Prediction of the Optimal Timing of Bone Marrow Reinfusion after High Dose Chemotherapy


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ABSTRACT

Autologous bone marrow transplantation allows the use of high dose chemotherapy by obviating dose limiting myelosuppression. The pharmacology of high dose chemotherapy has been inadequately explored, yet this information is critical to determine the timing of marrow infusion and assure that engraftment is not compromised. We have used the Salmonella mutagenesis test (SMT) and colony forming unit-granulocyte, erythrocyte, monocyte, megakaryocyte assay to evaluate the optimal time for marrow infusion after therapy with high dose combinations of alkylating agents (Solid Tumor Autologous Marrow Support Program) in seven patients. The SMT is sensitive, rapidly performed, and has been used to detect mutagenic activity in urine following administration of cyclophosphamide, cisplatin, and 1,3-bis(2-chloroethyl)-1-nitrosourea. In parallel, determination of colony forming ability of the patients own bone marrow (colony forming unit-granulocyte, erythrocyte, monocyte, megakaryocyte assay), when cocultured with autologous serum obtained before and after treatment, provided an assay for circulating marrow toxic drugs or metabolites.

The onset of mutagenic activity in the SMT and the in vitro appearance of myelotoxicity by autologous serum in the colony forming unit-granulocyte, erythrocyte, monocyte, megakaryocyte assay were concurrent, and these activities returned to base line at the time of marrow infusion (72h posttreatment). One patient of the seven was excreting mutagens (TA100 strain only) at the time of marrow reinfusion; he developed hepatic venoocclusive disease, and delayed engraftment. These observations suggest that as high dose regimens evolve the SMT may serve as a rapid, sensitive indicator of the circulation and excretion of toxic compounds, and thereby assist in predicting the optimum time of bone marrow reinfusion.

INTRODUCTION

The dose response curve for many effective nonhormonal chemotherapeutic agents has been shown to be steep for both therapeutic and toxic effects (1). For many agents used in the clinical setting, myelosuppression limits the ability to use what might be maximally effective cytotoxic therapy. The use of autologous or allogeneic bone marrow infusions offers the opportunity to exploit fully the principle of dose escalation. Critical to these efforts is the assurance that reinfused marrow (colony forming unit-granulocyte, erythrocyte, monocyte, megakaryocyte assay), when cocultured with autologous serum obtained before and after treatment, provided an assay for circulating marrow toxic drugs or metabolites.

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3 The abbreviations used are: CPA, cyclophosphamide; CFU-GEMM, colony forming unit-granulocyte, erythrocyte, monocyte, megakaryocyte; BCNU, 1,3-bis(2-chloroethyl)-1-nitrosourea; cDDP, cisplatin; L-PAM, melphalan.
RESULTS

The Solid Tumor Autologous Marrow Support Program uses high doses of multiple alkylating agents followed by reinfusion of autologous cryopreserved bone marrow (Fig. 1). To determine the kinetics of excretion of the alkylating agents or their metabolites our initial approach was to measure mutagenic enzyme of histidine biosynthesis. TA1535 and its R-factor derivative TA100 detect mutagens that cause base pair substitutions primarily at one of these GC pairs. TA1535 has a considerably lower spontaneous mutation frequency than TA100 and is thus convenient for the detection of mutagens that do not preferentially revert TA100 (5). The TA100 strain was grown on ampicillin containing agar. Frequent resoliation of the strain with continual growth on ampicillin containing media failed to show spontaneous reversion. Assay plates were made with 30 ml of minimum glucose agar medium, composed of 1.5% Bacto-Difco agar, and 2% glucose in Vogel-Bonner salts. Minimal glucose agar medium (30 ml) was placed in 100-mm Petri dishes and covered with a top layer containing 0.6% Difco agar, 0.5% sodium chloride, 0.5 ml L-histidine hydrochloride, 0.5 ml biotin, and the urine samples obtained. Urine was obtained prior to treatment, during drug therapy, and for three days following autologous bone marrow reinfusion. Urine samples were diluted 250-fold to obviate the bactericidal activity in the urine samples. Urine, β-glucuronidase, and top agar were mixed, placed in sterile Petri dishes, and incubated at 37°C for 48 h.

CFU-GEMM. This was performed by a modification of the technique described by Buick et al. (6). Aliquots of bone marrow obtained at the time of bone marrow harvest were thawed and washed in Hanks' balanced salt solution containing 2.5% heat inactivated AB serum and 0.1 mg of DNase/ml (Sigma Chemical Co., St. Louis, MO). Thawed cells were cultured in a concentration of 1 x 10^5 viable cells in 35-mm Petri dishes containing a modified Eagle's media (Flow Laboratories, McLean, VA), 15% heat inactivated fetal calf serum, 15% autologous serum, 10% media conditioned by the GTC cell line (Gibco, Grand Island, NY), and 0.3% Noble agar (Tip Laboratories, Detroit, MI). The assay plates were incubated in a humidified atmosphere and 5% CO<sub>2</sub> at 37°C. Clusters (groups of 10-50 cells) and colonies (>50 cells) were scored on days 7 and 14.

Morning serum samples were obtained from the day preceding therapy to 3 days after bone marrow reinfusion. Separate CFU-GEMM assays of the marrow were done for each day of serum collection using a 1:2 dilution of autologous serum with fetal calf serum. Base-line values were obtained by performing the assay with heat treated autologous serum. The control values were obtained by culturing the marrow cells without autologous serum. Each separate assay was performed in triplicate.

activity was observed with another strain, TA1535. The relative reversion frequencies of the tester strains differed in that strain TA100 was more sensitive to the mutagenic effect of the compounds under study than was TA1535. The clearance of mutagenic urinary metabolites was such that in all seven patients a
rapid decline was observed over the 24–48 h following the conclusion of chemotherapy.

