CONCISE REPORT

Amantadine Potentiates T Lymphocyte Killing by an Anti–Pan-T Cell (CD5) Ricin A-Chain Immunotoxin

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The studies described in this report demonstrate that 1-adamantanamine hydrochloride (amantadine) is a potent enhancer of the cytotoxic activity of the anti–pan-T lymphocyte (CD5) T101 monoclonal antibody conjugated to purified ricin A-chain (T101-immunotoxin; T101-IT). We also demonstrate that T101-IT in the presence of amantadine does not induce immunotoxin-mediated cytotoxicity in nontarget cells such as human marrow hematopoietic progenitor cells. These results provide further knowledge for the improvement of ex vivo purification of human bone marrow from normal or leukemic T cells prior to allogeneic or autologous stem cell transplantation, respectively. Furthermore, since amantadine has long been employed safely in human therapy, its use in conjunction with immunotoxins might be exploited in vivo.

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RESULTS

Unless otherwise specified, 10 mmol/L T101-IT was employed throughout the experiments presented here. In previous studies, this concentration was found to be optimal in terms of high immunotoxin activity and lack of cytotoxicity to human hematopoietic progenitors.

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Viability of PB as well as BM mononuclear cells was assessed by Trypan blue dye exclusion test at the end of four hours incubation. Treatment with 0.1 to 1.5 mmol/L amantadine and T101-IT did not affect cell viability. Higher concentrations, ie, 1.75 and 2 mmol/L amantadine either with or without T101-IT, reduced the number of total viable cells to 84.75% ± 13.36% and 27.45% ± 9.68% of medium controls, respectively.

In the absence of enhancer molecules, four-hour treatment with T101-IT did not change the mitogenic response of PB T lymphocytes. In contrast, the addition of amantadine to the incubation medium enhanced the activity of T101-IT in a dose-related manner (Fig 1). The most effective nontoxic concentration was 1 mmol/L amantadine which in the presence of 10 mmol/L T101-IT resulted in the inhibition of T lymphocyte mitogenic response to 2.18% ± 2.50% of medium controls (n = 4). The same amantadine concentration potentiated also the T101-IT inhibition of PB T cell response to allogeneic cells in MLC (11.72% ± 1.26%, 12.23% ± 1.32%, and 12.98 ± 1.40 of medium, amantadine, and T101-IT controls, respectively; n = 1).

In parallel experiments, T101-IT in the presence of 10 mmol/L ammonium chloride reduced the PB T cell proliferative response to PHA and to allogeneic cells in MLC to only 41.23% ± 21.60% (n = 3) and 32.5% ± 5.38% (n = 1) of controls, respectively. T101-IT in the presence of 20 mmol/L ammonium chloride, or 10 mmol/L methylamine, or 100 μmol/L chloroquine reduced the T cell proliferative response to PHA to 20.04% ± 9.70%, 42.30% ± 11.30%, and 49.00% ± 9.83% (n = 2) of controls, respectively.

The kinetics of the inhibition of PB T lymphocyte proliferative response to PHA by T101-IT in the presence of 1 mmol/L amantadine or 20 mmol/L ammonium chloride, is shown in Fig 2. Amantadine sped the rate at which T101-IT inhibited T lymphocyte proliferation more dramatically than did ammonium chloride. In addition, the overall enhancement of T101-IT activity was markedly higher with amantadine than with ammonium chloride. A two-hour incubation with T101-IT and 1 mmol/L amantadine was sufficient to inhibit lymphocyte proliferative response to 1.29% ± 0.41% (n = 2) of control medium.

To address the possibility that amantadine is toxic to an accessory cell(s) necessary for the in vitro T cell activation and proliferation, PB mononuclear cells treated with T101-IT and amantadine were mixed at different ratios with autologous medium-treated PB mononuclear cells and assayed for PHA proliferative response. The observed rate of 3H-TdR incorporation corresponded to the expected values calculated on the basis of dilution in the cell mixtures, thus ruling out a toxic effect of amantadine plus T101-IT on an accessory cell(s) (data not shown).

