toxicity by PSC-833 or its metabolites as a contributing factor to impaired host survival. This is unlikely, however, since the survival of tumor-free control mice was unimpaired by the combined VP-16/PSC-833 treatment regimens. Other possibilities include antagonism of VP-16 by PSC-833 or its metabolites or delivery vehicle. We can exclude the last possibility since control experiments comparing the survival of L1210 leukemic mice treated with VP-16 alone with mice treated with VP-16 in the corn oil-based vehicle used in the PSC-833 formulation showed no difference in survival. Since PSC-833 enhances VP-16 efficacy against sensitive L1210 leukemia in vitro, direct drug antagonism is also unlikely. The role of PSC-833 metabolites is unknown. Although the mechanism by which PSC-833 impairs in vivo VP-16 efficacy remains to be defined, our results suggest caution in the use of this compound in clinical trials in patients with drug-sensitive tumors.

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