The Ames assay is sensitive to mutagens other than alkylating agents. During the period of study the only medications administered to the patients were perphenazine, benztropine, or diphenhydramine, and urine from patients taking only these agents was not mutagenic (not shown). No patient received antibiotics or i.v. hyperalimentation during the study period.

A more direct determination of the presence of cytotoxic compounds that might affect marrow engraftment is obtained by measuring the effect of serum on autologous bone marrow stem cell proliferation and differentiation in vitro.

When autologous serum was assayed utilizing the CFU-GEMM technique, an inverse relationship was observed between colony forming ability and mutagenic activity in the urine. Sera obtained during chemotherapy and for 24 h thereafter resulted in a 50–100% reduction in colony formation (Fig. 3). In all cases studied the CFU-GEMM assay returned to within 90% of the base-line level at the time of marrow infusions and some individuals demonstrated an overshoot in the colony count. Resolution of myelotoxic activity from serum was slower than the decline in urinary mutagenic activity and suggests that the CFU-GEMM may be a more sensitive measure of inhibitory effects on the bone marrow (Fig. 3). The single patient who required a second marrow infusion demonstrated delayed excretion of mutagenic activity but exhibited no myelotoxic activity in autologous serum in assays from the time point (Fig. 4). Patient 25 demonstrated persistent excretion of a low level of mutagenic activity up to 24 h after marrow infusion when using the TA100 tester strain. The area under the curve of his free BCNU level was 491 µM · min after a 600-mg/m² dose, one of the highest in the series for which the curve of his free BCNU level was 491 µM · min after a 600-mg/m² dose.

DISCUSSION

Programs using escalating doses of chemotherapy are based on the assumption that a close relationship exists between dose, dose rate, and the resulting response in metastatic solid tumors (1, 8–11). Ablation of bone marrow function by many of these regimens limits the practicality of this approach, but overwhelming myelotoxicity can be obviated by cryopreservation of autologous bone marrow and reinfusion following administration of chemotherapy.

The timing of bone marrow reinfusion is of critical importance, yet there is no rapid, reliable assay to detect toxic substances in the plasma that may delay marrow recovery and have important clinical consequences. The pharmacology of high dose chemotherapy is not adequately developed, and it is possible that standard pharmacological assays will lack sufficient sensitivity to detect low levels of biologically active compounds. The tests used in this study are biological, rather than pharmacological. They provide no information regarding concentrations of a specific administered agent, but provide a high level of sensitivity for biological effects. The Salmonella mutagenesis assay is rapidly performed and sensitive to small amounts of alkylating agents or other mutagens. The assay yields results within 48 h and, therefore, the timing of marrow reinfusion could be scheduled to coincide with disappearance of mutagenic activity. A potential liability of the Ames test is its sensitivity which may result in the detection of mutagens other than chemotherapeutic agents. In our studies mutagenic activity returned to base line at the time of marrow reinfusion in all but one patient. The absence of mutagenic activity in the urine is consistent with the absence of delay in bone marrow engraftment in these patients. The single patient in whom the return to base-line values did not occur by the time of marrow reinfusion had prolonged leukopenia and thrombocytopenia. This patient also had prolonged exposure to BCNU. The association of delayed urinary excretion of mutagens, increased exposure to BCNU, and delayed marrow engraftment are suggestive, but the predictive value of the Ames test in determining the optimal timing for bone marrow infusion must await the detailed study of a large number of patients.

The CFU-GEMM assay performed in the presence of serum obtained during treatment is a specific test for suppressive effects on bone marrow stem cells. Postchemotherapy inhibition by serum presumably relates to potential myelotoxicity, and recovery of the ability to form colonies in the presence of autologous serum is consistent with the loss of cytotoxic activity. These changes were observed in five patients treated with the Solid Tumor Autologous Marrow Support Program regimens. The serum samples used were drawn in the morning.

Fig. 3. CFU-GEMM colonies at day 14 per 10⁶ cells plated. CFU-GEMM were cocultured with 15% autologous serum drawn from pretreatment daily through 2 days after marrow reinfusion. Poisson distribution, 0.95 confidence intervals.
before CPA administration, and the survival of any colonies cultured with this serum reflects predominantly the effects ofDDP and long lived metabolites of CPA only (12). L-PAM and BCNU would not be present at this time4 (7, 10, 13).

Technical problems caution against reliance on this test for clinical decision making. Scoring colonies and clusters requires a period of 7–14 days from the time the assay is begun, making it impractical as an ongoing monitor in an individual patient. Second, the assay is expensive, and requires technical expertise. However, in the setting of a phase I trial it can provide important complementary information to pharmacological data and to information obtained from the mutagenesis assay.

Of the two assays studied, our observations indicate that the Salmonella mutagenesis test is a rapid, inexpensive bioassay that can document the clearance of mutagenic metabolites from serum and appears to correlate with successful engraftment after bone marrow infusions. Its ultimate utility in planning novel treatment strategies in the autologous transplant setting can be firmly established by developing correlations between the pharmacokinetics of drug clearance, the appearance and disappearance of mutagenic activity in the urine, and hematological recovery.

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