Based on the results of the experiments in which PB lymphocytes were the target of the immunotoxin cytotoxicity, human BM mononuclear cells at 20 × 10⁶/mL were incubated for two hours with T101-IT alone or T101-IT plus either amantadine or ammonium chloride. The frequency of BM T lymphocytes was evaluated by a limiting dilution microculture assay which is the most sensitive method for the detection and enumeration of very low numbers of residual BM T cells. In the presence of 1 mmol/L amantadine, a two-log depletion of BM T lymphocytes was achieved. In contrast, when 10 mmol/L ammonium chloride was employed instead of amantadine, only one log reduction of BM T lymphocytes was achieved. In all instances, the treatment with T101-IT and/or either enhancer molecule did not significantly alter the recovery of CFU-GEMM, BFU-E, and CFU-GM hematopoietic progenitor cells (Table 1).

### DISCUSSION

The data presented in this report demonstrate that amantadine enhances T101-IT cytotoxic effect and that it is
superior to ammonium chloride both in terms of rapidity of action and level of T101-IT cytotoxicity against PB T lymphocytes. In 1984, Casellas et al reported that the degree of enhancement of T101-IT cytotoxicity against T leukemia CEM cells by 1 mmol/L amantadine is inferior to 100 μmol/L chloroquine, 10 mmol/L ammonium chloride, or 10 mmol/L methylamine. The same authors chose ammonium chloride in their subsequent work. The present study shows that when targets of T101-IT cytotoxicity are normal T cells, the most effective potentiator is amantadine followed by ammonium chloride, methylamine, and chloroquine. The diversity of our results compared to those of Casellas et al supports the notion that A-chain immunotoxins exhibit variability in target cell toxicity.

The immediate application of these data is the use of amantadine as optimal potentiator of T101-IT for ex vivo depletion of mature T lymphocytes from bone marrow grafts prior to allogeneic transplantation. Recently, Kernan et al have shown that the number of clonable residual T lymphocytes measured by a limiting dilution assay correlates with the development of GVHD in 32 leukemia patients following transplantation of histocompatible donor T cell-depleted BM. In particular, these authors found that 24 patients who received a median of 3.72 × 10^6 (0.92 to 16.0 × 10^6) residual BM T cells per kg of recipient body weight did not develop GVHD. Of the 8 patients who received a median of 23.99 × 10^6 (18.63 to 43.93 × 10^6) T cells per kg, 4 had no GVHD, and 4 developed grade I-II (skin only) GVHD. Employing the same limiting dilution assay for quantitation of residual BM T cells, we show in this report that T101-IT in the presence of amantadine induces a 2 log reduction of BM T lymphocytes without adversely affecting multipotential hematopoietic progenitor cells. Typical untreated BM grafts contain 1.0 × 10^6 (0.7 to 1.4 × 10^6) clonable T lymphocytes. Following a 2 log T cell reduction, an average 50 kg patient would receive 2 × 10^6 (1.4 to 2.8 × 10^6) T cells per kg. Thus, based on Kernan's studies, data presented here suggest that depletion of BM T cells by T101-IT plus amantadine may be effective in preventing severe grade III-IV GVHD.

Amantadine is a licensed drug employed for prophylaxis of influence A virus infections in humans. The in vitro concentrations found to be active in present experiments cannot be achieved in the blood of patients treated with amantadine. For this reason we are now investigating whether amantadine analogues will prove active at clinically occurring concentrations. T101-IT in the presence of non-toxic amantadine concentrations prompts its immediate use for a more efficient ex vivo depletion of human bone marrow T lymphocytes.

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REFERENCES


13. Royston I, Meyda JA, Boird SM, Meserve BL, Griffins JC: Human T-cell antigens defined by monoclonal antibodies: The 65,000 dalton antigen on T-cells (T65) is also found on chronic lymphocyte leukemia cells bearing surface immunoglobulin. J Immunol 125:7255, 1980


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